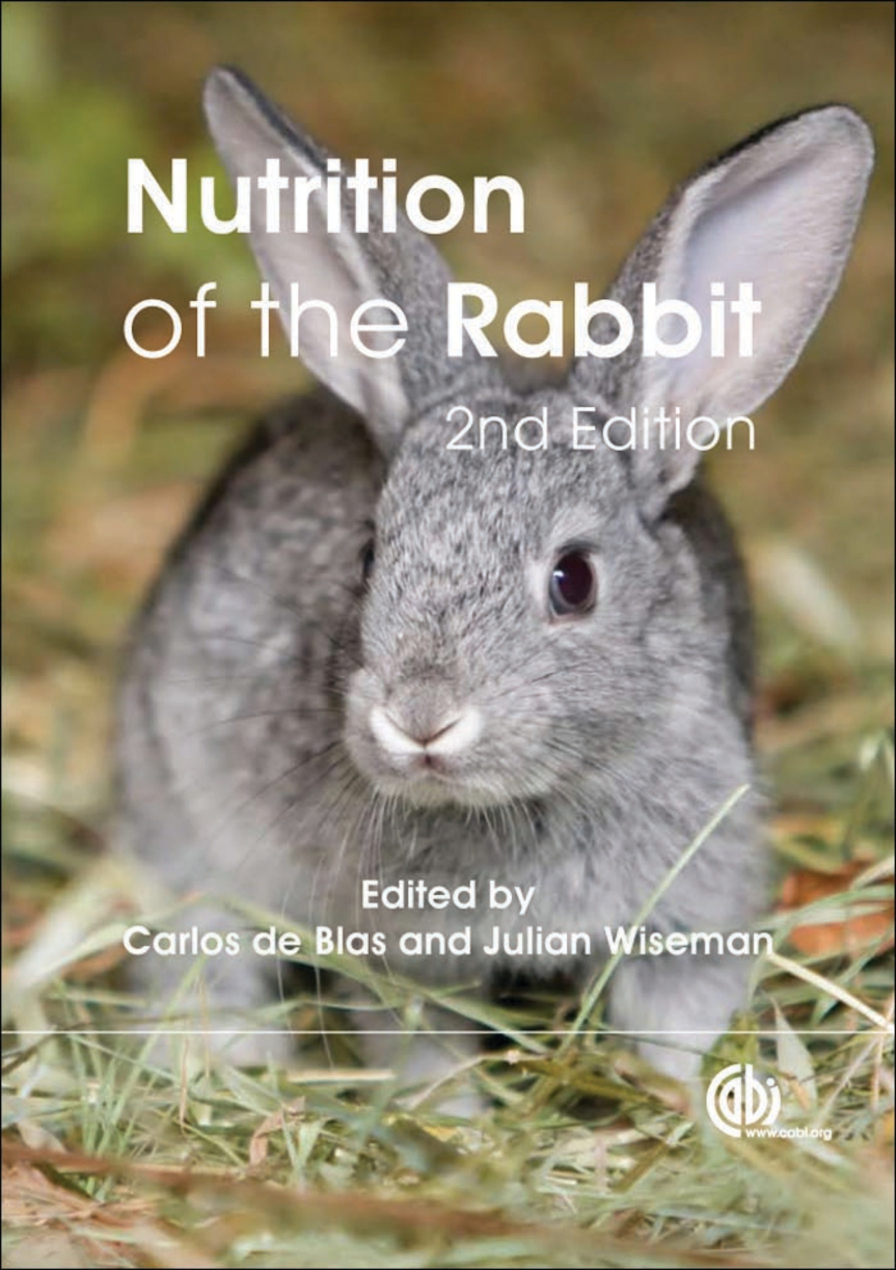
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of the Rabbit
2nd Edition
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1 The Digestive System of the Rabbit

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1.1 Introduction

The digestive system of the rabbit is characterized by the relative importance of the caecum and colon when compared with other species (Portsmouth, 1977). As a consequence, the microbial activity of the caecum is of great importance for the processes of digestion and nutrient utilization, but also in the control of digestive pathologies. Furthermore, caecotrophy, the behaviour of ingestion of soft faeces of caecal origin, makes microbial digestion in the caecum more important for the overall utilization of nutrients by the rabbit. Additionally, the rabbit has developed a strategy of high feed intake (65–80 g kg^{-1} body weight (BW)) and a rapid transit of feed through the digestive system to meet nutritional requirements.

To reach its full functional capacity, the digestive system of the growing rabbit must go through a period of adaptation from milk-base feeding to the sole dependence on solid feed. This adaptation process not only affects the digestion processes, but also microbiota colonization and the development of gut barrier mechanisms that protect the animal against digestive pathologies. This chapter: (i) gives a general and brief description of the morphological and functional characteristics of the digestive system of the rabbit that may be important for understanding the digestive processes explained in the following chapters; and (ii) explains how these characteristics change from the time of weaning until attainment of maturity.

1.2 The Digestive System of the Rabbit

The first important compartment of the digestive system of the rabbit is the stomach; this has a very weak muscular layer and is always partially filled. After caecotrophy the fundic region of the stomach acts as a storage cavity for caecotrophes. Thus, the stomach is continuously secreting and the pH is acid. The stomach pH ranges from 1 to 5, depending on site of determination (fundus versus cardiac-pyloric region) (Gutiérrez *et al.*, 2002, 2003; Chamorro *et al.*, 2007; Orengo and Gidenne, 2007; Gómez-Conde *et al.*, 2009), the presence or absence of soft faeces (Griffiths and Davies, 1963), the time from feed intake (Alexander and Chowdhury, 1958) and the age of the rabbit (Grobner, 1982). The lowest figures (from 1 to 2.5) are determined in the cardiac region, in the absence of soft faeces, after 4 h of diet ingestion and in rabbits older than 3 weeks with low presence of milk (Orengo and Gidenne, 2007). The capacity of the stomach is about 0.34 of the total capacity of the digestive system (Portsmouth, 1977). The stomach is linked with a coiled caecum by a small intestine approximately 3 m long, where the secretion of bile, digestive enzymes and buffers occurs. The pH of the small intestine is close to 7 (Vernay and Raynaud, 1975; Nicodemus *et al.*, 2002). The small intestine is the site where the greater part of digestion and absorption take place by passive or active transportation throughout the mucosa. Digestibility at the end of the ileum accounts for 0.8–1 of the total dietary amino acid and starch digestibility (Gutiérrez *et al.*, 2002; García *et al.*, 2005; Carabaño *et al.*, 2009).

The caecum is characterized by a weak muscular layer and contents with a dry matter (DM) of 200 g kg^{-1}. The caecal contents are slightly acid (pH 5.4–6.8) (García *et al.*, 2002). The capacity of the caecum is approximately 0.49 of the total capacity of the digestive tract (Portsmouth, 1977). The colon can be divided in two portions: the proximal colon (approximately 35 cm long) and the distal colon (80–100 cm long). The proximal colon can be further divided into three segments: the first segment possesses three taeniae with haustra between them; the second segment has a single taenia covering half of the circumference of the digestive tube; and the third segment or fusus coli has no taeniae or haustra, but is densely enervated. Thus, it acts as a pacemaker for the colon during the phase of hard faeces formation (Snipes *et al.*, 1982).

Other tissues are also associated with the gut. Gut-associated lymphoid tissue (GALT) and specialized cells (goblet or Paneth cells, responsible for mucus and antimicrobial peptide secretion, respectively) regulate the interaction of the gut mucosa with the microbiota and develop the mechanisms of tolerance and protection against pathogens. The gut barrier function has been recently reviewed by Forthun-Lamothe and Boullier (2007) and Carabaño *et al.* (2008).

1.3 Age-related Changes in the Morphology and Function of the Digestive System

The different segments of the digestive system of the rabbit grow at different rates until reaching maturity. The development of the digestive tract begins in the fetal stage; at birth, the stomach and small intestine are the main components of digestive tract. According to Toofanian and Targowski (1982) and Sabatakou *et al.* (1999), the stomach glands are evident in late fetuses (26 days' gestation) and true villi and intestinal glands (crypts of Lieberkühn) are observed at 29 days' gestation. At birth, however, the intestine of the newborn does not possess all of the mucosal constituents that are present in the adult. These appear in the first week of age (Brunner's glands in the duodenum) and the adult morphology is not completed until 20 days of age.

The developmental pattern follows a cranio-caudal gradient. The early development of these two segments is important to ensure the survival of the newborn (Fig. 1.1a). From birth to 18–20 days of age, kits drink large amounts of milk during a once-daily nursing, an amount that can reach 0.12 of their BW. This explains the importance of the relative weight of the stomach when its contents are also recorded (Fig. 1.1b). At around 18 days of age the suckling rabbit begins to eat solid food and decrease its milk intake (see Chapter 13 for more details) and the caecum and colon develop faster than the rest of the digestive tract (Fig. 1.1a). The fast growth of the caecum during this period is more evident if the caecal contents are included (Fig. 1.1b). From 3 to 7 weeks of age the caecum is filled by digesta and microbiota, and its contents reach a peak of about 0.06 to total BW at 7–9 weeks of age. The pH of the caecum is also affected by age and decreases from 6.8 at 15 days of age to 5.6 at 50 days of age (Padilha *et al.*, 1995).

The study of the evolution of the functionality of the intestinal mucosa and pancreas is important to understand the ability of the animals (mainly around weaning) to digest substrates other than milk. There has been much effort in the last 10 years to clarify this subject, but some discrepant results remain (Tables 1.1 and 1.2). Differences in the management of animals before sampling (fast or free access to feed, type of diet), the interval of age studied, type of sample analysed (digesta, tissue or serum), place in the intestinal tract (duode-

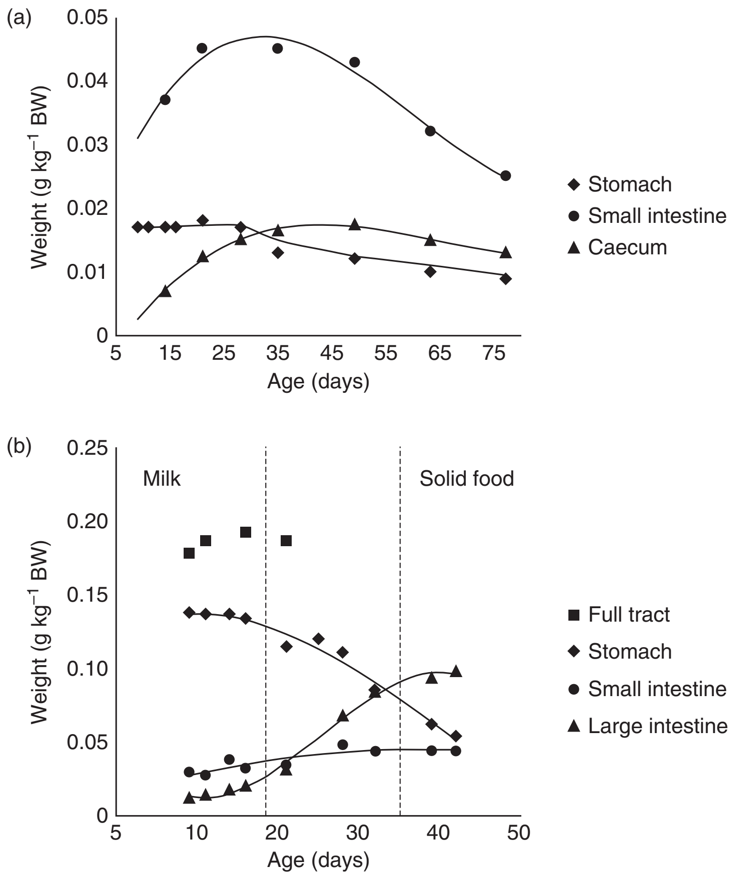

Fig. 1.1. Development of different segments of the digestive tract of the rabbit (weaned at 35 days) from 9 to 77 days of age. (a) The relative weight of empty segments with respect to body weight; (b) the relative weight of the segments and their contents with respect to body weight (Lebas and Laplace, 1972; García Rebollar *et al*., 2004; Gallois *et al*., 2005). BW, body weight.

num, jejunum or ileum), time of slaughter (morning or evening), enzymatic methodology (specificity of substrate, time, pH and temperature of the reaction) and unit used to express the activity (IU per mg of protein or mg of tissue or mg digestive content, etc.) may partially explain these discrepancies and make it difficult to give clear conclusions to formulate adequate post-weaning diets.

During the suckling period, the mucosal glands are able to produce enzymes to digest the main components of the milk, while the maturity and functionality of the pancreas are limited when compared with the adult. In this period, gastric lipase represents most of the lipolytic activity of the whole digestive tract, whereas this activity is not detectable in the 3-month-old rabbit (Marounek *et al*., 1995). Lactase activity is highest until 25 days of age, and sucrase and maltase rise until reaching the adult level at around 28–32 days (Gutiérrez *et al*., 2002; García Rebollar *et al*., 2004; Gallois *et al*., 2008b). The main proteolytic activity is also localized in the stomach of the young rabbit and its importance decreases with age as proteolytic activity in the caecum, colon and pancreas increases (Marounek *et al*., 1995). There is common agreement that the functionality of the digestive tract is limited from 21 to 42 days of age for amylase and lipase secreted by the pancreas and some enzymes of the gastric or intestinal mucosa; however,

Table 1.1. Evolution with the age of pancreatic enzymes, according to several authors.

Enzyme	Sampling place	Age (days) 7–21	21–45	45–90	90–180	Unit	Ref.
Amylase	Pancreas	=	▲			IU g^{-1} tissue	1, 2, 3
			▲			IU g^{-1} protein	
	Pancreas	=	=	▲	=	IU g^{-1} protein	5
	Pancreas		▲			IU g^{-1} protein	7
	Pancreas		▲			IU g^{-1} protein	8
	Intestinal content		▲	▲	▲	IU g^{-1}	4
	Intestinal content						
	Jejunum		▲			IU g^{-1}	6
	Ileum		=				
	Intestinal content	=	▲			IU g^{-1}	9
	Serum	=	=	▲	=	IU l^{-1}	5
	Serum		▲			IU l^{-1}	10
Lipase	Pancreas	=	▲			IU g^{-1} tissue	1, 2
		=	▲			IU g^{-1} protein	
	Pancreas	▼	▼	=	=	IU g^{-1} protein	5
	Pancreas		▲			IU g^{-1} protein	7
	Intestinal content		=	=	=	IU g^{-1}	4
	Intestinal content		▲			IU g^{-1}	10
Trypsin	Pancreas	▼	▲			IU g^{-1} tissue	1, 2
		▼	=			IU g^{-1} protein	
	Pancreas	▲	=	=	=	IU g^{-1} protein	5
	Pancreas		=			IU g^{-1} protein	7
	Intestinal content		▼			IU g^{-1}	10
Chymotrypsin	Pancreas	▼	▲			IU g^{-1} tissue	1, 2
		▼	=			IU g^{-1} protein	
	Pancreas	▲	▲	▼	▼	IU g^{-1} protein	5
	Pancreas		=			IU mg^{-1} protein	7
Proteases	Intestinal content		=	=	=	IU g^{-1}	4

=, ▲,▼: enzyme activity remains constant, increases or decreases, respectively, in each period.
1, Lebas *et al.* (1971); 2, Corring *et al.* (1972); 3, Blas (1986); 4, Marounek *et al.* (1995); 5, Dojanã *et al.* (1998); 6, Scapinello *et al.* (1999); 7, Gutiérrez *et al.* (2002); 8, Debray *et al.* (2003); 9, Sabatakou *et al.* (2007); 10, Gallois *et al.* (2008b).

protease activity is unclear. These findings are in line with the evolution of the pancreas, which greatly increases in weight when the animals begin to eat solid feed (Lebas *et al.*, 1971), and the development of intestinal morphology (Gallois *et al.*, 2008a). However, this limited enzymatic capacity allows young rabbits (35 days old) to digest 0.9–0.96 of starch by the end of the ileum (Gutierrez *et al.*, 2002; Gómez-Conde *et al.*, 2007).

Other enzyme activities that increase markedly with the age of the rabbit are those due to the presence of microorganisms that will determine the ability of the rabbit to utilize fibre sources. Cellulase, pectinase, xylanase and urease are some of the main enzymes provided by the intestinal microflora.

The major age-related changes in the morphologic and functional maturation of the digestive tract seem to be associated with the change from milk to solid food in the feeding pattern of the young rabbit. Furthermore, a higher solid-feed intake in this transition period leads to better growth performance and lower mortality in the growing period (Pascual, 2001). This has increased the interest in studying the effect of an improvement in solid-feed intake by different management techniques such as weaning or modulating the litter size. The

Table 1.2. Evolution with age of gastric and intestinal mucosa, according to several authors.

Enzyme	Sampling place	Age (days) 7–21	21–45	45–90	90–180	Unit	Ref.
Pepsin	Gastric mucosa	▲	▲	▲	=	IU g^{-1} tissue	2
		=	=	=	=	IU g^{-1} protein	
	Stomach content		=	=	=	IU g^{-1}	1
Lactase	Mucosa jejunum		▼			IU g^{-1} protein	3
	Mucosa jejunum	=				IU g^{-1} tissue	5
Maltase	Mucosa						
	Duodenum	=	=	▼	=	IU g^{-1} protein	2
	Jejunum	=	=	▲	=		
	Ileum	=	=	▲	=		
	Mucosa jejunum		=			IU g^{-1} protein	3
	Mucosa jejunum		▲			IU mg^{-1} tissue	4
	Mucosa						6
	Duodenum	=	▲				
	Jejunum	=	▲				
	Intestinal content		▲	▲	▲	IU g^{-1}	1
Sucrose	Mucosa jejunum		▲			IU g^{-1} protein	3
	Mucosa jejunum	▲				IU g^{-1} protein	5
	Duodenum	▲	=			IU g^{-1} protein	6
	Jejunum	▲	▲				

=, ▲,▼: enzyme activity remains constant, increases or decreases, respectively, in each period.
1, Marounek *et al.* (1995); 2, Dojanã *et al.* (1998); 3, Gutiérrez *et al.* (2002); 4, Debray *et al.* (2003); 5, García Rebollar *et al.* (2004); 6, Gallois *et al.* (2008b).

effect of age at weaning (from 21 to 35 days) seems to have little influence on the morphology and enzymatic activity in the upper tract (stomach and small intestine) (Corring *et al.*, 1972; Scapinello *et al.*, 1999; Gallois *et al.*, 2005, 2008b). On the other hand, some authors (Gutierrez *et al.*, 2002; Gómez-Conde *et al.*, 2007) have observed villous atrophy accompanied by a reduction of brush border enzymes in 35-day-old rabbits weaned at 25 days compared with suckling rabbits of the same age. However, these problems seem to be dependent on the composition of the weaning diet. The inclusion of moderate levels of soluble fibre in the diet seems to be enough to avoid these problems (Gallois *et al.*, 2005, 2008b; Alvarez *et al.*, 2007; Gómez-Conde *et al.*, 2007). The effects of weaning on the maturation of the caecum and colon seem to be positive. Early weaning increases the weight of the organs and their contents, encourages microbiota colonization (quantity and type of bacteria), promotes fermentative activity and accelerates the maturation of GALT (Piattoni and Maertens, 1999; Niza *et al.*, 2001; Gutierrez *et al.*, 2002; Xiccato *et al.*, 2003; Gallois *et al.*, 2005, 2008a; Carabaño *et al.*, 2008; Kovács *et al.*, 2008).

1.4 Development of the Immune Response: the Gut-associated Lymphoid Tissue

At birth, the rabbit immune system is immature. B-cell lymphogenesis begins in the fetal liver and omentum before switching to the bone marrow, where immunoglobulin (Ig) rearrangement has been described at around 12 days' gestation (Mage *et al.*, 2006). As maternal Ig levels decrease (at a few weeks after birth), B cells are transported from the liver and the bone marrow to the GALT, where somatic diversification and expansion of Ig genes takes place. In the rabbit, the GALT comprises an organized lymphoid tissue that consists of Peyer's patches, the appendix and the sacculus rotundus, and a diffuse form represented by the lamina propria and intraepithelial lymphocytes (Carabaño *et al.*, 2008). Therefore, the primary antibody repertoire is generated and developed between 4 and 8 weeks of age in the GALT (especially in the appendix) in response to the host interaction with the intestinal microbiota (Lanning *et al.*, 2004; Mage *et al.*, 2006). Intestinal bacteria are thus necessary for the rabbit immune system as they promote GALT development and the somatic diversification of Ig (Rhee *et al.*, 2004). However, the bacterial species that trigger immune system development and the mechanisms they use remain to be elucidated. In this regard, research has identified *Bacteroides fragilis* and *Bacillus subtilis* (two members of the normal rabbit intestinal microflora) as inducers of GALT development and primary antibody repertoire diversification (Mage *et al.*, 2006). Moreover, the mechanisms used by these bacterial species to drive GALT development and somatic diversification of Ig genes are not mediated by antigen interactions (Rhee *et al.*, 2004). They seem to be induced by a B-cell superantigen through a direct interaction with the B-cell receptor or through stimulation of the innate immune system via Toll-like receptors (Rhee *et al.*, 2004). In addition, the cytokine environment produced after the activation of macrophages and dendritic cells by bacterial products (e.g. lipopolysaccharide) may be another possible way of developing the immune repertoire (Rhee *et al.*, 2004).

Bacterial translocation occurs spontaneously in the newborn rabbit, reaching a maximum at 6 days of age. By this time, the GALT begins to develop limited bacterial passage (Urao *et al.*, 1996). The commensal flora starts to develop during the lactation stage, but the fermentative area only begins to grow after feed consumption. Dasso *et al.* (2000) reported that the follicular area in the appendix increases between 3 and 6 weeks after birth, and maintains the same relative importance until the adult stage. The proliferative area of the follicles also reached a maximum at 6 weeks and the proportion of total lymphocytes and the proportion of B cells in lamina propria increased in rabbits between 19 and 26 days of age (Campín *et al.*, 2003; Carabaño *et al.*, 2008). In this regard, weaning can accelerate the maturation of the immune system and its functionality, as a larger follicular area in the appendix and increased lymphocyte numbers in lamina propria have been recorded in rabbits weaned at 25 days (Carabaño *et al.*, 2008). However, it is important to take into account that, at weaning, the immune system is not fully developed. An immature immune system together with the stress associated with weaning and the decrease in milk intake that confers protective Ig and bactericidal nutrients (peptides, short-chain fatty acids) might challenge rabbit's health status (Gallois *et al.*, 2007; Skrivanová *et al.*, 2008; Romero *et al.*, 2009).

Specific to the rabbit is suppression of B lymphopoiesis with age. This is reversibly arrested by 4 months (Kalis *et al.*, 2007). Thus, the secondary antibody repertoire of the adult rabbit is developed by the expansion of specific B cells and somatic mutation of Ig genes in response to specific antigens at the secondary lymphoid organs (Lanning *et al.*, 2000).

1.5 The Role of the Intestinal Flora in the Digestion and Absorption of Nutrients

The presence of the microbial population in the caecum, together with caecotrophy, permits the rabbit to obtain additional energy, amino acids and vitamins. The main genus of the microbial population in the caecum of the adult rabbit is *Bacteroides* (Gouet and Fonty, 1973), which comprises 10^9–10^{10} bacteria g^{-1}. Other genera such as *Bifidobacterium*, *Clostridium*, *Streptococcus* and *Enterobacter* complete this population to give a bacterial load of 10^{10}–10^{12} bacteria g^{-1} (Bonnanfous and Raynaud, 1970; Gouet and Fonty, 1979; Forsythe and Parker, 1985; Penney *et al.*, 1986; Cortez *et al.*, 1992). However, more recent research, based on molecular techniques, has shown that the complexity of the gut microbiota is greater than was previously described with classical approaches (Bennegadi *et al.*, 2003; Badiola *et al.*, 2004; Abecia *et al.*, 2005). With these studies, it can be inferred that, as expected, the majority (0.66) of the components of the rabbit microbiota is unknown/uncultured and that bacteria only represent 0.40 of the total microbiota in young rabbits (18 days old), increasing their importance (0.80–0.90) when the animals begin to eat solid food. The use of molecular techniques has allowed an understanding of the importance of the transmission of the whole microbiota to the youngster through the mother and its evolution with age or the influence of nutrients in the development of digestive diseases (Carabaño *et al.*, 2006; Gidenne *et al.*, 2008). However, more information is needed to describe the bacterial community in the gastrointestinal tract of rabbits.

The role of the whole microflora community in the digestive processes can also be evaluated by its enzymatic activity or the end products of fermentation. The presence of cellulolytic bacteria in the caecum of the rabbit had been indicated by Hall (1952) and Davies (1965). Later, Emaldi *et al.* (1979) studied the enzymatic activities of the microflora and indicated that the main activities were, in decreasing order, ammonia use, ureolytic, proteolytic and cellulolytic. The great importance of other activities (i.e. xylanolytic, pectinolytic, mucolytic) has been indicated in studies conducted by Forsythe and Parker (1985), Marounek *et al.* (1995) and Sirotek *et al.* (2003). Forsythe and Parker (1985) estimated populations of 10^8 and 10^9 xylanolytic and pectinolytic bacteria g^{-1}, respectively.

The composition of the microflora does not remain constant throughout the life of the rabbit and is strongly influenced by the time of weaning (Padilha *et al.*, 1996). During the first week of age, the digestive system of the rabbit is colonized by strict anaerobes, predominantly *Bacteroides*. At 15 days of age, the numbers of amylolytic bacteria seem to stabilize, whereas those of colibacilli decrease as the numbers of cellulolytic bacteria increase (Padilha *et al.*, 1995). Milk intake may delay the colonization by cellulolytic flora, but does not seem to affect the evolution of the colibacilli population (Padilha *et al.*, 1996). As a consequence of age-related changes in the microbial population, the production of volatile fatty acids (VFAs) increases with age (Bellier *et al.*, 1995; Padilha *et al.*, 1995). Moreover, as caecotrophy is initiated, the presence of bacteria of caecal origin can be detected. Smith (1965) and Gouet and Fonty (1979) were able to detect precaecal microbial flora after only 16 and 17 days of age, respectively. The presence of these precaecal microbes is dependent on caecotrophy, with high counts after caecotrophy and no viable cells after 5–6 h (Jilge and Meyer, 1975). The composition of the microflora does not remain constant during the life of the rabbit.

As a result of the fermentative activity of the microflora, VFAs are produced in the proportion of 60–80 mol of acetate, 8–20 mol of butyrate and 3–10 mol of propionate 100 mol^{-1} of VFAs (García *et al.*, 2002). However, these proportions change with the time of the day, as described in the caecotrophy section of this chapter (see section 1.6), and with the developmental stage of the rabbit, with increases in the acetate concentration from 15 to 25 days of age and a reversal of the propionate to butyrate ratio from 15 to 29 days of age (Padilha *et al.*, 1995). The potential of modification of VFA production by dietary changes will be described in the following chapters of this book. According to Marty and Vernay (1984), VFAs can be metabolized in the hindgut tissues, with butyrate being the preferred substance for the colonocytes. The liver is the main organ metabolizing absorbed propionate and butyrate. However, acetate is available for extrahepatic tissue metabolism. It is estimated that the rabbit obtains up to 0.40 of its maintenance energy requirements from VFAs produced by fermentation in the hindgut (Parker, 1976; Marty and Vernay, 1984).

1.6 Caecotrophy

1.6.1 Patterns of daily feed intake and soft faeces excretion

Soft faeces are excreted according to a circadian rhythm, which is the opposite to that of feed intake and hard faeces excretion. Caecotrophy occurs mainly during the light period, whereas feed intake and hard faeces excretion occur during darkness (Lebas and Laplace, 1974, 1975; Fioramonti and Ruckebush, 1976; Ruckebush and Hörnicke, 1977; Battaglini and Grandi, 1988; Merino, 1994; Bellier *et al.*, 1995; Bellier and Gidenne, 1996; El-Adawy, 1996; Orengo and Gidenne, 2007). Figure 1.2 shows the pattern of soft faeces excretion and feed intake for adult rabbits under a schedule of 12 h light/12 h dark and *ad libitum* access to feed (Carabaño and Merino, 1996). Most of the rabbits showed monophasic patterns of soft faeces excretion from 08.00 to 17.00 h, with a maximum at 12.00 h. However, 0.25 of rabbits showed a diphasic pattern, with a second period of excretion during the night. The occurrence of diphasic patterns is more frequent when the length of the light period is reduced. Under continuous light conditions (24 h) caecotrophy runs freely and monophasically (Jilge, 1982). During the caecotrophy period, lasting from 7 to 9 h, there is an absence of hard faeces excretion and the feed intake is low.

Feed intake and hard faeces excretion occur along the complementary period, showing two phases. Feed intake increases from 15.00 to 18.00 h and then remains high until 24.00 h (Fig. 1.2). After this period, rabbits reduce feed intake until 02.00 h and then a new phase starts, with a maximum at 06.00 h. The second phase finishes at 08.00 h. Hard faeces excretion (from 18.00 to 08.00 h) shows a similar pattern, with two maxima at 24.00 and 06.00 h.

Age, physiological status and restricted access to feed can alter this pattern. Bellier *et al.* (1995) observed that weaned rabbits (6 weeks old) show a greater incidence of diphasic patterns and a longer caecotrophy period than adults (14 weeks old): from 04.00 to 12.00 h and from 22.00 to 24.00 h versus from 08.00 to 14.00 h, respectively. Lactating does show a different pattern of excretion from that described previously for non-lactating adult rabbits. During the lactation period, does exhibit an alternated rhythm of soft and hard faeces excretion. Caecotrophy occurs during two periods, from 02.00 to 09.00 h (0.40 of total excretion) and from 13.00 to 17.00 h (0.60 of total excretion), with a lack of excretion from 09.00 to 13.00 h (Lorente *et al.*, 1988). This pattern could be mainly related to the maternal behaviour of does through the morning rather than to physiological status.

All of the experiments described above were carried out with *ad libitum* access to feed. When the feeding regime is changed from *ad libitum* to restricted access to feed the rhythm of excretion is profoundly altered, whatever the length of the light

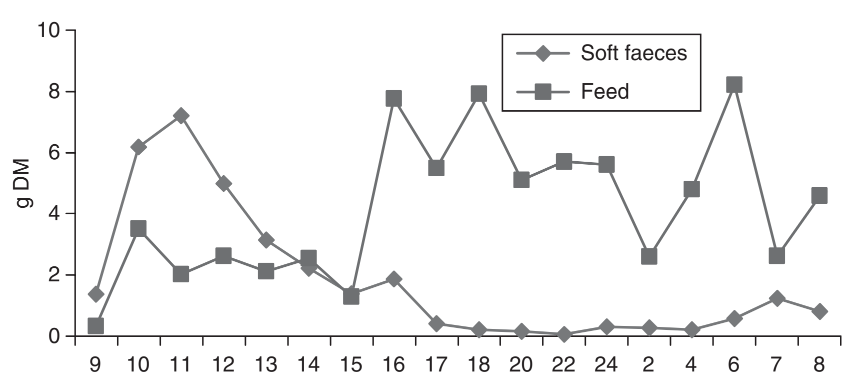

Fig. 1.2. Soft faeces excretion and dry matter (DM) intake throughout the day (Carabaño and Merino, 1996).

period. In this situation, the time of soft faeces excretion depends on the time of feed distribution (Fioramonti and Ruckebush, 1976). Disruption of the internal cycle may have important practical implications. Lebas and Laplace (1975) recommended distributing the feed once per day late in the afternoon. In other scenarios (e.g. one meal at 09.30 h or two meals at 09.30 and 16.30 h), changes in faecal excretion patterns and a lower growth rate should be expected.

1.6.2 Determination of soft faeces excretion and consumption

Several authors have tried to explain the physiological mechanisms that determine the differentiation and recognition of the two types of faeces in rabbits, according to the circadian patterns described above. The results obtained allow a partial understanding of the complex regulation of this behaviour.

Differentiation between soft and hard faeces begins during the transit of digesta through the caecum and proximal colon. From the results obtained by Björnhag (1972) and Pickard and Stevens (1972) it can be assumed that the formation of hard faeces is not by resorption of some components of caecal contents in the colon, but by mechanical separation of the different components of digesta. During hard faeces excretion, water-soluble substances and fine particles (<0.3 mm in diameter, including microorganisms) are brought back to the caecum by means of antiperistaltic movements and retrograde flow. Coarse particles (>0.3 mm in diameter) pass to the distal part of the colon. In contrast, the motility of both the caecal base and the proximal colon decreases during the formation of soft faeces (Ruckebush and Hörnicke, 1977). Endogenous prostaglandins (PGs) play an important role in the motor function involved in soft faeces formation. The infusion of both PGE_2 and $PGF_{2\alpha}$ inhibits proximal colon movements, stimulates the distal colon and is followed by soft faeces production (Pairet *et al.*, 1986). Changes in VFA concentrations and caecal pH occurring after a meal have been proposed as primary signals leading to a period of soft faeces excretion. Ruckebush and Hörnicke (1977) observed soft faeces excretion after an intracaecal infusion of VFAs in rabbits with restricted access to feed. However, postprandial VFA variations are not so evident in rabbits fed *ad libitum*, and therefore factors other than those mentioned above could also be implicated. Structures that are typically involved in feed intake regulation, such as the lateral hypothalamus and hypothalamic ventromedial nodes, do not seem to have the same roles as those described in other nonruminant species. Damage to these structures does not imply changes in feed intake behaviour in rabbits (Gallouin, 1984).

During soft faeces excretion, the caecal contents are covered by a mucous envelope secreted at the proximal colon according to the described circadian rhythms. Therefore, the soft faeces consist of small pellets of 5 mm diameter that rabbits can recognize. Soft faeces are taken directly from the anus, swallowed without mastication and stored intact in the fundus of the stomach for 3–6 h (Gidenne and Poncet, 1985). The mechanisms of recognition are unclear. The special smell of soft faeces compared with that of hard faeces or the existence of mechanoreceptors in the rectum have been proposed as factors involved in the reingestion of soft faeces. However, results obtained from rabbits deprived of olfactory bulbs and with an artificial anus that bypasses the rectum show that rabbits are still able to recognize and reingest soft faeces (Gallouin, 1984).

1.6.3 Nutritional implications

Caecotrophy in rabbits does not occur as a response to a nutritional imbalance, but represents a specialized digestive strategy. Caecotrophy begins at 3–4 weeks of age, when rabbits begin to consume solid food. In postweaned rabbits (4 weeks old), soft faeces production linearly increases with age, reaching a maximum at 63–77 days old (25 g DM day^{-1}). This period corresponds to the maximum growth requirements and to the greatest increment in feed intake. From 77 to 133

Table 1.3. Average chemical composition of caecal contents and soft and hard faeces.

	Caecum	Soft faeces	Hard faeces	Ref.
Dry matter (g kg^{-1})	200	340	470	3, 4, 5, 6, 7
Crude protein (g kg^{-1} DM)	280	300	170	3, 4, 5, 6, 7
Crude fibre (g kg^{-1} DM)	170	180	300	3, 4, 5, 6, 7
MgO (g kg^{-1} DM)	–	12.8	8.7	2
CaO (g kg^{-1} DM)	–	13.5	18.0	2
Fe_2O_3 (g kg^{-1} DM)	–	2.6	2.5	2
Inorganic phosphorous (g kg^{-1} DM)	–	10.4	6.0	2
Organic phosphorous (g kg^{-1} DM)	–	5.0	3.5	2
Cl^- (mmol kg^{-1} DM)	–	55	33	2
Na^+ (mmol kg^{-1} DM)	–	105	38	2
K^+ (mmol kg^{-1} DM)	–	260	84	2
Bacteria (10^{10} g^{-1} DM)	–	142	31	2
Nicotinic acid (mg kg^{-1})	–	139	40	1
Riboflavin (mg kg^{-1})	–	30	9	1
Panthotenic acid (mg kg^{-1})	–	52	8	1
Cyanocobalamin (mg kg^{-1})	–	3	1	1

1, Kulwich *et al.* (1953); 2, adapted from Hörnicke and Björnhag (1980); 3, Carabaño *et al.* (1988); 4, Carabaño *et al.* (1989); 5, Fraga *et al.* (1991); 6, Motta-Ferreira *et al.* (1996); 7, Carabaño *et al.* (1997).

days old (2.5 versus 3.9 kg, respectively) growth rate decreases, feed intake increases slightly and soft faeces excretion stabilizes (20 g DM day^{-1}) (Gidenne and Lebas, 1987). Similar figures (21.8 g DM day^{-1}) have been reported for adult females during pregnancy. However, lactating does show greater soft faeces production (34 g DM day^{-1}) related to their higher feed intake (Lorente *et al.*, 1988). In these situations caecotrophy represents from 0.09 to 0.15 of the total DM intake (feed intake plus soft faeces). The importance of caecotrophy also varies with the nutritive characteristics of the diet, as will be discussed in the following chapters.

As a consequence of the mechanical separation of digesta at the caecum and proximal colon, the chemical composition of soft faeces is similar to that of the caecal contents but quite different from that of hard faeces (Table 1.3). Soft faeces contain greater proportions of protein, minerals and vitamins than hard faeces, while hard faeces are higher in fibrous components compared with soft faeces. As far as nutrient supply through soft faeces is concerned, protein represents from 0.15 to 0.22 of the total daily protein intake in growing rabbits and lactating does. The protein of soft faeces is high in essential amino acids such as lysine, sulphur amino acids and threonine (Proto, 1976; Spreadbury, 1978; Nicodemus *et al.*, 1999; García *et al.*, 2004), which represent from 0.10 to 0.23 of total intake. Belenguer *et al.* (2005) and Abecia *et al.* (2007) reported similar contributions of microbial lysine in body and milk proteins. This contribution came through the intake of soft faeces and a small direct intestinal absorption was observed. The importance of these amino acids depends on the efficiency of microbial protein synthesis. The proportion of microbial protein with respect to total protein of soft faeces varies with the diet from 0.30 to 0.68 (Spreadbury, 1978; García *et al.*, 1995; García *et al.*, 2005). Microbial activity is also responsible for the high content of K and B vitamins in soft faeces.

In conclusion, caecotrophy could overcome poor quality protein or low vitamin diets in traditional rearing conditions, but it is necessary to supply extra B vitamins, minerals and limiting amino acids in intensive rearing conditions.

1.7 Methodological Implications of Caecotrophy on Physiological Research Work

The marked circadian rhythms of caecotrophy and feed intake imply changes in both organ content weights and the chemical composition of their contents throughout the day. These circumstances make it necessary to take into account the sampling time in experimental procedures to obtain reliable digestibility data. The lack of homogeneity in the sampling procedures between different studies leads to difficulties in making comparisons and considerable misunderstanding. The diurnal variations of the main physiological parameters will now be summarized.

1.7.1 Weight and chemical composition of the organ contents

The weight of the stomach and caecal contents reflects the diurnal rhythm of intake and soft faeces production. Stomach contents show greater weights during the morning than during the night. The opposite is found for the weight of caecal contents. Diurnal differences in the weight of caecal and stomach contents of up to 20% and 30%, respectively, can be observed (Fraga *et al.*, 1984; Gidenne and Lebas, 1987).

Differences in the stomach contents are explained by diurnal changes in the chemical composition of stomach, duodenum, jejunum and ileum contents. Intact soft faeces in the stomach have been detected from 09.00 to 18.00 h (Gidenne and Poncet, 1985; Carabaño *et al.*, 1988), representing about a half of the total weight of the stomach contents. During the complementary period, the stomach only contains food. The protein content of precaecal digesta is the chemical parameter most affected by sampling time, showing greater values (from 0.50 to 1.00) during the soft faeces excretion period (Catala, 1976; Gidenne and Poncet, 1985; Merino, 1994). The same tendency has been observed for the chemical composition of colonic and rectal contents. However, the protein concentration of caecal contents remains stable throughout the day.

1.7.2 Ileal digestibility

The use of cannulated animals to determine ileal digestibility requires markers to estimate the ileal flow of DM and an ileal sample that is representative of that present throughout the day. Merino (1994) and Blas *et al.* (2003) observed, in cannulated animals, a diurnal variation in the crude protein (CP) content of ileal digesta, with greater values during the soft faeces excretion period than during the hard faeces excretion period (180 versus 120 g CP kg^{-1} DM). When caecotrophy was prevented, no variation was detected in the protein content of ileal digesta (average value 120 g kg^{-1} DM) (Merino and Carabaño, 2003). These results suggest that it is essential to take samples throughout the day to estimate the average composition of ileal digesta. Diurnal changes were detected in the marker concentration or fibre content of ileal digesta. However, sampling during the evening period (hard faeces excretion) does not produce significant differences when the ileal digestibility of the diet is determined by avoiding caecotrophy (Merino and Carabaño, 2003).

1.7.3 Fermentation patterns

The results obtained by Fioramonti and Ruckebush (1976) and Gidenne and Bellier (1992) in adult animals showed that the VFA concentration in caecal contents depends on the time of feeding, rising to a maximum 5 h after feeding. In weaned (4 weeks old) or growing (9 weeks old) rabbits fed *ad libitum*, diurnal differences in VFA concentrations and caecal pH of 50% and 10%, respectively, can be observed (Gidenne, 1986; Bellier *et al.*, 1995; Bellier and Gidenne, 1996). Caecal VFA concentrations are greater during the hard faeces than during the soft faeces excretion period. According to Bellier *et al.* (1995), this increment could have two causes: (i) the greater flow of substrate to the caecum related to an increase in feed intake during this period; and (ii) enrichment of the microbial population as a consequence of antiperistaltic movements of the proximal colon. Caecal pH varies inversely to the increase in VFA concentration. Smaller values of caecal pH have been observed during hard faeces excretion. Consequently, it is preferable to take the caecal samples during the hard faeces excretion period.

1.7.4 Transit time

Giving a marker as simple doses is the most frequent procedure used in transit time studies. This raises the question as to when the doses should be administered. According to Laplace and Lebas (1975), doses given before the caecotrophy period lead to a higher mean retention time (3–4 h) compared with doses given after caecotrophy. This effect can be explained by an increase in time before the first appearance of the marker in hard faeces. According to Jilge (1974), the time for the first appearance of the marker in faeces is the same (4 or 5 h) for doses given before or after the caecotrophy period. However, depending on the time of administration, the marker changes the site of its first appearance (soft or hard faeces) and, as a consequence, its detection will change.

According to these results, and taking into account the fact that feed intake starts just after the caecotrophy period, the hard faces excretion period is recommended as the best time for marker administration.

1.8 Rate of Passage

The capacity of the rabbit to digest its feed depends not only on endogenous enzyme activities and digestion by the microbial population, but also on the rate of passage of the feed. The passage of feed through the stomach of the rabbit and caecum is relatively slow and varies between 3–6 and 4–9 h, respectively, as measured by the technique of comparative slaughter (Gidenne and Poncet, 1985). However, transit is very fast in the small intestine. Estimated retention times in the jejunum and ileum are 10–20 and 30–60 min, respectively (Lebas, 1979). Taking into account the entire digestive tract, the mean retention time varies from 9 to 30 h, with an average of 19 h (Laplace and Lebas, 1975, 1977; Udén *et al.*, 1982; Fraga *et al.*, 1984; Ledin, 1984). More recently, with rabbits cannulated at the ileum, the mean retention times for the ileo-rectal and oro-ileal segments, and for the stomach, have been calculated as 7–24, 4–9 and 1–3 h, respectively (Gidenne and Ruckebush, 1989; Gidenne *et al.*, 1991; Gidenne and Pérez, 1993; Gidenne, 1994).

The wide variability in the results obtained might be related to factors such as the methodology used (e.g. type of marker, time and route of administration of the marker, mathematical calculations), characteristics of the animal (e.g. age, physiological status) and feeding variables (e.g. feed intake, particle size and fibre concentration of the diet, caecotrophy allowed or not). It has been reported that the marker ytterbium is retained for 3 h longer than chromium (Gidenne and Ruckebush, 1989) and that liquid-phase markers are retained for longer than solid-phase markers (Laplace and Lebas, 1975; Sakaguchi *et al.*, 1992). Preventing caecotrophy reduces the mean retention time by 0–7 h, depending on the type of diet fed (Fraga *et al.*, 1991; Sakaguchi *et al.*, 1992), whereas restricting feed intake to 0.50 and 0.60 of *ad libitum* levels increases mean retention time by 7 and 13 h, respectively (Ledin, 1984). Increasing the dietary fibre content from 220 to 400 g kg^{-1} decreases the total mean retention time by 12 h (an 11 h reduction in the ileo-rectal mean retention time) (Gidenne, 1994). Particle size can also modify the rate of passage, with longer times being obtained using diets with smaller particle size (Laplace and Lebas, 1977; Auvergne *et al.*, 1987).

References

Abecia, L., Fondevila, M., Balcells, J., Edwards, J.E., Newbold, C.J. and Mcewan, N.R. (2005) Molecular profiling of bacterial species in the rabbit caecum. *Fems Microbiological Letters* 244, 111–115.

Abecia, L., Balcells, J., Fondevila, M., Belenguer, A., Holtrop, G. and Lobley, G.E. (2007) Contribution of gut microbial lysine to liver and milk amino acids in lactating does. *British Journal of Nutrition* 100, 977–983.

Alexander, F. and Chowdhury, A.K. (1958) Digestion in the rabbit's stomach. *British Journal of Nutrition* 12, 65–73.

Alvarez, J.L., Marguenda, I., García-Rebollar, P., Carabaño, R., de Blas, J.C., Corujo, A. and García-Ruiz, A.I. (2007) Effects of type and level of fibre on digestive physiology and performance in reproducing and growing rabbits. *World Rabbit Science* 15, 9–17.

Auvergne, A., Bouyssou, T., Pairet, M., Bouillier-Oudot, M., Ruckebusch, Y. and Candau, M. (1987) Nature de l'aliment, finesse de mouture et données anatomo-fonctionnelles du tube digestif proximal du lapin. *Reproduction Nutrition Development* 27, 755–768.

Badiola, I., Pérez De Rozas, A.M., Roca, M., Carabaño, R., Gómez, M., García, J. and de Blas, J.C. (2004) Characterization of the microbial diversity of rabbit intestinal tract by restriction fragment length polymorphism. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 746–751.

Battaglini, M.A. and Grandi, A. (1988) Some observations of feeding behavior of growing rabbits. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 3. Sandor Holdas, Hercegalom, Budapest, Hungary, pp. 79–87.

Belenguer, A., Balcells, J., Guada, J.A., Decoux, M. and Milne, E. (2005) Protein recycling in growing rabbits: contribution of microbial lysine to amino acid metabolism. *British Journal of Nutrition* 94, 763–770.

Bellier, R. and Gidenne, T. (1996) Consequences of reduced fibre intake on digestion, rate of passage and caecal microbial activity in the young rabbits. *British Journal of Nutrition* 75, 353–363.

Bellier, R., Gidenne, T. and Collin, M. (1995) *In vivo* study of circadian variations of the caecal fermentation pattern in postweaned and adult rabbits. *Journal of Animal Science* 73, 128–135.

Bennegadi, N., Fonty, G., Millet, L., Gidenne, T. and Licois, D. (2003) Effects of age and dietary fibre level on caecal microbial communities of conventional and specific pathogen-free rabbits. *Microbial Ecology in Health and Disease* 5, 23–32.

Björnhag, G. (1972) Separation and delay contents in the rabbit colon. *Swedish Journal of Agricultural Research* 2, 125–136.

Blas, E. (1986) El almidón en la nutrición del conejo: utilización digestiva e implicaciones prácticas. Doctoral thesis, Universidad de Zaragoza, Spain.

Blas, E., Falcao, L., Gidenne, T., Scapinello, C., Pinheiro, V., García, A.I. and Carabaño, R. (2003) Interlaboratory study on ileal digestibility in rabbits: the effect of digesta collection time and a simplification of the procedure. *World Rabbit Science* 11, 101–111.

Bonnafous, R. and Raynaud, P. (1970) Recherches sur le variation de la densité des microorganismes dans le colon du lapin domestique. *Experientia* 26, 52.

Campín, J., Eiras, P., Rebollar, P.G. and Carabaño, R. (2003) Estudio del tejido linfoide asociado a intestino en gazapos en torno al destete. *ITEA* 24, 660–662.

Carabaño, R. and Merino, J.M. (1996) Effect of ileal cannulation on feed intake, soft and hard faeces excretion throughout the day in rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 121–126.

Carabaño, R., Fraga, M.J., Santomá, G. and de Blas, J.C. (1988) Effect of diet on composition of caecal contents and on excretion and composition of soft and fard feces of rabbits. *Journal of Animal Science* 66, 901–910.

Carabaño, R., Fraga, M.J. and de Blas, J.C. (1989) Effect of protein source in fibrous diets on performance and digestive parameters of fattening rabbits. *Journal of Applied Rabbit Research* 12, 201–204.

Carabaño, R., Motta-Ferreira, W., de Blas, J.C. and Fraga, M.J. (1997) Substitution of sugarbeet pulp for alfalfa hay in diets for growing rabbits. *Animal Feed Science and Technology* 65, 249–256.

Carabaño, R., Badiola, I., Licois, D. and Gidenne, T. (2006) The digestive ecosystem and its control through nutritional or feeding strategies. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Sciences*. Melle, Belgium, pp. 211–228.

Carabaño, R., Badiola, I., Chamorro, S., García, J., García-Ruiz, A.I.,García-Rebollar, P., Gómez-Conde, M.S., Gutiérrez, I., Nicodemus, N., Villamide, M.J. and de Blas, J.C. (2008) Review. New trends in rabbit feeding: influence of nutrition on intestinal health. *Spanish Journal of Agricultural Research* 6 (special issue), 15–25.

Carabaño, R., Villamide, M.J., García, J., Nicodemus, N., Llorente, A., Chamorro, S., Menoyo, D., García-Rebollar, P., García-Ruiz, A.I. and de Blas, J.C. (2009) New concepts and objectives for protein-amino acid nutrition in rabbits: a review. *World Rabbit Science* 17, 1–14.

Catala, J. (1976) Étude sur les répartitions hydriques, pondérals et azotées dans le material digestif chez le lapin, en relation avec la dualité de l'élimination fécale. In: Lebas, F. (ed.) *Proceedings of the 1st World Rabbit Congress*. WRSA, Dijon, France.

Chamorro, S., Gómez-Conde, M.S., Pérez de Rozas, A.M., Badiola, I., Carabaño, R. and de Blas, J.C. (2007) Effect on digestion and performance of dietary protein content and of increased substitution of lucerne hay with soya-bean protein concentrate in starter diets for young rabbits. *Animal* 1, 651–659.

Corring, T., Lebas, F. and Courtot, T. (1972) Contrôle de l'evolution de l'equipment enzymatique du pancreas exocrine du lapin de la naissance a six semaines. *Annales de Biologie Animale, Biochmie et Biophysique* 12, 221–231.

Cortez, S., Brandenburger, H., Greuele, E. and Sundrum, A. (1992) Undersuchungen zur Darmflora des Kaninchens in Abbängigkeit von der Fütterrung und dem Gesund-heitsstatus. *Tierärztliche Umschau* 47, 544–549.

Dasso, J.F., Obiakor, H., Bach, H., Anderson, A.O. and Mage, Rg. (2000) A morphological and immunohistological study of the human and rabbit appendix for comparison with the avian bursa. *Developmental and Comparative Immunology* 24, 797–814.

Davies, M.E. (1965) Cellulolytic bacteria in some ruminants and herbivores as shown by fluorescent antibodies. *Journal of General Microbiology* 39, 139–141.

Debray, L., Le Huërou-Luron, I., Gidenne, T. and Fortun-Lamothe, L. (2003) Digestive tract development in rabbit according to the dietary energetic source: correlation between whole tract digestion, pancreatic and intestinal enzymatic activities. *Comparative Biochemistry and Physiology Part A* 135, 443–455.

Dojanã, N., Costache, M. and Dinischiotu, A. (1998) The activity of some digestive enzymes in domestic rabbits before and after weaning. *Animal Science* 66, 501–507.

El-Adawy, M.M. (1996) The influence of caecotomy on composition and excretion rate of soft and hard faeces, feed and water intake in rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 145–149.

Emaldi, O., Crociani, F., Matteuzi, D. and Proto, V. (1979) A note on the total viable counts and selective enumeration of anaerobic bacteria in the caecal content, soft, and hard faeces of rabbit. *Journal of Applied Bacteriology* 46, 169–172.

Fioramonti, J. and Ruckebush, Y. (1976) La motrici'te caecale chez le lapin. 3. Dualité de l'excretion fécale. *Annales de Recherches Vétérinaires* 7, 281–295.

Forsythe, S.J. and Parker, D.S. (1985) Nitrogen metabolism by the microbial flora of the rabbit caecum. *Journal of Applied Bacteriology* 58, 363–369.

Forthun-Lamothe, L. and Boullier, S. (2007) A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. *Livestock Science* 107, 1–18.

Fraga, M.J., Barreno, C., Carabaño, R., Méndez, J. and de Blas, J.C. (1984) Efecto de los niveles de fibra y proteína del pienso sobre la velocidad de crecimiento y los parámetros digestivos de los conejos. *Anales del INIA Serie Ganadera* 21, 91–110.

Fraga, M.J., Pérez de Ayala, P., Carabaño, R. and de Blas, J.C. (1991) Effect of type of fibre on rate of passage and on the contribution of soft feces to nutrient intake of fattening rabbits. *Journal of Animal Science* 69, 1566–1574.

Gallois, M., Gidenne, T., Fortun-Lamothe, L., Le Huerou-Luron, I. and Lallès, J.P. (2005) An early stimulation of solid feed intake slightly influences the morphological gut maturation in the rabbit. *Reproduction Nutrition and Development* 45, 109–122.

Gallois, M., Gidenne, T., Tasca, C.I., Caubet, C., Coudert, C., Milon, A. and Boullier, S. (2007) Maternal milk contains antimicrobial factors that protect young rabbits from enteropathogenic *Escherichia coli* infection. *Clinical and Vaccine Immunology* 14, 585–592.

Gallois, M., Le Huërou-Luron, I., Fortun-Lamothe, L., Lallès, J.P. and Gidenne, T. (2008a) Adaptability of the digestive function according to age at weaning in the rabbit: I. Effect on feed intake and digestive functionality. *Animal* 2, 525–535.

Gallois, M., Fortun-Lamothe, L., Michelan, A. and Gidenne, T. (2008b) Adaptability of the digestive function according to age at weaning in the rabbit: II. Effect on nutrient digestion in the small intestine and in the whole digestive tract. *Animal* 2, 536–547.

Gallouin, F. (1984) Le comportement de caecotrophie chez lae lapin. In: Finzi, A. (ed.) *Proceedings of the 3rd World Rabbit Congress*, Vol. 2. Rome, Italy, pp. 363–408.

García, A.I., de Blas, J.C. and Carabaño, R. (2004) Effect of type of diet (casein-based or protein-free diet) and caecotrophy on ileal endogenous nitrogen and amino acid flow in rabbits. *Animal Science* 79, 231–240.

García, A.I., de Blas, J.C. and Carabaño, R. (2005) Comparison of different methods for nitrogen and amino acid evaluation in rabbit diets. *Animal Science* 80, 169–178.

García, J., de Blas, J.C., Carabaño, R. and García, P. (1995) Effect of type of lucerne hay on caecal fermentation and nitrogen contribution through caecotrophy in rabbits. *Reproduction Nutrition Development* 35, 267–275.
García, J., Gidenne, T., Falcao E Cunha, L. and de Blas, J.C. (2002) Identification of the main factors that influence caecal fermentation traits in growing rabbits. *Animal Research* 51, 165–173.
García Rebollar, P., Espinosa, A., Lorenzo, P.L. and Carabaño, R. (2004) Transitory disturbances in growing lactating rabbits after transient doe-litter separation. *Reproduction Nutrition and Development* 44, 437–447.
Gidenne, T. (1986) Évolution nycthémérale des produits de la fermentation bactérienne dans le tuve digestif du lapin en croissance. Relations avec la teneur en lignines de la ration. *Annales de Zootechnie* 35, 121–136.
Gidenne, T. (1994) Effets d'une reduction de la tener en fibres alimentaires sur le transit digestif du lapin. Comparaison et validation de modéles d'ajustement des cinétiques d'excrétion fécale des marqueurs. *Reproduction Nutriton Development* 34, 295–307.
Gidenne, T. and Bellier, R. (1992) Étude *in vivo* de l'activité fermentaire caecale chez le lapin. Mise au point et validation d'une nouvelle technique de canulation caecale. *Reproduction Nutrition Development* 32, 365–376.
Gidenne, T. and Lebas, F. (1987) Estimation quantitative de la caecotrophie chez le lapin en croissance: variations en fonction de l'âge. *Annales de Zootechnie* 36, 225–236.
Gidenne, T. and Perez, J.M. (1993) Effect of dietary starch origin on digestion in the rabbit. 2. Starch hydrolysis in the small intestine, cell wall degradation and rate of passage measurements. *Animal Feed Science and Technology* 42, 249–257.
Gidenne, T. and Poncet, C. (1985) Digestion chez le lapin en croissance, d'une ration à taux élevé de constituants pariétaux: etude méthodologique pour le calcul de digestilité apparetne, par segment digestif. *Annales de Zootechnie* 34, 429–446.
Gidenne, T. and Ruckebusch, Y. (1989) Flow and passage rate studies at the ileal level in the rabbit. *Reproduction Nutrition Development* 29, 403–412.
Gidenne, T., Carre, B., Segura, M., Lapanouse, A. and Gomez, J. (1991) Fibre digestión and rate of passage in the rabbit: effect of particle size and level of lucerne meal. *Animal Feed Science and Technology* 32, 215–221.
Gidenne, T., Combes, S., Licois, D., Carabaño, R., Badiola, I. and García, J. (2008) Ecosystème caecal et nutrition du lapin: interactions avec la santé digestive. *INRA Productions Animales* 21, 239–250.
Gómez-Conde, M.S., Garcia, J., Chamorro, S., Eiras, P., Rebollar, P.G., de Rozas, A.P., Badiola, I., de Blas, C. and Carabaño, R. (2007) Neutral detergent-soluble fiber improves gut barrier function in twenty-five-day-old weaned rabbits. *Journal of Animal Science* 85, 3313–3321.
Gómez-Conde, M.S., Pérez de Rozas, A., Badiola, I., Pérez-Alba, L., de Blas, C., Carabaño, R. and García, J. (2009) Effect of neutral detergent soluble fibre on digestion, intestinal microbiota and performance in twenty-five-day-old weaned rabbits. *Livestock Science* 125, 192–198.
Gouet, P. and Fonty, G. (1973) Evolution de la microflore digestive du lapin holoxénique de la naissance au sevrage. *Annales de Biologie Animale, Biochimie et Biophysique* 13, 733–735.
Gouet, P. and Fonty, G. (1979) Changes in the digestive microflora of holoxenic rabbits from birth until adulthood. *Annales de Biologie Animale, Biochimie et Biophysique* 19, 553–556.
Griffiths, M. and Davies, D. (1963) The role of soft pellets in the production of lactic acid in the rabbit stomach. *Journal of Nutrition* 80, 171–180.
Grobner, M.A. (1982) Diarrhea in the rabbit. A review. *Journal of Applied Rabbit Research* 5, 115–127.
Gutiérrez, I., Espinosa, A., García, J., Carabaño, R. and de Blas, J.C. (2002) Effect of levels of starch, fiber, and lactose on digestion and growth performance of early-weaned rabbits. *Journal of Animal Science* 80, 1029–1037.
Gutiérrez, I., Espinosa, A., García, J., Carabaño, R. and de Blas, J.C. (2003) Effect of protein source on digestion and growth performance of early-weaned rabbits. *Animal Research* 52, 461–471.
Hall, E.R. (1952) Investigations on the microbiology of cellulose utilization in domestic rabbits. *Journal of General Microbiology* 7, 350–357.
Hörnicke, H. and Björnhag, G. (1980) Coprophagy and related strategies for digesta utilization. In: Ruckebusch, Y. and Thievend, P. (eds) *Digestive Physiology and Metabolism in Ruminants*. MTP Press, Lancaster, UK, pp. 707–730.
Jilge, B. (1974) Soft faeces excretion and passage time in the laboratory rabbit. *Laboratory Animals* 8, 337–346.

Jilge, B. (1982) Monophasic and diphasic patterns of the circadian caecotrophy rhythm of rabbits. *Laboratory Animals* 16, 1–6.

Jilge, B. and Meyer, H. (1975) Coprophagy dependent changes of the anaerobic bacterial flora in the stomach and small intestine of the rabbit. *Zeitung Versuchstierkd* 17, 308–314.

Kalis, S.L., Zhai, S.K., Yam, P.C., Witte, P.L. and Knight, K.L. (2007) Suppression of B lymphopoiesis at a lymphoid progenitor stage in adult rabbits. *International Immunology* 19, 801–811.

Kovács, M., Milisits, G., Szendro, Z.S., Lukács, H., Bónai, A., Pósa, R., Tornyos, G., Kovács, F. and Horn, P. (2008) Effect of different weaning age (days 21, 28 and 35) on caecal microflora and fermentation in rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 701–704.

Kulwich, R., Struglia, L. and Pearson, P.B. (1953) The effect of coprophagy in the excretion of B vitamins by the rabbit. *Journal of Nutrition* 49, 639–645.

Lanning, D., Zhu, X., Zhai, S. and Knight, K.L. (2000) Development of the antibody repertoire in rabbit: gut-associated lymphoid tissue, microbes, and selection. *Immunological Reviews* 175, 214–228.

Lanning, D., Osborne, B. and Knight, K.L. (2004) Immunoglobulin genes and generation of antibody repertoires in higher vertebrates: a key role for GALT. In: Alt, F.W., Honjo, T. and Neuberger, M.S. (eds) *Molecular Biology of B Cells*. Elsevier, Amsterdam, The Netherlands, pp. 433–448.

Laplace, J.P. and Lebas, F. (1975) Le transit digetif chez le lapin. III. Influence de l'heure et du mode d'administration sur l'excrétion du cérium–141 chez le lapin alimenté ad libitum. *Annales de Zootechnie* 24, 255–265.

Laplace, J.P. and Lebas, F. (1977) Le transit digestif chez le lapin. VII. Influence de la finesse du broyage des constituents d'un aliment granulé. *Annales de Zootechnie* 26, 413–420.

Lebas, F. (1979) La nutrition du lapin: mouvement des digesta et transit. *Cuniculture* 6, 67–68.

Lebas, F. and Laplace, J.P. (1972) Mensurations viscérales chez le lapin. I. Croissance du foie, des reins et des divers segments intestinaux entre 3 et 11 semaines d'âge. *Annales de Zootechnie* 21, 337–347.

Lebas, F. and Laplace, J.P. (1974) Note sur l'excretion fécale chez le lapins. *Annales de Zootechnie* 23, 577–581.

Lebas, F. and Laplace, J.P. (1975) Le transit digestif chez le lapin. 5. Evolution de l'excretion fécale en fonction de l'heure de distribution de l'aliment et du niveau de rationnement durante les 5 jours qui suivent l'application de ce denier. *Annales de Zootechnie* 24, 613–627.

Lebas, F., Corring, T. and Courtot, D. (1971) Equipment enzymatique du pancreas exocrine chez le lapin, mise en place en evolution de la naissance au sévrage. Relation avec la composition du régime alimentaire. *Annales de Biologie Animale, Biochimie et Biophysique* 11, 399–413.

Ledin, I. (1984) Effect of restricted feeding and realimentation on compensatory growth, carcass composition and organ growth in rabbit. *Annales de Zootechnie* 33, 33–50.

Lorente, M., Fraga, M.J., Carabaño, R. and de Blas, J.C. (1988) Coprophagy in lactating does fed different diets. *Journal of Applied Rabbit Research* 11, 11–15.

Mage, R.G., Lanning, D. and Knigh, K.L. (2006) B cell and antibody repertoire development in rabbits: the requirement of gut-associated lymphoid tissues. *Developmental and Comparative Immunology* 30, 137–153.

Marounek, M., Vovk, S.J. and Skrinova, V. (1995) Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *British Journal of Nutrition* 73, 463–469.

Marty, J. and Vernay, M. (1984) Absorption and metabolism of the volatile fatty-acids in the hind-gut of the rabbit. *British Journal of Nutrition* 51, 265–277.

Merino, J.M. (1994) Puesta a punto de una técnica de canulación ileal en el conejo para el estudio del aprovechamiento de los nutrientes de la dieta. Doctoral thesis, Universidad Complutense de Madrid, Spain.

Merino, J.M. and Carabaño, R. (2003) Efecto de la coprofagía sobre la composición química de la digesta y sobre la digestibilidad ileal. *ITEA* 24, 657–659.

Motta-Ferreira, W., Fraga, M.J. and Carabaño, R. (1996) Inclusion of grape pomace, in substitution for lucerne hay, in diets for growing rabbits. *Animal Science* 63, 167–174.

Nicodemus, N., Mateos, J., de Blas, J.C., Carabaño, R. and Fraga, M.J. (1999) Effect of diet on amino acid composition of soft faeces and the contribution of soft faeces to total amino acid intake, through caecotrophy in lactating doe rabbits. *Animal Science* 69, 167–170.

Nicodemus, N., García, J., Carabaño, R. and de Blas, J.C. (2002) Effect of inclusion of sunflower hulls in the diet on performance, disaccharidase activity in the small intestine and caecal traits of growing rabbits. *Animal Science* 75, 237–243.

Niza, A., Di Meo, C., Stanco, G. and Cutrignelli, M.I. (2001) Influence of solid feed intake and age at weaning on caecal content characteristics and post-weaning performance. *World Rabbit Science* 9, 149–153.

Orengo, O. and Gidenne, T. (2007) Feeding behavior and caecotrophy in the young rabbit before weaning: An approach by analysing the digestive contents. *Applied Animal Behaviour Science* 102, 106–118.

Pascual, J.J. (2001) Early weaning of young rabbits: a review. *World Rabbit Science* 9, 165–170.

Padilha, M.T.S., Licois, D., Gidenne, T., Carré, B. and Fonty, G. (1995) Relationships between microflora and caecal fermentation in rabbits before and after weaning. *Reproduction Nutrition Development* 35, 375–386.

Padilha, M.T.S., Licois, D., Gidenne, T., Carré, B., Coudert, P. and Lebas, F. (1996) Caecal microflora and fermentation pattern in exclusively milk-fed young rabbit. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 247–251.

Pairet, M., Bouyssou, T. and Ruckebusch, Y. (1986) Colonic formation of soft feces in rabbits: a role for endogenous prostaglandins. *American Journal of Physiology* 250, G302–G308.

Parker, D.S. (1976) The measurement of production rates of volatile fatty acid production in rabbits. *British Journal of Nutrition* 36, 61–78.

Penney, R.L., Folk, G.E., Galask, R.P. and Petzold, C.R. (1986) The microflora of the alimentary tract of rabbits in relation to pH, diet and cold. *Journal of Applied Rabbit Research* 9, 152–156.

Piattoni, F. and Maertens, L. (1999) Effect of weaning age and solid feed distribution before weaning on the caecal fermentation pattern of young rabbits. In: *Proceedings of the Arbeitstagung über Krankheiten der Kaninchen, Pelztiere und Heimtiere*. Deutschen Vet. Med. Gesellschaft e. V., Giessen, Germany, pp. 97–105.

Pickard, D.W. and Stevens, C.E. (1972) Digesta flow through the rabbit large intestine. *American Journal of Physiology* 222, 1161–1166.

Portsmouth, J.I. (1977) The nutrition of the rabbits. In: Haresign, W., Swan, H. and Lewis, D. (eds) *Nutrition and the Climatic Environment*. Butterworths, London, UK, pp. 93–111.

Proto, V. (1976) Fisiologia della nutrizione del coniglio con particolare riguardo alla ciecotrofia. *Rivista di Conigliocoltura* 7, 15–33.

Rhee, K.J., Sethupathi, P., Driks, A., Lanning, D.K. and Knight, K.L. (2004) Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. *The Journal of Immunology* 172, 1118–1124.

Romero, C., Nicodemus, N., García-Rebollar, P, García-Ruiz, A.I., Ibáñez, M.A. and de Blas, J.C. (2009) Dietary level of fibre and age at weaning affect the proliferation of *Clostridium perfringens* in the caecum, the incidence of epizootic rabbit enteropathy and the performance of fattening rabbits. *Animal Feed Science and Technology* 153, 131–140.

Ruckebush, Y. and Hörnicke, H. (1977) Motility of the rabbit's colon and caecotrophy. *Physiology and Behavior* 18, 871–878.

Sabatakou, O., Xylouri-Frangiadaki, E., Paraskevakou, E. and Papatonakis, K. (1999) Scanning electron microscopy of stomach and small intestine of rabbit during foetal and post natal life. *Journal of Submicroscopic Cytology and Pathology* 31, 107–114.

Sabatakou, A.O., Xylouri, M.E., Sotirakoglou, A.K., Fragkiadakis, G.M. and Noikokyris, N.P. (2007) Histochemical and biochemical characteristics of weaning rabbit intestine. *World Rabbit Science* 15, 173–178.

Sakaguchi, E., Kaizu, K. and Nakamichi, M. (1992) Fibre digestion and digesta retention from different physical forms of the feed in the rabbit. *Comparative Biochemistry and Physiology* 102A, 559–563.

Scapinello, C., Gidenne, T. and Fortun-Lamothe, L. (1999) Digestive capacity of the rabbit during the post-weaning period, according to milk/solid feed intake pattern before weaning. *Reproduction Nutrition Development* 39, 423–432.

Sirotek, K., Santos, E., Benda, V. and Marounek, M. (2003) Isolation, identification and characterization of rabbit caecal mucinolytic bacteria. *Acta Veterinaria Brno* 72, 365–370.

Skrivanová, E., Molatová, Z. and Marounek, M. (2008) Effects of caprylic acid and triacylglycerols of both caprylic and capric acid in rabbits experimentally infected with enteropathogenic *Escherichia coli* O103. *Veterinary Microbiology* 126, 372–376.

Smith, H.W. (1965) The development of the flora of the alimentary tract in young animals. *Journal of Pathology and Bacteriology* 90, 495–513.

Snipes, R.L., Clauss, W., Weber, A. and Hörnicke, H. (1982) Structural and functional differences in various divisions of rabbit colon. *Cell and Tissue Research* 225, 331–346.

Spreadbury, D. (1978) A study of the protein and amino acid requirements of the growing New Zealand White rabbit, with emphasis on lysine and the sulphur containing amino acids. *British Journal of Nutrition* 39, 601–613.

Toofanian, F. and Targowski, S.P. (1982) Morphogenesis of rabbit small intestinal mucosa. *American Journal of Veterinarian Research* 43, 2213–2219.

Udén, P., Rounsaville, T.R., Wiggans, G.R. and Van Soest, P.J. (1982) The measurement of liquid and solid digesta retention in ruminants, equines and rabbits given timothy (*Phleum pretense*) hay. *British Journal of Nutrition* 48, 329–339.

Urao, M., Teitelbaum, D.H., Drongowski, R.A. and Coran, Ag. (1996) The association of gut-associated lymphoid tissue and bacterial translocation in the newborn rabbit. *Journal of Pediatric Surgery* 31, 1482–1487.

Vernay, M. and Raynaud, P. (1975) Répartition des acides gras volatils dans le tube digestif du lain domestique. II. Lapin soumis au jeûne. *Annales Recherches Vétérinaries* 6, 369–377.

Xiccato, G., Trocino, A., Sartori, A. and Queaque, P.I. (2003) Effect of weaning diet and weaning age on growth, body composition and caecal fermentation of young rabbits. *Animal Science* 77, 101–111.

2 Digestion of Sugars and Starch

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It is possible to classify the carbohydrate fractions of plants incorporated into animal feed into two groups: (i) those that are hydrolysable by the endogenous intestinal enzymes of the animal (these polysaccharides are located predominantly within the plant cell); and (ii) those that are hydrolysable only by enzymes produced by the microbiota (these are principally cell wall polysaccharides and are considered in more detail in Chapter 5). The former can be further separated into two main groups: first, the simple sugars and oligosaccharides, which are present at low levels (<50 g kg^{-1}) in rabbit feeds; and second, the polysaccharides represented mainly by the starches (contributing 100–250 g kg^{-1}). The structure and digestion of starch are described later in this chapter. First, the digestion of simple sugars and oligosaccharides are considered.

2.1 Simple Sugars and Oligosaccharides

These two types of carbohydrate are often classed simply under one general term, 'the sugars'. Nevertheless, from a biochemical point of view, it is convenient to distinguish clearly between simple sugars and oligosaccharides because they are not digested by the same processes. For instance, the α-galactosides are only degraded by bacterial enzymes, whereas simple sugars are very rapidly hydrolysed by endogenous enzymes of the host and absorbed from the small intestine.

2.1.1 Definition, structure and analysis

Sugars are generally found at low concentrations in animal feeds, although the level of sucrose can reach 500 g kg^{-1} in some raw materials such as molasses (Table 2.1). Among the sugars found in common raw materials, glucose and fructose are the two major types, found as monosaccharides or as sucrose (a disaccharide based on a combination of the two). Furthermore, compared with other mammals, lactose (glucose + galactose, $\alpha[1\rightarrow4]$) is at a very low level (50 g kg^{-1} dry matter (DM)) in the milk of the rabbit female (Maertens *et al.*, 2006) and thus is not added to the pelleted feed for the young rabbit. Other disaccharides can also occur in the feed: maltose (two glucose units, $\alpha[1\rightarrow4]$), which mainly originates from starch hydrolysis, and melibiose (galactose + glucose, $\alpha[1\rightarrow6]$) which is found in some roots.

Oligosaccharides are defined as molecules with a low degree of polymerization (dp). Maltotriose corresponds to three units of glucose linked by $\alpha(1\rightarrow4)$ bonds and

Table 2.1. Level of total sugars[a] in some raw materials (INRA, 2004).

Raw material	Sugars (g kg^{-1} air dry)
Beet molasses	466
Sugarbeet	160
Sugarbeet pulp	66
Citrus pulps	200
Apple pomace	140
Sweet lupin	64
Soybean meal	83
Sunflower meal	57
Brewer's grain	9
Wheat bran	67
Barley	21
Winter pea	39

[a]Analysed as total sugars soluble in ethanol (80%, v/v).

originates from starch hydrolysis. The α-galactosides (dp 3–5) are a group of oligosaccharides (raffinose, stachyose, verbascose and ajugose) that are not digestible by the endogenous enzymes of the animal, but are rapidly degraded and fermented by the caecal microbiota. They are found mainly in legume seeds or extracted legume meals. Pea seeds contain 50 g kg^{-1}, lupin seeds 40 g kg^{-1} and soybean meal 50 g kg^{-1}, all on a DM basis (Thivend, 1981).

Simple sugars and oligosaccharides are solubilized in boiling ethanol (80%, v/v). Following acid hydrolysis (with hot concentrated sulphuric acid), the total sugars can be quantified (as monosaccharides) either through colorimetric or chromatographic methods. The choice of extraction process is important when analysing the sugars in a raw material or a feed. It is probable that the cold extraction (ethanol 40%, v/v, at ambient temperature) recommended in the European Community (EC) method (AFNOR, 1985) is not adequate for extracting all sugars. This would explain the unexpected low values sometimes found for total sugars in soybean meals and legume seeds. Attention must be paid to the colorimetric determination of sugar in digesta and faeces because of interference by bile pigment (particularly for the glucose determination in a starch analysis procedure). It is thus recommended to avoid the glucose-oxidase/peroxidase technique and to use the hexokinase/glucose-6-phosphate-dehydrogenase system (Kozlowski, 1994).

2.1.2 Digestion

Compared with starch, glucose and fructose are readily absorbed in the small intestine. However, fructose has been observed to be absorbed more slowly than glucose in pigs (Carré, 1992). In contrast with glucose, fructose is probably not absorbed through an energy-dependent mechanism. The level of sugars (soluble in 80% ethanol) in the ileal contents, including therefore ethanol-soluble α-glucosides and glucose resulting from digestion of starch, may reach 25 g kg^{-1} DM for adult rabbits fed a standard commercial diet (Gidenne and Ruckebusch, 1989), indicating that the flow of sugars entering the caecum is not negligible. Further studies are necessary to evaluate the digestion of sugars, especially in the young animal.

Complete digestion of α-galactosides has been observed in rats and pigs (Goodlad and Mathers, 1990, 1991). It is assumed that they are also totally digested by the caecal microbiota in rabbits, although this has not yet been measured.

2.2 Starch

2.2.1 Definition, structure and analysis

Starch (α-glucan) is a major reserve polysaccharide of green plants and probably the second most abundant carbohydrate in nature next to cellulose. In some cases, the reserve polysaccharides of the plant are α-fructans, such as inulin (linear α-fructan, dp ≈30) in the Jerusalem artichoke (*Helianthus tuberosus*) or levan (branched α-fructan, dp ≈100) in some grasses. Starch is found in nature as granules either in seeds, roots or tubers. The shape of the starch granule depends on the botanical source and many different sizes and forms are found – from tiny granules in oats or rice (5–6 μm) to larger granules in banana (38–50 μm). The interior of a granule is composed of alternating crystalline and amorphous regions. The disruption of this organization is the basis of gelatinization. The starch granule is modified by either chemical or physical treatment (e.g. heat, pressure). A prerequisite for digestion is that the enzymes are adsorbed onto the starch granule. Hydrolysis may then proceed either through surface erosion or penetration via pinholes. The physicochemical and functional aspects of starch have been reviewed by Eliasson and Gudmundsson (1996).

From a biochemical point of view, starch is a polysaccharide composed simply of D-glucose units. Starch basically consists of a mixture of two types of chains: (i) amylose, a linear chain of glucose ($\alpha[1\rightarrow4]$ links); and (ii) amylopectin, a branched chain ($\alpha[1\rightarrow4]$ + $\alpha[1\rightarrow6]$ links). However, the polymeric structure of starch is more complicated, and its primary structure is not yet fully understood. Starch is the subject of many investigations because of its multiple uses in chemistry, the food industry, fermentation processes and so on.

It is now recognized that some amylose molecules have several branches. In addition, the presence of materials intermediate between amylose and amylopectin has been suggested in amylomaize (maize rich in amylose) and wrinkled-pea starches (Hizukuri, 1996). The relative proportion of amylose and amylopectin may vary considerably according to the plant source, and this may significantly affect its digestion (Table 2.2). For instance, maize rich in amylose has a lower digestibility than standard maize. In addition, the starch granule is sometimes encapsulated in a protein matrix that reduces its accessibility to enzymes. Thus, starch degradation is dependent on the biochemical and physical structure of the granule.

Numerous processes currently used in animal feed manufacturing are able to modify the starch granule, either slightly by steaming in the pelleting process or strongly by using temperature combined with hydration and pressure in the extrusion process. The interaction between starch and water is well known: the starch structure is strongly hydrophilic and the ratio of starch to water is inversely correlated to the gelatinization temperature (Champ and Colonna, 1993). In general, with an excess of water and temperatures over 55°C, the granules swell and solubilize (disorganization/dispersion of the structure); this is the gelatinization step (in the case of pure starch, a viscous solution results). Following cooling, the chains of glucose can reassociate. This is termed retrogradation and can lead to forms of starch that are resistant to amylases. Complete gelatinization of the starch granule is essential for the correct determination of the starch using enzymatic procedures.

Starch is usually determined in animal feeds or raw materials through the Ewers EC (optical rotation determination) or enzymatic methods (hydrolysis followed by glucose determination). The two techniques provide, in general, very well correlated data, with slightly higher values for the Ewers EC method (+0.5–4%). The differences between the two methods are greater for legume than for cereal materials. The difference lies in the fact that the Ewers EC method may interfere with unextracted sugars or acid-labile polysaccharides. For example, the recommended starch method for beet pulp is the enzymatic one. Specific

Table 2.2. Starch and amylose concentrations in some raw materials, and respective faecal digestibility for the rat.

Source	Starch (g kg^{-1} DM)	Amylose (proportion of starch)	Faecal digestibility of starch in the rat[a]
Soft wheat	650–700	0.25–0.30	0.98–1.00
Maize	650–800	0.20–0.24	0.98–1.00
Maize rich in amylose	500–650	0.60–0.65	0.66–0.77
Smooth pea	430–480	0.31–0.35	0.99
Fava bean	300–430	0.31–0.34	0.99
Banana (green)	150–250	0.15–0.18	0.49
Cassava roots	800–850	0.17	0.95–0.97
Potato (uncooked)	600–650	0.20	0.27–0.28

[a]Champ and Colonna (1993).

procedures are also recommended for starch determination in digesta and faeces (Kozlowski, 1994). If no previous ethanol extraction of samples has been carried out when using enzymatic procedures, starch, ethanol-soluble α-glucosides and glucose are considered as a whole.

2.2.2 Digestion of starch in the different parts of the gastrointestinal tract

Starch is almost completely digested in the digestive tract of rabbits, as in other livestock species. For this reason, the faecal excretion of starch is generally minimal (less than 0.02 of intake), although in some cases it can reach 0.10 of intake, depending mainly on the age of the rabbit and the source of the starch, both of which will be discussed later.

It is acknowledged that starch digestion takes place mainly in the small intestine. However, starch may also be degraded to some extent in other parts of the digestive tract, such as the stomach and large intestine. It would be of particular interest to evaluate the degradation of starch (or of intermediate α-glucosides and glucose not absorbed in the small intestine) by the microbiota in the large intestine and to review the factors affecting the ileal flow of starch and the possible consequences on caeco-colic fermentative activity, as well as on the stability of the microbial ecosystem and digestive health. Therefore, the following section discusses various aspects of the process of starch digestion in the different parts of the gastrointestinal tract of rabbits.

Gastric digestion

There are no reliable measurements of the extent of starch hydrolysis in the stomach. It has been observed that the starch concentration in the gastric digesta is clearly less than in the diet (Fraga *et al.*, 1984; Blas, 1986). Wolter *et al.* (1980) observed that, for restricted-fed rabbits slaughtered 4 h after feeding, 0.31 of the starch ingested had been hydrolysed in the stomach. However, this is probably an overestimate because of dilution of the diet with soft faeces recycled through caecotrophy; moreover, the intake of marker through soft faeces was not taken into account.

Amylase in the stomach originates essentially from the soft faeces and saliva, and remains at a constant level from week 4 of life independently of starch intake (Blas, 1986). The gastric pH is the main factor limiting its activity. Marounek *et al.* (1995) did not find amylase activity in the contents of the stomach of 4-week-old and 3-month-old rabbits, with enzyme-substrate incubations undertaken at pH 2.5. On the other hand, Sequeira *et al.* (2000) observed amylase activity in the gastric contents of 9-week-old rabbits with incubations carried out at pH 6.9.

In fact, the amylase activity of the stomach contents disappears completely if the pH is lower than 3.2 (Blas, 1986), and the gastric pH in the antrum is usually around 2 (see Chapter 1). However, the buffering capacity of the diet, soft faeces and saliva probably prevents immediate acidification. For instance, Blas (1986) found a pH of 4–4.5 in the stomach contents of growing rabbits 150 min after feeding following a 24-h fast; and Herrmann (1989) even reported a pH of >5 in certain areas of the stomach after high feed intake. However, rabbits that are fed *ad libitum* have 20–30 ‘voluntary meals’ a day, and the gastric pH is thus normally <2.5. Nevertheless, as a consequence of physiological hypochlorhydria in young rabbits, the gastric pH is >5 in 3-week-old rabbits and still >4 in 4-week-old rabbits, as reviewed by Gidenne and Fortun-Lamothe (2002). On the other hand, while soft faeces are being stored the pH of the fundus can rise to 4.0–5.1, whereas the pH of the antrum always remains very acidic (Griffiths and Davies, 1963).

Under these less acidic conditions, the amylase in the stomach contents, especially that of microbial origin from soft faeces, maintains appreciable activity (Alexander and Chowdhury, 1958; Griffiths and Davies, 1963; Hörnicke and Mackiewicz, 1976; Blas, 1986; Vernay, 1986). Table 2.3 illustrates this process of gastric fermentation (originating

Table 2.3. Effect of caecotrophy prevention on lactate concentration in the gastric contents and blood of rabbits (Vernay, 1986).

	Control	Caecotrophy prevention for 4 days
Feed intake (g day^{-1})	124	127
Gastric contents (mM)		
Fundus	4.7	1.9
Corpus	3.4	1.9
Antrum	2.4	1.6
Blood (mM in plasma)		
Venous		
Gastric	3.4	1.9
Ileal	3.8	2.1
Caecal	2.8	1.9
Portal	3.6	2.5
Arterial (abdominal aorta)	3.0	1.8

from starch, sugars and perhaps other carbohydrates), demonstrating that the concentration of lactate in both the gastric digesta (and not in that of the other parts of the digestive tract) and the blood falls significantly when caecotrophy is prevented.

Intestinal digestion

As stated above, it is acknowledged that starch digestion takes place mainly in the small intestine, and the most important enzyme involved is pancreatic amylase. Other enzymes of the epithelial cells of the intestinal mucosa are also necessary (maltase, amyloglucosidase), resulting finally in the release of glucose, which in principle is absorbed *in situ*.

Studies to assess the capacity of rabbits to digest starch in the small intestine vary widely in methodological aspects, such as: (i) target samples (pancreatic tissue or secretion, intestinal mucosa, intestinal contents); (ii) time of sampling (evening, morning, without or with a previous fasting period); (iii) conditions in assaying the enzyme activity (with or without considering optimal kinetic parameters, concerning substrate and enzyme concentrations, incubating period, temperature, pH); and (iv) units used to express enzyme activity (as relative to protein in pancreatic tissue or secretion, as relative to pancreatic tissue or secretion, as relative to intestinal mucosal protein or tissue, as relative to intestinal contents, as total in pancreatic tissue, intestinal mucosa or intestinal contents kg^{-1} live weight). Logically, these methodological variations make it difficult to evaluate the age-dependent evolution of capacity to digest starch intestinally and, especially, the possible role of starch intake in modulating this capacity.

Despite these difficulties, the ontogenic development of such digestive capacity is well established. Amylase activity increases rapidly between weeks 2 and 7 of life (Corring *et al.*, 1972; Blas, 1986; Scapinello *et al.*, 1999; Gutiérrez *et al.*, 2002a; Toral *et al.*, 2002; Debray *et al.*, 2003; Gidenne *et al.*, 2007; Gallois *et al.*, 2008a) and is still increasing in 3-month-old rabbits (Marounek *et al.*, 1995; Dojanã *et al.*, 1998); similarly, the amyloglucosidase activity of the jejunal mucosa generally increases between 37 and 60 days of age (Otutumi *et al.*, 2005). However, the ontogenic development of intestinal maltase activity remains controversial. According to Toofanian (1984) and Gallois *et al.* (2008a), maltase activity increases very rapidly between weeks 2 and 4 of life, but not afterwards; others have reported increasing maltase between 32 and 42 days of life (Debray *et al.*, 2003; Gidenne *et al.*, 2007), and even between 1 and 3 months (Marounek *et al.*, 1995). Still further studies have reported no changes in maltase activity between 25- and 35-day-old rabbits (Gutiérrez *et al.*, 2002a) or between 32- and 42-day-old rabbits (Scapinello *et al.*, 1999). Finally, Otutumi *et al.* (2005) found similar maltase activity in the jejunal mucosa of 37- and 60-day-old rabbits, while it was higher in jejunal contents for 60- than for 37-day-old rabbits, maltase activity being expressed per mg of, respectively, mucosa or contents.

Modulating the intestinal capacity for starch digestion according to diet is usually approached by varying starch intake. In many species, it is acknowledged that the digestive potential of the small intestine adapts to higher starch intake by increasing pancreatic amylase, intestinal maltase and amyloglucosidase secretion. Blas (1986) found higher amylase activity in the pancreatic secretions of both growing (28- and 42-day-old) and adult rabbits with a higher starch intake in samples obtained 150 min after feeding following a 24-h fast (no differences were found in basal samples taken following a 24-h fast). Similar results have been observed in adult rabbits in both pancreatic and intestinal tissue, and also in the intestinal contents (Abbas *et al.*, 1991).

However, more recent studies that have investigated differences in the starch intake of young rabbits by modifying the dietary starch concentration (consequently changing other components, such as fibre) seem to disagree with the above-mentioned adaptability of the digestive potential. For instance, Debray *et al.* (2003) found no differences either in pancreatic amylase or in intestinal mucosal or intraluminal maltase in 42-day-old rabbits consuming twice the amount of starch than controls. Furthermore, Gidenne *et al.* (2007) reported higher amylase activity in the total intestinal contents in 42-day-old rabbits consuming one-third less starch than controls, while Gutiérrez *et al.* (2002a) reported no changes in pancreatic amylase in 35-day-old rabbits consuming twice the amount of starch (by replacing lactose) than controls and higher pancreatic amylase and jejunal mucosal maltase when consuming one-third less starch.

Finally, studies in young rabbits inducing differences in starch intake by stimulating feed intake through early weaning or milk restriction have reported contradictory results. Corring *et al.* (1972) found that pancreatic amylase activity in 30-day-old rabbits was higher in those weaned at 21 days than in those remaining suckling. Gutiérrez *et al.* (2002a) found even wider differences in 35-day-old rabbits depending on whether they were weaned at 25 days old or remained suckling, although jejunal mucosal maltase decreased in those that were early weaned as a consequence of impairing mucosal morphology. Conversely, higher feed and starch intake before weaning in milk-restricted rabbits had no effect on amylase and maltase activities in the intestinal contents at weaning or 10 days later (Scapinello *et al.*, 1999). Gallois *et al.* (2008a) reported lower amylase activity in the intestinal contents but higher maltase activity in the intestinal mucosa of 28-day-old rabbits that were still suckling than in those early weaned at 21 days of age (without negative effects of early weaning on mucosal morphology).

Caecal fermentation

Starch undigested in the small intestine is in principle very quickly hydrolysed and fermented by the microbiota in the caeco-colic segment to lactate and volatile fatty acids (VFAs), absorbed *in situ*. Different studies have demonstrated the presence of amylase activity in this part of the digestive tract (Yoshida *et al.*, 1968; Blas, 1986; Makkar and Singh, 1987; Marounek *et al.*, 1995). Some data suggest that amylase could be of microbial origin and also from the ileal digesta flow. For instance, amylase activity in the caecum and the colon is even greater in germ-free rabbits than in normal rabbits (Yoshida *et al.*, 1968), and is more than twice as high in the rabbit caecum than in the rumen of steers (Makkar and Singh, 1987). Blas (1986) observed that amylase activity in the caecal contents hardly varied with age in 4- to 8-week-old rabbits, but was four times greater with a diet rich in starch than with a low-starch diet. On the other hand, Marounek *et al.* (1995) found amylase activity in caecal contents to be five times greater in 4-week-old rabbits than in 3-month-old rabbits.

Stable high counts of amylolytic bacteria in the caecal contents of 2- to 7-week-old rabbits have been reported (Padilha *et al.*, 1995). Different strains of rabbit caecal bacteria (*Actinomyces israelii*, *Dichelobacter nodosus*, *Mitsuokella multiacidus*, *Bacteroides* spp., *Eubacterium* spp., *Clostridium* spp.) have been shown to produce extracellular or membrane-bound α-amylases (Sirotek *et al.*, 2006). Paradoxically, a study on the *in vitro* fermentation of soluble potato starch used as a substrate for determining α-amylase, by inocula prepared from caecal contents of 36- or 78-day-old rabbits, indicated slow and poor fermentation (long lag phase, long time to reach maximum fermentation rate, low maximum fermentation rate), especially in the younger rabbits (Laurenčič, 2007). This suggests that starch fermentation could be negligible if considering the usual mean retention time of digesta in this digestive segment (6–12h); it could be hypothesized that a low availability of glucose exo-splitting enzymes (β-amylase, amyloglucosidase) is a limiting factor. Nevertheless, the differences between ileal and faecal digestibility of starch shown later seem to clearly indicate *in vivo* fermentation of starch passing the ileo-caecal junction.

2.2.3 Factors affecting starch digestibility

As stated above, starch digestion is primarily affected by the age of the rabbit and by the dietary level and origin of the starch. Other factors may also have some influence, such as the feed manufacturing process or the use of exogenous enzymes as dietary supplements.

Age and starch in the diet

ADULT RABBITS. Table 2.4 shows starch digestibility data obtained from different studies in adult rabbits. Faecal losses of starch are always very low (<0.01 of intake). These losses are greater for maize than for other sources (Gidenne and Perez, 1993b; Amber, 1997), although the differences are quantitatively of little relevance. For adults, the faecal digestibility of starch is also largely independent of starch intake. Accordingly, de Blas *et al.* (1995) found no variation between lactating and non-lactating rabbit does, with the former consuming more than twice the amount of starch. As expected, almost all of the starch is hydrolysed before reaching the caecum: its ileal digestibility is about 0.97 and is largely independent of both the starch intake and the source of starch. Only Pinheiro (2002) detected a statistically significant but minor reduction in the ileal digestibility of starch when replacing half of the starch from wheat with purified potato

Table 2.4. Ileal and faecal digestibility of starch in adult rabbits fitted with an ileal cannula.

Source of starch	Dietary level of starch (g kg^{-1} DM)	Digestibility of starch: Ileal	Digestibility of starch: Faecal	Reference
Purified maize starch	158	0.945	0.993	Gidenne (1992)
	353	0.947	0.996	
Barley	306	0.982	0.995	Merino and Carabaño (1992)
Purified maize starch	256	–	0.997	Gidenne and Perez (1993b)
Maize	292	–	0.990	Gidenne and Perez (1993b)
Barley	283	–	0.998	Gidenne and Perez (1993b)
Pea	280	–	0.996	Gidenne and Perez (1993b)
Purified maize starch	280	0.992	0.998	Amber (1997)
	353	0.991	0.999	
Maize	103	0.972	0.989	Amber (1997)
	255	0.971	0.990	
Barley	102	0.970	0.990	Amber (1997)
	251	0.984	0.994	
Wheat, wheat bran	116	0.987	0.992	Gidenne *et al.* (2000)
Wheat	329	0.930	0.997	Gidenne *et al.* (2000)
Wheat, wheat bran	220	0.983	0.997	Pinheiro (2002)
Purified potato starch, wheat	223	0.967	0.996	Pinheiro (2002)

starch (0.983 versus 0.967), the latter being very resistant to *in vitro* digestion with thermostable amylase during 270 min at pH 4.5 and 37°C (Pinheiro and Gidenne, 2000). Consequently, the ileal flow of starch is low: 1.0–3.2 g day^{-1} (Gidenne, 1992), 0.3–1.3 g day^{-1} (Amber, 1997), 0.2–2.3 g day^{-1} (Gidenne *et al.*, 2000) and 0.5–1.1 g day^{-1} (Pinheiro, 2002). The amount of starch fermented in the caeco-colic segment of adult rabbits is between 0.01 and 0.07 of the starch intake.

GROWING RABBITS. A review of 30 studies involving 65 different diets, from 1982 to 2009, reveals that faecal digestibility in growing rabbits (4–11 weeks old) is higher than 0.98 (averaging 0.99) in most cases, independent of the age of the rabbits, starch intake and starch source (barley, wheat, maize, oats, triticale, cassava, purified maize starch or purified potato starch). There are some exceptions that will be discussed later, essentially suggesting an interaction between the age of rabbit and some starch sources.

The effect of rabbit age is very limited for the majority of starch sources. In fact, faecal digestibility of starch during week 4 of life, in still-suckling young rabbits beginning to consume feed, is almost total (0.98–0.99) with starch from barley, wheat or pea, with minor but statistically significant reduction when including pea (Blas, 1986; Gidenne *et al.*, 2007). However, as shown in Table 2.5 and Fig. 2.1, faecal losses of starch can increase in some cases, usually for maize and particularly for the youngest rabbits. The resistance of maize starch to digestion is also seen in pigs and ruminants, but not in poultry. The endosperm structure of maize seeds and their resistance to grinding are considered the main factors behind this lower degradation (Rooney and Pflugfelder, 1986). These disappear in the process of manufacturing purified maize starch. Nevertheless, some studies using maize as the only or main starch source have reported faecal digestibility of starch similar to that from other sources, from 0.98 to 1.00 (Toral *et al.*, 2002; Xiccato *et al.*, 2002; Furlan *et al.*, 2003). It must be stated that the differences between varieties of a particular grain, with special reference to the amylose-amylopectin ratio, may affect faecal losses of starch. This may also help to explain some values of <0.98 of faecal digestibility of starch from wheat, barley, oats and wheat bran (Parigi-Bini *et al.*, 1990; Cossu *et al.*, 2004; Volek and Marounek, 2008) (see Table 2.5).

In different studies with both growing and adult rabbits, comparing various dietary starch levels (Blas, 1986; Blas *et al.*, 1990; Parigi-Bini *et al.*, 1990; Gidenne, 1992; de Blas *et al.*, 1995; Amber, 1997; Gidenne and Perez, 2000; de Arruda *et al.*, 2002; Gutiérrez *et al.*, 2002a), the faecal digestibility of starch has tended to decrease systematically in diets

Table 2.5. Faecal digestibility of starch from several studies on growing rabbits showing values of <0.98.

Source of starch	Age (weeks)	Faecal digestibility of starch	Reference
Barley, wheat bran	8	0.967	Parigi-Bini *et al.* (1990)
Wheat bran, barley	8	0.945	Parigi-Bini *et al.* (1990)
Maize	7–9	0.945	de Arruda *et al.* (2002)
Wheat, wheat bran	8–11	0.950	Cossu *et al.* (2004)
Maize, wheat bran	7	0.961	de Faria *et al.* (2004)
Maize, purified maize starch	11	0.973	dos Santos *et al.* (2004)
Maize	6–9	0.970	Otutumi *et al.* (2005)
Sorghum	6–9	0.979	Otutumi *et al.* (2005)
Maize, wheat bran	8	0.971	Furlan *et al.* (2006)
Buckwheat, maize, wheat bran	8	0.979	Furlan *et al.* (2006)
Maize, wheat bran	9	0.957	Michelan *et al.* (2006)
Maize, cassava hulls, wheat bran	9	0.972	Michelan *et al.* (2006)
Oats, barley, wheat bran	8	0.966	Volek and Marounek (2008)

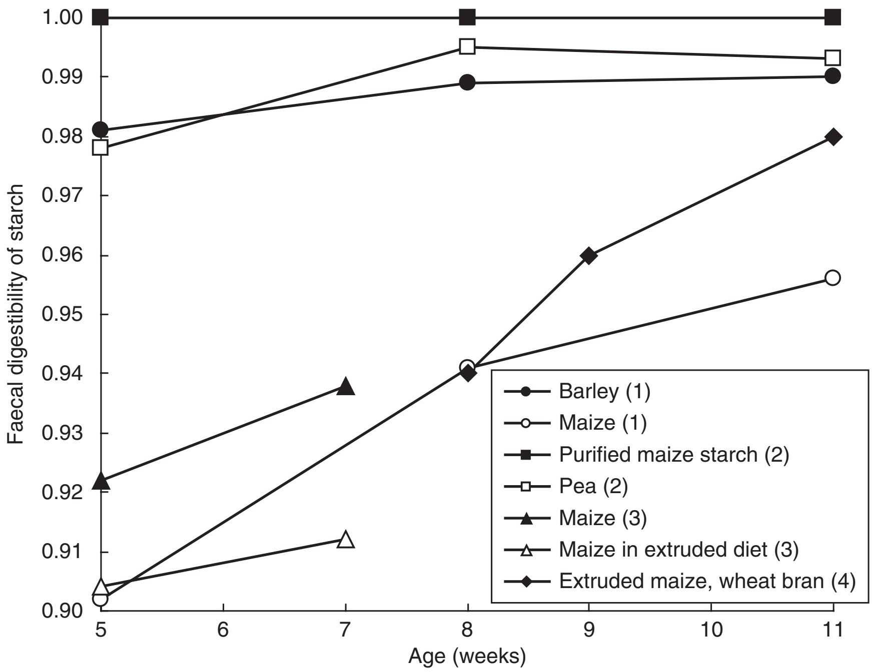

Fig. 2.1. Effect of age and starch source on the faecal digestibility of starch in growing rabbits. 1, Blas *et al.* (1990); 2, Gidenne and Perez (1993a); 3, Maertens and Luzi (1995); 4, Cossu *et al.* (2004).

with lower starch content in comparison with those of higher starch content (even with the same source of starch). Although statistically significant, this decrease remains small and may often be considered irrelevant. There is no clear explanation for these results. In fact, a lower dietary starch level corresponds to a higher fibre level, and thus it can be hypothesized that a faster rate of passage leads to a lower efficiency in starch degradation. A presence of endogenous α-linked glucose polymers (e.g. dextrans in the microbial reserves) being proportionally more important in diets lower in starch can also be hypothesized.

Results on the ileal digestibility of starch in growing rabbits are summarized in Table 2.6, with values ranging between 0.88 and 0.98 and averaging 0.94. No clear evidence of effect of the dietary starch level or source on its ileal digestibility has been detected, although studies with sources of more resistant starch (e.g. maize) are not available. However, some other factors can be considered as affecting the efficiency of starch digestion in the small intestine. In this context, the ileal digestibility of starch in 35-day-old rabbits is significantly lower than in 42-day-old rabbits when the dietary starch level is increased enough (Soler *et al.*, 2006). It is also decreased significantly when the dietary neutral detergent-soluble fibre is reduced in isostarch diets by using different fibre sources (oat hulls, lucerne hay, beet-apple pulp), and this seems to be associated with an impaired intestinal mucosal morphology (Gómez-Conde *et al.*, 2007). On the other hand, the ileal flow of starch in 35-day-old rabbits has been found to range between 0.6 and 2.3 g day^{-1} (Gutiérrez *et al.*, 2002a), 0.8 g day^{-1} (Nicodemus *et al.*, 2003), 0.1 and 1.5 g day^{-1} (Soler *et al.*, 2006), 0.5 and 1.2 g day^{-1} (Gómez-Conde *et al.*, 2007) or 0.1 and 0.4 g day^{-1} (Sánchez-Martínez, 2009). The amount of starch fermented in the caeco-colic segment of 4- to 6-week-old rabbits is between 0.03 (Gallois *et al.*, 2008b) and 0.11 (Gutiérrez *et al.*, 2002a) of the starch intake, without excluding possible higher proportions if

Table 2.6. Ileal digestibility of starch in growing rabbits slaughtered between 19.00 and 22.00 h.

Source of starch	Dietary level of starch (g kg^{-1} DM)	Age (days)	Ileal digestibility of starch	Reference
Wheat, wheat bran	75	35	0.918	Gutiérrez *et al.* (2002a)
Wheat, wheat bran	103	35	0.916	Gutiérrez *et al.* (2002a)
Wheat	168	35	0.914	Gutiérrez *et al.* (2002a)
Wheat	226	35	0.884	Gutiérrez *et al.* (2002a)
Wheat	215	35	0.955	Nicodemus *et al.* (2003)
Wheat, wheat flour, wheat bran	279	39	0.968	Nicodemus *et al.* (2004)
Wheat bran	67	35	0.974	Soler *et al.* (2006)
		42	0.975	
Wheat	186	35	0.895	Soler *et al.* (2006)
		42	0.955	
Heat-treated wheat + oat hulls	211	35	0.932	Gómez-Conde *et al.*(2007)
Heat-treated wheat + lucerne hay	208	35	0.950	Gómez-Conde *et al.* (2007)
Heat-treated wheat + beet-apple pulp	205	35	0.968	Gómez-Conde *et al.* (2007)
Wheat, wheat bran	113	28	0.941	Gallois *et al.* (2008b)
		42	0.954	
No starchy ingredients	24	35	0.957	Sánchez-Martínez (2009)
Wheat	116	35	0.954	Sánchez-Martínez (2009)

sources of more resistant starch are used. As a reference, in the study of Gallois *et al.* (2008b), with a diet containing 113 and 147 g kg^{-1} DM of starch (wheat and wheat bran) and cellulose, respectively, the amount of starch fermented in the caeco-colic segment of 21-day-weaned rabbits at 28 days old was only 0.1 g day^{-1}, while the amount of cellulose digested in the whole gastrointestinal tract was 0.9 g day^{-1} (using the mean value of faecal digestibility for cellulose (0.17) reported by Gidenne, 2003). In contrast, in the study of Gutiérrez *et al.* (2002a), with a diet containing 226 and 141 g kg^{-1} DM of starch (wheat) and cellulose, respectively, the amount of starch fermented in the caeco-colic segment of 25-day-weaned rabbits at 35 days old was about 20 times higher (2.2 g day^{-1}), while the amount of cellulose digested in the whole gastrointestinal tract was only about twice as high (2.1 g day^{-1}, using the mean value of faecal digestibility for cellulose given above).

Other studies have indirectly approached the efficiency of the small intestine for starch digestion by measuring the starch concentration in the terminal ileum of growing rabbits, as summarized in Fig. 2.2. Within these studies, large variations in sampling times make it difficult to establish clear conclusions, because the composition of the ileal contents varies according to the feed-intake pattern of the animal, including caecotrophy. Nevertheless, these results suggest that the amount of starch reaching the caecum increases at earlier ages when the rabbit is fed with high-starch diets, and that an interaction with the starch source cannot be excluded. Thus, purified potato starch (uncooked), known to be resistant to intestinal digestion in the piglet, seems to be highly digested in the intestine of the young rabbit; the starch concentration in the ileum with a diet including a high proportion of this starch source was around 10 g kg^{-1} DM (Pinheiro, 2002), while it reached 110–130 g kg^{-1} DM with maize-rich diets (Blas *et al.*, 1994; Gidenne *et al.*, 2005a). Similarly, Gutiérrez *et al.* (2002b) found a higher starch concentration in the terminal ileum of early-weaned 35-day-old rabbits when using pea instead of wheat (60 and 36 g kg^{-1} DM, respectively). It has also been found that,

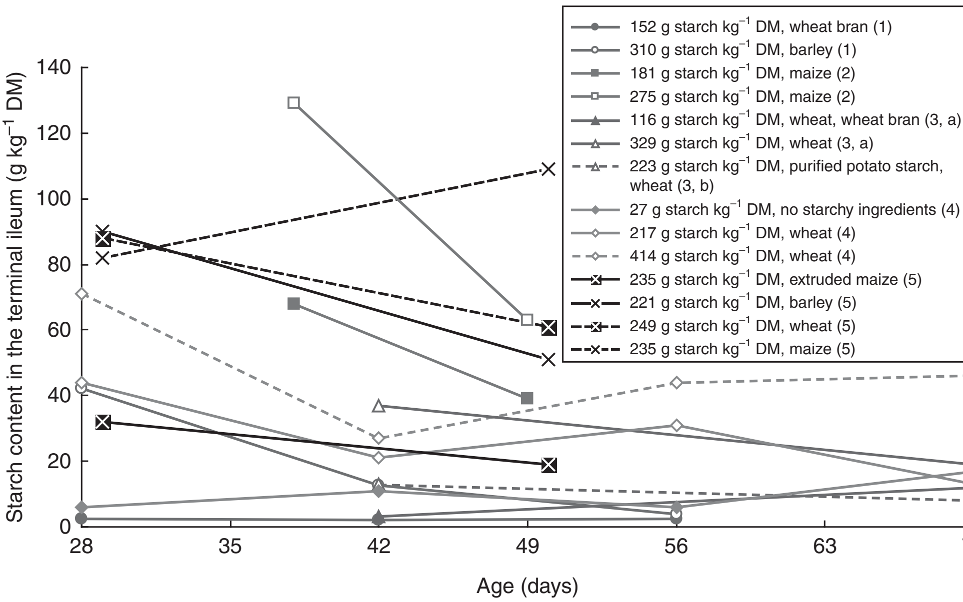

Fig. 2.2. Effect of age and dietary starch level or source on starch concentration in the terminal ileum of growing rabbits. 1, Sampling at 150 min after feeding following a 24-h fast (Blas, 1986); 2, sampling at 20.00 h, fed *ad libitum* (Blas *et al.*, 1994); 3, sampling at 10.00 and 18.00 h (a) or at 13.00 h (b), fed *ad libitum* (Pinheiro, 2002); 4, sampling at 13.00 h, fed *ad libitum* (Gidenne *et al.*, 2004a); 5, sampling at 13.00 h, fed *ad libitum* (Gidenne *et al.*, 2005a). DM, dry matter.

compared with ileal digesta, caecal digesta with a maize-rich diet contains 50% less starch in 38-day-old rabbits and 32% less in 49-day-old rabbits, while these figures are 24% and 3%, respectively, with a diet containing less maize (Blas *et al.*, 1994). Finally, as mentioned above, amylase activity in the caecal contents does not differ throughout the growing period, whereas pancreatic amylase secretion increases during this period. This may be interpreted as the result of an increment in the microbial contribution to the pool of amylase in the caecal contents of younger rabbits.

Feed manufacturing process

The oral administration of cooked purified maize starch in adult rabbits causes a clear post-prandial response of glycaemia in peripheral blood. This is similar to that produced by glucose, but somewhat later and more prolonged (Fig. 2.3). However, uncooked purified maize starch hardly affects basal glycaemia. This is due to slower digestion leading to a prolonged but much less pronounced increase in glycaemia in the portal blood, which has little impact on glycaemia in the peripheral blood. It is unlikely that slower digestion results in greater faecal losses of starch, but it could affect the amount of starch fermented in the large intestine.

In practice, it would be interesting to clarify whether, under normal feeding conditions, the feed manufacturing process (involving heating, moisture and pressure) affects starch digestion, especially in young rabbits. Unfortunately, there is little information available on this matter.

All of the results presented so far have been obtained with pelleted diets, as pelleting is the usual process in rabbit-feed manufacturing. Maertens and Luzi (1995) observed that extrusion of feed (which involves more intensive processing of the diet at a higher temperature, moisture and pressure) improved the *in vitro* solubility of dietary starch, but failed to reduce faecal losses of starch in 5- or 7-week-old rabbits fed on a maize-rich diet. On the other

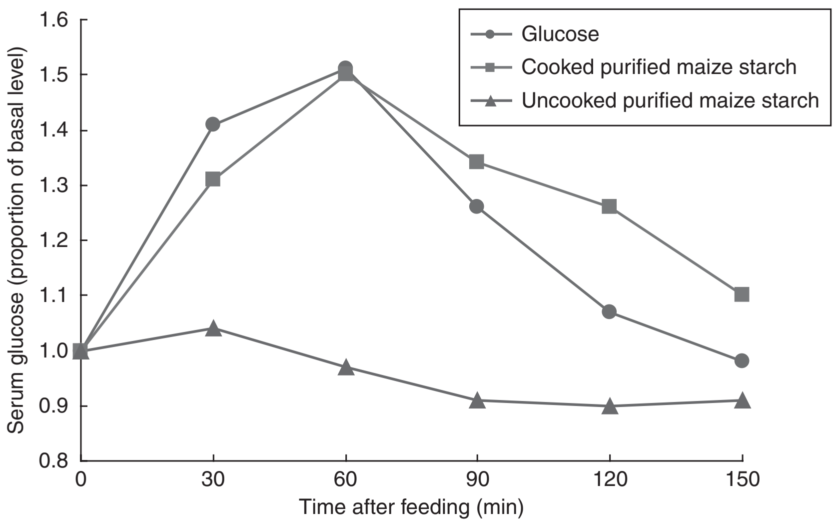

Fig. 2.3. Serum glucose in peripheral blood (marginal ear vein) of adult rabbits after oral administration of glucose or purified maize starch (0.5 g kg^{-1}) following a 12-h fast (Lee *et al.*, 1985).

hand, these losses increased with the extruded diet, and the authors suggested that starch may be retrograded after cooling. The alternative is the inclusion of previously extruded or cooked starch sources in pelleted diets. Otutumi *et al.* (2005) reported improved faecal digestibility of starch from both extruded maize and sorghum in 5- or 9-week-old rabbits. Obviously, no effect of extrusion or cooking on faecal digestibility of starch was found when it was already 0.99 when using raw maize, wheat, triticale, pea or cassava (Gutiérrez *et al.*, 2002b; Furlan *et al.*, 2003, 2004, 2005; Otutumi *et al.*, 2005). On the other hand, these processes could reduce the amount of starch escaping from the small intestine. Significant decreases in the ileal starch concentration in 35-day-old rabbits have been reported by cooking pea or wheat (Gutiérrez *et al.*, 2002b), and especially by extruding maize in 29- or 50-day-old rabbits (Gidenne *et al.*, 2005a).

Enzyme supplementation

It is well established that the effectiveness of exogenous enzymes depends on their capacity to resist gastric pH and proteolytic attack by host digestive enzymes, as well as to survive the feed manufacturing process (Inborr, 1989; Bedford, 1995). Yu and Tsen (1993) observed that the incubation of thermostable amylase with rabbit intestinal contents at pH 7.5 did not greatly reduce its activity, while the activity fell to 0.2 in 10 min and reached negligible values in 30 min when the incubation was performed with the contents of the stomach at pH 2–3.2.

Mahagna *et al.* (1995) observed that the addition of amylase to a sorghum-based diet in meat-type chicks did not improve the faecal digestibility of starch and reduced the amount of amylase present in the intestinal contents, suggesting that the addition of an enzyme having activity similar to that of the pancreatic enzyme appears to have no benefit. Logically, enzyme supplements including α-amylase, even if thermostable, are ineffective in increasing the faecal digestibility of starch when it is already >0.99 in control diets (Fernández *et al.*, 1996; Sequeira and Villamide, 1999; Gutiérrez *et al.*, 2002b). However, a significant reduction of the ileal starch concentration in 35-day-old rabbits fed pea- or wheat-including diets has been reported (Gutiérrez *et al.*, 2002b).

2.2.4 Consequences of starch digestion on fermentative activity in the caeco-colic segment

As discussed earlier, the ileal flow of starch is low. However, since it varies depending on the age of the rabbit and the intake or origin of starch, the activity of the caeco-colic microbiota can potentially be modified. As variations in the level of starch intake are classically linked to inverse variations in fibre intake, the possible effect of undigested starch on microbial activity can only be elucidated by comparing diets with negligible differences in the diverse fibrous constituents, as some diets formulated to compare different starch sources. Gidenne *et al.* (2005a) found that an increase in the starch content of digesta in the terminal ileum of 7-week-old rabbits (from 19 to 109 g kg^{-1} DM), as a consequence of replacing exclusively extruded maize with maize, was associated with a reduction of faecal digestibility of cellulose (from 0.24 to 0.15), no changes in the total VFA concentration in the caecal contents and slight changes in the fermentation pattern (increasing the butyrate proportion at the expense of propionate). In 10-week-old rabbits, the effect of dietary starch source (maize versus barley) on caecal fermentation was weak and limited to a higher proportion of isovalerate (Belenguer *et al.*, 2000) or valerate (Xiccato *et al.*, 2002), both minor VFAs being linked to amylolytic microbiota (Padilha *et al.*, 1995). In 12-week-old rabbits, Belenguer *et al.* (2008) reported a lower caecal VFA concentration but a higher butyrate proportion at the expense of acetate and propionate, for rabbits fed a ‘maize’ diet in comparison with a ‘wheat’ diet, but only when the fibre source was lucerne and not sugar beet pulp. *In vitro* fermentation induced by the corresponding inocula produced a higher butyrate proportion at the expense of acetate, as well as higher gas production at any time, with inocula from rabbits fed a ‘maize’ diet in comparison with a ‘wheat’ diet, whatever the fibre source. According to Bird *et al.* (2007), a higher amount of resistant starch from maize reaching the hindgut may lead to butyrogenic fermentation in piglets. In adult rabbits, the starch concentration in the ileum ranged from 3 to 27 g kg^{-1} DM, depending on the source of starch (purified maize starch, barley, pea, maize), while the faecal digestibility of hemicelluloses ranged from 0.37 to 0.54, although changes in the nature of dietary fibre were appreciable because of the high level of inclusion of starch sources (Gidenne and Perez, 1993b). However, Amber (1997) found that both ileal flow and the amount of starch fermented in the caeco-colic segment were slightly greater with a maize-rich diet than with a barley-rich diet, and no relevant differences in the faecal digestibility of the fibres were observed.

In any case, fibre remains the main factor determining fermentative activity. The influence of starch is negligible, although relevant in young animals fed starch-rich diets, especially containing resistant starch.

2.2.5 Role of starch on digestive health

Suckling rabbits

Reviewing a total of 17 studies involving 40 different diets, from 1995 to 2006, it appears that dietary starch levels (also linked to fibre or fat changes) do not greatly affect the mortality rate of the young rabbits, from the time they begin to consume feed until weaning. In fact, the consumption of milk represents an important part of nutrient intake and contributes to health protection, thus explaining that the health status of the suckling rabbit is largely independent of the feed. The protective role of milk intake has been observed (Fortun-Lamothe and Boullier, 2007; Gallois *et al.*, 2007). In the context of epizootic rabbit enteropathy (ERE), Martínez-Paredes *et al.* (2009) reported very high mortality during week 6 of age in rabbits weaned at 28 days old, but very low mortality in those that remained suckling until 42 days old, where the mortality rate clearly increased during week 8 of age.

Table 2.7. Mortality rates after weaning in growing rabbits fed on diets differing in starch and fibre levels.

Dietary fibre										
g kg^{-1} DM				Ratios						
Lignin (ADL)	Cellulose (ADF-ADL)	Hemicelluloses (NDF-ADF)	Water-insoluble pectins[a]	Lignin to cellulose	DF[b] to ADF	Starch (g kg^{-1} DM)	Mortality (%)	Comments	No. of rabbits involved	Reference
51	146	131	44	0.35	0.89	264	6.7	32–49 days	808	Perez *et al*. (2000)
46	167	181	82	0.28	1.23	136	2.4			
56	162	134	70	0.35	0.94	127	24.8	28–42 days	1600	Soler *et al*. (2004)
56	162	154	87	0.35	1.11	67	13.6	ERE		
79	150	148	62	0.53	0.92	142	11.3	36–64 days	7203	Fabre *et al*. (2006)[c]
71	191	151	86	0.37	0.90	47	5.3	ERE		
34	148	140	63	0.23	1.12	211	43.1	27–76 days	246	Carraro *et al*. (2007)
39	168	152	75	0.23	1.10	151	6.9	ERE		
56	173	204	69	0.33	1.19	87	42.8	28–49 days	594	Martínez-Vallespín (2008)
96	195	211	63	0.49	0.94	30	29.2	ERE		
45	170	153	54	0.26	0.96	211	31.7	25–70 days	240	Xiccato *et al*. (2008)
43	188	177	96	0.23	1.18	108	11.5	ERE		

ADF, acid detergent fibre; ADL, acid detergent lignin; DF, detergent fibre; DM, dry matter; ERE, epizootic rabbit enteropathy; NDF, neutral detergent fibre.

[a]Calculated as uronic acids + galactose + arabinose + rhamnose in insoluble dietary fibre, from values reported by Brillouet *et al.* (1988), Bach Knudsen (1997) and Llobera and Cañellas (2007).

[b]Digestible fibre as hemicellulose + water-insoluble pectins.

[c]Nutrient content calculated according tables of FEDNA (2003), 6571 and 632 rabbits for the high- and low-starch diet, respectively.

Growing rabbits

It is well established that the susceptibility of rabbits to digestive disorders is greater after weaning, on account of the many physiological changes occurring around this time. A former hypothesis suggested that an overload of rapidly fermentable carbohydrates in the large intestine increases the likelihood of digestive disorders in weaned rabbits (Cheeke and Patton, 1980). The previous sections indicate the limited flow of starch at the ileum. Furthermore, two large-scale studies separating the effects of starch and fibre have revealed a low impact of starch intake on the incidence of digestive disorders in growing rabbits (Gidenne *et al.*, 2004b, 2005a, b). Thus, the effects on digestive health are mainly linked to changes in fibre intake (see Chapters 5 and 10). Accordingly, Gidenne and García (2006) proposed that the restriction of the dietary starch level could be higher than the usual one of 150–155 g kg^{-1} DM, or even removed.

Table 2.7 summarizes the mortality rates in the post-weaning or full-growing periods in six different studies, with diets varying in starch and fibre content. In all cases, the high-starch diets were maintained above the fibre requirements with the exception of lignin and lignin to cellulose ratio, which are generally lower than the recommended values (≥61 g kg^{-1} DM and >0.40 respectively). A reduction in the starch to fibre ratio resulted in decreased mortality. In practice, this means that, with respect to digestive health and especially in the context of ERE, fibre requirements should be increased at the expense of starch content.

In the young rabbit (before 6 weeks old), however, the role of starch in digestive troubles may not be excluded, taking into account the already-mentioned variations in the ileal flow of starch (by two, four or 15 times) and its potential impact on caecal microbiota. Nevertheless, Remois *et al.* (1996) found the inclusion of thermostable amylase and/or amyloglucosidase in a rabbit diet to have no effect on mortality rate. Conversely, Gutiérrez *et al.* (2002b) reported lower mortality as a consequence of the inclusion of an enzymatic supplement containing α-amylase and reducing the starch concentration at the ileum.

Adult rabbits

The relationship of starch intake to the incidence of digestive disorders in adult rabbits seems to be very limited within the usual dietary starch levels. de Blas *et al.* (1995) have suggested a trend towards an increase in the replacement rate of rabbit does (associated with more diarrhoea and sudden death at parturition) as the starch content of the diet increases while the fibre content decreases. However, other studies with changes in the starch content, at the expense of those of fibre or fat, have not reported relevant differences in the replacement rate of does (Lebas and Fortun-Lamothe, 1996; Pascual *et al.*, 1998, 1999; Quevedo *et al.*, 2006).

References

Abbas, T.A.K., Abdel-Latif, A.M. and Osman, A.M. (1991) Responses of rabbit digestive enzymes amylase and lipase to food composition and intermittent heat exposure. *World Review of Animal Production* 26, 81–86.

AFNOR (1985) *Aliments des animaux: méthodes d'analyse Françaises et communautaires*. AFNOR, Paris – La Défense, France.

Alexander, F. and Chowdhury, A.K. (1958) Digestion in the rabbit's stomach. *British Journal of Nutrition* 12, 65–73.

Amber, K.A. (1997) Efecto de la fibra y el almidón del pienso sobre la digestibilidad fecal e ileal en conejos adultos. Doctoral thesis, Universidad Politécnica de Valencia, Spain.

Bach Knudsen, K.E. (1997) Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology* 67, 319–338.

Bedford, M.R. (1995) Mechanism of action and potential environmental benefits from the use of feed enzymes. *Animal Feed Science and Technology* 53, 145–155.

Belenguer, A., Fondevila, M., Balcells, J. and Torre, C. (2000) Effect of the source and level of cereal in diet on the rabbit caecal environment and microbial population. *World Rabbit Science* 8 (Suppl. 1, Vol. C), 95–100.

Belenguer, A., Fondevila, M., Balcells, Abecia, L., Lachica, M. and Carro, M.D. (2008) *In vivo* and *in vitro* study of caecal fermentation pattern and methanogenesis in rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 535–539 (electronic edition).

Bird A.R., Vuaran, M., Brown, I. and Topping, D.L. (2007) Two high-amylose starches with different amounts of resistant starch vary in their effects on fermentation, tissue and digesta mass accretion, and bacterial populations in the large bowel of pigs. *British Journal of Nutrition* 97, 134–144.

Blas, E. (1986) El almidón en la nutrición del conejo: utilización digestiva e implicaciones prácticas. Doctoral thesis, Universidad de Zaragoza, Spain.

Blas, E., Fandos, J.C., Cervera, C., Gidenne, T. and Perez, J.M. (1990) Effet de la nature et du taux d'amidon sur l'utilisation digestive de la ration chez le lapin au cours de la croissance. In: *Proceedings 5èmes Journeés de la Recherche Cunicole*, Vol. 2. Comm. no. 50. INRA-ITAVI, Paris, France.

Blas, E., Cervera, C. and Fernández-Carmona, J. (1994) Effect of two diets with varied starch and fibre levels on the performances of 4–7 weeks old rabbits. *World Rabbit Science* 2, 117–121.

Brillouet, J.M., Rouau, X., Hoebler, C., Barry, J.L., Carré, B. and Lorta, E. (1988) A new method for determination of insoluble cell walls and soluble nonstarchy polysaccharides from plant materials. *Journal of Agricultural and Food Chemistry* 36, 969–979.

Carraro, L., Trocino, A., Fragkiadakis, M., Xiccato, G. and Radaelli, G. (2007) Digestible fibre to ADF ratio and starch level in diets for growing rabbits. *Italian Journal of Animal Science* 6 (Suppl. 1), 752–754.

Carré, B. (1992) Factors affecting the digestibility of non-starch carbohydrates in monogastric animals. *Poultry Science* 61, 1257–1269.

Champ, M. and Colonna, P. (1993) Importance de l'endommagement de l'amidon dans les aliments pour animaux. *INRA Production Animale* 6, 185–198.

Cheeke, P.R. and Patton, N.M. (1980) Carbohydrate-overload of the hindgut. A probable cause of enteritis. *Journal of Applied Rabbit Research* 3, 20–23.

Corring, T., Lebas, F. and Courtout, D. (1972) Contrôle de l'évolution de l'équipement enzymatique du pancréas exocrine de la naissance à 6 semaines. *Annales de Biologie Animale, Biochemie et Biophysique* 12, 221–231.

Cossu, M.E., Cumini, M.L. and Lazzari, G. (2004) Effect of corn processing and level of inclusion on growth of meat rabbits. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Mexico, pp. 785–791 (electronic edition).

de Arruda, A.M.V., Lopes, D.C., Ferreira, W.M., Rostagno, H.S., Queiroz, A.C., Pereira, E.S., Albino L.F.T. and da Silva, J.F. (2002) Digestibilidade aparente dos nutrientes de raçoes contendo diferentes fontes de fibra e níveis de amido com coelhos em crescimento. *Revista Brasileira de Zootecnia* 31, 1166–1175.

de Blas, C., Taboada, E., Mateos, G.G., Nicodemus, N. and Méndez, J. (1995) Effect of substitution of starch for fiber and fat in isoenergetic diets on nutrient digestibility and reproductive performance of rabbits. *Journal of Animal Science* 73, 1131–1137.

Debray, L., Le Huërou-Luron, I., Gidenne, T. and Fortun-Lamothe, L. (2003) Digestive tract development in rabbit according to the dietary energetic source: correlation between whole tract digestion, pancreatic and intestinal enzymatic activities. *Comparative Biochemistry and Physiology Part A* 135, 443–455.

de Faria, H.G., Scapinello, C., Peralta, R.M., Gidenne, T., Furlan, A.C. and Andreazzi, M.A. (2004) Digestibilidade e desempenho de coelhos oriundos de quatro padrões de alimentação até a desmama alimentados com dietas contendo diferentes níveis de amido após a desmama. *Revista Brasileira de Zootecnia* 33, 1172–1180.

Dojanã, N., Costache, M. and Dinischiotu, A. (1998) The activity of some digestive enzymes in domestic rabbits before and after weaning. *Animal Science* 66, 501–507.

dos Santos, E.A., Lui, J.F. and Scapinello, C. (2004) Efeito dos níveis de fibra em detergente ácido sobre os coeficientes de digestibilidade das dietas e desempenho de coelhos em crescimento. *Acta Scientiarum, Animal Sciences* 26, 79–86.

Eliasson, A.C. and Gudmundsson, M. (1996) Starch: physicochemical and functional aspects. In: Eliasson, A.C. (ed.) *Carbohydrates in Food*. Marcel Dekker Inc, Basel, Switzerland, pp. 431–503.

Fabre, C., Juvero, M.A., Blas, E., Fernández-Carmona, J. and Pascual, J.J. (2006) Utilización de un pienso rico en fibra digestible e indigestible y pobre en almidón en conejos de engorde: ensayo en condiciones de campo. In: *Proceedings XXXI Symposium de Cunicultura, Lorca*. ASESCU, Canet de Mar, Spain, pp. 67–72.

FEDNA (2003) Tablas de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos (de Blas, C., Mateos, G.G. and Rebollar, P.G., eds). FEDNA, Madrid, Spain.

Fernández, C., Merino, J. and Carabaño, R. (1996) Effect of enzyme complex supplementation on diet digestibility and growth performance in growing rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse,* Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 163–166.

Fortun-Lamothe, L. and Boullier, S. (2007) A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. *Livestock Science* 107, 1–18.

Fraga, M.J., Barreno, C., Carabaño, R., Méndez, J. and de Blas, C. (1984) Efecto de los niveles de fibra y proteína del pienso sobre la velocidad de crecimiento y los parámetros digestivos de los conejos. *Anales del INIA Serie Ganadera* 21, 91–110.

Furlan, A.C., Monteiro, R.T., Scapinello, C., Moreira, I., Murakami, A.E., Otutumi, L.K. and Santolin, M.L.R. (2003) Valor nutritivo e desempenho de coelhos em crescimento alimentados con rações contendo milho extrusado. *Revista Brasileira de Zootecnia* 32, 1157–1165.

Furlan, A.C., Monteiro, R.T., Scapinello, C., Moreira, I., Murakami, A.E. and Martins, E.N. (2004) Avaliação nutricional do triticale extrusado ou não para coelhos em crescimento. *Acta Scientiarum, Animal Sciences* 26, 49–55.

Furlan, A.C., Scapinello, C., Moreira, I., Murakami, A.E., Santolin, M.L.R. and Otutumi, L.K. (2005) Avaliação nutricional da raspa integral de mandioca extrusada ou não para coelhos em crescimento. *Acta Scientiarum, Animal Sciences* 27, 99–103.

Furlan, A.C., Santolin, M.L.R., Scapinello, C., Moreira, I. and de Faria, H.G. (2006) Avaliação nutricional do trigo mourisco (*Fagopyrum esculentum* Moench) para coelhos em crescimento. *Acta Scientiarum, Animal Sciences* 28, 21–26.

Gallois, M., Gidenne, T., Tasca, C., Caubet, C., Coudert, C., Milon, A. and Boullier, S. (2007) Maternal milk contains antimicrobial factors that protect young rabbits from enteropathogenic *E. coli* infection. *Clinical and Vaccine Immunology* 14, 585–592.

Gallois, M., Le Huërou-Luron, I., Fortun-Lamothe, L., Lallès, J.P. and Gidenne, T. (2008a) Adaptability of the digestive function according to age at weaning in the rabbit: I. Effect on feed intake and digestive functionality. *Animal* 2, 525–535.

Gallois, M., Fortun-Lamothe, L., Michelan, A. and Gidenne, T. (2008b) Adaptability of the digestive function according to age at weaning in the rabbit: II. Effect on nutrient digestion in the small intestine and in the whole digestive tract. *Animal* 2, 536–547.

Gidenne, T. (1992) Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. *British Journal of Nutrition* 67, 133–146.

Gidenne, T. (2003) Fibres in rabbit feeding for digestive troubles prevention: respective role of low-digested and digestible fibre. *Livestock Production Science* 81, 105–117.

Gidenne, T. and Fortun-Lamothe, L. (2002) Feeding strategy for young rabbits around weaning: a review of digestive capacity and nutritional needs. *Animal Science* 75, 169–184.

Gidenne, T. and García, J. (2006) Nutritional strategies improving the digestive health of the weaned rabbit. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Sciences*. ILVO, Melle, Belgium, pp. 229–238.

Gidenne, T. and Perez, J.M. (1993a) Effect of dietary starch origin on digestion in the rabbit. 1. Digestibility measurements from weaning to slaughter. *Animal Feed Science and Technology* 42, 237–247.

Gidenne, T. and Perez, J.M. (1993b) Effect of dietary starch origin on digestion in the rabbit. 2. Starch hydrolysis in the small intestine, cell wall degradation and rate of passage measurements. *Animal Feed Science and Technology* 42, 249–257.

Gidenne, T. and Perez, J.M. (2000) Replacement of digestible fibre by starch in the diet of the growing rabbit. I. Effects on digestion, rate of passage and retention of nutrients. *Annales de Zootechnie* 49, 357–368.

Gidenne, T. and Ruckebusch, Y. (1989) Flow and rate of passage studies at the ileal level in the rabbit. *Reproduction Nutrition Development* 29, 403–412.

Gidenne, T., Pinheiro, V. and Falcao e Cunha, L. (2000) A comprehensive approach of the rabbit digestion: consequences of a reduction in dietary fibre supply. *Livestock Production Science* 64, 225–237.

Gidenne, T., Jehl, N., Lapanouse, A. and Segura, M. (2004a) Inter-relationship of microbial activity, digestion and gut health in the rabbit: effect of substituting fibre by starch in diets having a high proportion of rapidly fermentable polysaccharides. *British Journal of Nutrition* 92, 95–104.

Gidenne, T., Mirabito, L., Jehl, N., Perez, J.M., Arveux, P., Bourdillon, A., Briens, C., Duperray, J. and Corrent, E. (2004b) Impact of replacing starch by digestible fibre, at two levels of lignocellulose, on digestion, growth and digestive health of the rabbit. *Animal Science* 78, 389–398.

Gidenne, T., Segura, M. and Lapanouse, A. (2005a) Effect of cereal sources and processing in diets for the growing rabbits. I. Effects on digestion and fermentative activity in the caecum. *Animal Research* 54, 55–64.

Gidenne, T., Jehl, N., Perez, J.M., Arveux, P., Bourdillon, A., Mousset, J.L., Duperray, J., Stephan, S. and Lamboley, B. (2005b) Effect of cereal sources and processing in diets for the growing rabbits. II. Effects on performance and mortality by enteropathy. *Animal Research* 54, 65–72.

Gidenne, T., Debray, L., Fortun-Lamothe, L. and Le Huërou-Luron, I. (2007) Maturation of the intestinal digestion and of microbial activity in the young rabbit: impact of the dietary fibre:starch ratio. *Comparative Biochemistry and Physiology Part A* 148, 834–844.

Gómez-Conde, M.S., García, J., Chamorro, S., Eiras, P., Rebollar, P.G., Pérez de Rozas, A., Badiola, I., de Blas, C. and Carabaño, R. (2007) Neutral detergent-soluble fiber improves gut barrier function in twenty-five-day-old weaned rabbits. *Journal of Animal Science* 85, 3313–3321.

Goodlad, J.S. and Mathers, J.C. (1990) Large bowel fermentation in rats given diets containing raw peas (*Pisum sativum*). *British Journal of Nutrition* 64, 569–587.

Goodlad, J.S. and Mathers, J.C. (1991) Digestion by pigs of non starch polysaccharides in wheat bran and raw peas (*Pisum sativum*) fed in mixed diets. *British Journal of Nutrition* 65, 259–270.

Griffiths, M. and Davies, D. (1963) The role of the soft pellets in the production of lactic acid in the rabbit stomach. *Journal of Nutrition* 80, 171–180.

Gutiérrez, I., Espinosa, A., García, J., Carabaño, R. and de Blas, C. (2002a) Effects of levels of starch, fiber, and lactose on digestion and growth performance of early-weaned rabbits. *Journal of Animal Science* 80, 1029–1037.

Gutiérrez, I., Espinosa, A., García, J., Carabaño, R. and de Blas, C. (2002b) Effects of starch and protein sources, heat processing, and exogenous enzymes in starter diets for early-weaned rabbits. *Animal Feed Science and Technology* 98, 175–186.

Herrmann, A. (1989) Untersuchungen über die Zusammensetzung des Chymus im Magen-Darm-Kanal von Jungkaninchen in Abhängigkeit vom Rohfaser- und Stärkegehalt des Futters. Inaugural dissertation, doctor medicinae veterinariae, Tierärztliche Hochschule, Hannover, Germany.

Hizukuri, S. (1996) Starch: analytical aspects. In: Eliasson, A.C. (ed.) *Carbohydrates in Food*. Marcel Dekker Inc., Basel, Switzerland, pp. 347–429.

Hörnicke, H. and Mackiewicz, A. (1976) Production d'amylase, décomposition de l'amidon et formation des acides D- et L- lactiques par les caecotrophes. In: Lebas, F. (ed.) *Proceedings of the 1st World Rabbit Congress, Dijon*. Communication no.56. WRSA, Dijon, France.

Inborr, J. (1989) Pig diet: enzymes in combination. *Feed International* 10, 16–27.

INRA (2004) Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage, 2ème edition, revue et corrigée (Sauvant, D., Perez, J.M. and Tran, G., eds). INRA Editions, Paris, France.

Kozlowski, F. (1994) L'amidon, quel dosage pour quel échantillon? *Cahiers Techniques de l'INRA* 35, 5–22.

Laurenčič, A. (2007) The effect of rabbit age on *in vitro* caecal fermentation of starch, pectin, xylan, cellulose, compound feed and its fibre. *Animal* 1, 241–248.

Lebas, F. and Fortun-Lamothe, L. (1996) Effects of dietary energy level and origin (starch *vs* oil) on performance of rabbit does and their litters: average situation after 4 weanings. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse,* Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 217–222.

Lee, P.C., Brooks, S.P., Kim, O., Heitlinger, L.A. and Lebenthal, E. (1985) Digestibility of native and modified starches: *in vitro* studies with human and rabbit pancreatic amylases and *in vivo* studies in rabbits. *Journal of Nutrition* 115, 93–103.

Llobera, A. and Cañellas, J. (2007) Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): pomace and stem. *Food Chemistry* 101, 659–666.

Maertens, L. and Luzi, F. (1995) The effect of extrusion in diets with different starch levels on the performance and digestibility of young rabbits. In: *Proceedings 9th Symposium on Housing and Diseases of Rabbits, Furbearing Animals and Pet Animals*. DVG, Celle, Germany, pp. 131–138.

Maertens, L., Lebas, F. and Szendrö, Z. (2006) Rabbit milk: a review of quantity, quality and non-dietary affecting factors. *World Rabbit Science* 14, 205–230.

Mahagna, M., Nir, I., Larbier, M. and Nitsan, Z. (1995) Effect of age and exogenous amylase and protease on development of the digestive tract, pancreatic enzyme activities and digestibility of nutrients in young meat-type chicks. *Reproduction Nutrition Development* 35, 201–212.

Makkar, H.P.S. and Singh, B. (1987) Comparative enzymatic profiles or rabbit caecum and bovine rumen contents. *Journal of Applied Rabbit Research* 10, 172–174.

Marounek, M., Vook, S.J. and Skrivanová, V. (1995) Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *British Journal of Nutrition* 73, 463–469.

Martínez-Paredes, E., Martínez-Vallespín, B., Ródenas, L., Pascual, J.J., Blas, E. and Cervera, C. (2009) Mortalidad de gazapos de cebo en función de la alimentación y de la edad al destete. In: *Proceedings XXXIV Symposium de Cunicultura, Sevilla*. ASESCU, Canet de Mar, Spain, pp. 113–137.

Martínez-Vallespín, B. (2008) Efectos del contenido en fibra, almidón y proteína del pienso de peridestete sobre los gazapos y las conejas. Masters thesis, Universidad Politécnica de Valencia, Spain.

Merino, J.M. and Carabaño, R. (1992) Effect of type of fibre on ileal and faecal digestibilities. *Journal of Applied Rabbit Research* 15, 931–937.

Michelan, A.C., Scapinello, C., Furlan, A.C., Martins, E.N., de Faria, H.G. and Andreazzi, M.A. (2006) Utilização da casca de mandioca desidratada na alimentação de coelhos. *Acta Scientiarum, Animal Sciences* 28, 31–37.

Nicodemus, N., Gómez-Conde, M.S., Espinosa, A., García, J., Carabaño, R. and de Blas, C. (2003) Efecto de la utilización de bacitracina de zinc y sulfato de apramicina sobre la digestión en gazapos destetados precozmente. In: *Proceedings XXVIII Symposium de Cunicultura, Alcañiz*. ASESCU, Canet de Mar, Spain, pp. 163–170.

Nicodemus, N., Pérez-Alba, L., Carabaño, R., de Blas, C., Badiola, I., Pérez de Rozas, A. and García, J. (2004) Effect of level of fibre and level of ground of fibre sources on digestion and ileal and caecal characterization of microbiota of early weaned rabbits. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Mexico, pp. 928–929 (electronic edition).

Otutumi, L.K., Furlan, A.C., Scapinello, C., Martins, E.N., Peralta, R.M., de Souza, D.L. and Santolin, M.L.R. (2005) Digestibilidade e atividade enzimática intestinal de coelhos em crescimento alimentados com diferentes fontes de amido procesadas ou não por extrusão. *Revista Brasileira de Zootecnia* 34, 557–567.

Padilha, M.T.S., Licois, D., Gidenne, T., Carré, B. and Fonty, G. (1995) Relationship between microflora and caecal fermentation in rabbits before and after weaning. *Reproduction Nutrition Development* 35, 375–386.

Parigi-Bini, R., Xiccato, G. and Cinetto, M. (1990) Influenza del contenuto di amido alimentare sulla produttività, sulla digeribilità e sulla composizione corporea di conigli in accrescimento. *Zootecnica e Nutrizione Animale* 16, 271–282.

Pascual, J.J., Cervera, C., Blas, E. and Fernández-Carmona, J. (1998) Effect of high fat diets on the performance and food intake of primiparous and multiparous rabbit does. *Animal Science* 66, 491–499.

Pascual, J.J., Tolosa, C., Cervera, C., Blas, E. and Fernández-Carmona, J. (1999) Effect of diets with different digestible energy content on the performance of rabbit does. *Animal Feed Science and Technology* 81, 105–117.

Perez, J.M., Gidenne, T., Bouvarel, I., Arveux, P., Bourdillon, A., Briens, C., Le Naour, J., Messager, B. and Mirabito, L. (2000) Replacement of digestible fibre by starch in the diet of the growing rabbit. II. Effects on performances and mortality by diarrhoea. *Annales de Zootechnie* 49, 369–377.

Pinheiro, V. (2002) Contributo para o studio da digestão nos coelhos: efeito do tenor em fibra e da natureza do amido da dieta. Doctoral thesis, Universidade de Trás-os-Montes e Alto Douro, Portugal.

Pinheiro, V. and Gidenne, T. (2000) Substitution of wheat by potato starch for growing rabbits: effect on performances, digestion and health. *World Rabbit Science* 8 (Suppl. 1, Vol. C), 391–398.

Quevedo, F., Cervera, C., Blas, E., Baselga, M. and Pascual, J.J. (2006) Long-term effect of selection for litter size and feeding programme on the performance of reproductive rabbit does 1. Pregnancy of multiparous does. *Animal Science* 82, 739–750.

Remois, G., Lafargue-Hauret, P. and Rouillere, H. (1996) Effect of amylase supplementation in rabbit feed on growth performance. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse,* Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 289–292.

Rooney, L.W. and Pflugfelder, R.L. (1986) Factors affecting starch digestibility with special emphasis on sorghum and corn. *Journal of Animal Science* 63, 1607–1623.

Sánchez-Martínez, J. (2009) Efectos del contenido en fibra, almidón y proteína del pienso sobre el desarrollo del tracto gastrointestinal, la digestibilidad ileal y el ambiente cecal en gazapos. Degree thesis, Universidad Politécnica de Valencia, Spain.

Scapinello, C., Gidenne, T. and Fortun-Lamothe, L. (1999) Digestive capacity of the rabbit during the post-weaning period, according to milk/solid feed intake pattern before weaning. *Reproduction Nutrition Development* 39, 423–432.

Sequeira, J. and Villamide, M.J. (1999) Evaluación nutritiva de dos variedades de trigo (duro y blando) en conejos. Efecto de la adición de enzimas. *ITEA* 20, 463–465.

Sequeira, J., Nicodemus, N., Carabaño, R. and Villamide, M.J. (2000) Effect of type of wheat and addition of enzymes on some digestive parameters at different sampling time. *World Rabbit Science* 8 (Suppl. 1, Vol. C), 437–444.

Sirotek, K., Marounek, M. and Suchorská, O. (2006) Activity and cellular location of amylases of rabbit caecal bacteria. *Folia Microbiologica* 51, 309–312.

Soler, M.D., Blas, E., Cano, J.L., Pascual, J.J., Cervera, C. and Fernández Carmona, J. (2004) Effect of digestible fibre/starch ratio and animal fat level in diets around weaning on mortality rate of rabbits. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Mexico, pp. 996–1001 (electronic edition).

Soler, M.D., Blas, E., Biglia, S., Casado, C., Moya, J. and Cervera, C. (2006) Digestibilidad ileal de piensos con distinto contenido en fibra digestible, almidón y grasa animal en gazapos en postdestete. In: *Proceedings XXXI Symposium de Cunicultura, Lorca*. ASESCU, Canet de Mar, Spain, pp. 73–77.

Thivend, P. (1981) Les constituants glucidiques des aliments concentrés et de leurs dérivés. In: Demarquilly, C. (ed.) *Prévision de la Valeur Nutritive des Aliments des Ruminants*. INRA, Paris, France, pp. 219–235.

Toofanian, F. (1984) The fetal and postnatal development of small intestinal disaccharidases in the rabbit. *Laboratory Animal Science* 34, 268–271.

Toral, F.L.B., Furlan, A.C., Scapinello, C., Peralta, R.M. and Figueiredo, D.F. (2002) Digestibilidade de duas fontes de amido e atividade enzimática em coelhos de 35 e 45 dias de idade. *Revista Brasileira de Zootecnia* 31, 1434–1441.

Vernay, M. (1986) Influence de la caecotrophie sur la production, l'absorption et l'utilisation des acides organiques chez le lapin. *Reproduction Nutrition Development* 26, 1137–1149.

Volek, Z. and Marounek, M. (2008) White lupin (cv. Amiga) seeds as a protein source in diet for growing rabbits: effect on growth performance, digestibility of nutrients and carcass traits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 831–835 (electronic edition).

Wolter, R., Nouwakpo, F. and Durix, A. (1980) Étude comparative de la digestion d'un aliment complet chez le poney et le lapin. *Reproduction Nutrition Development* 20, 1723–1730.

Xiccato, G., Trocino, A., Sartori, A. and Queaque, P.I. (2002) Effect of dietary starch level and source on performance, caecal fermentation and meat quality in growing rabbits. *World Rabbit Science* 10, 147–157.

Xiccato, G., Trocino, A., Carraro, L., Fragkiadakis, M. and Majolini, D. (2008) Digestible fibre to starch ratio and antibiotic treatment time in growing rabbits affected by epizootic rabbit enteropathy. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 847–851 (electronic edition).

Yoshida, T., Pleasants, J.R., Reddy, B.S. and Wostmann, B.S. (1968) Efficiency of digestion in germ-free and conventional rabbits. *British Journal of Nutrition* 22, 723–737.

Yu, B. and Tsen, H.Y. (1993) An *in vitro* assessment of several enzymes for the supplementation of rabbits diets. *Animal Feed Science and Technology* 40, 309–320.

3 Protein Digestion

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3.1 Some Characteristics of the Main Protein Sources Included in Rabbit Diets

Proteins are macromolecules made up of long chains of amino acid residues covalently linked by peptide bonds to form polypeptide chains. In each protein, these polypeptide chains are folded in three dimensions to form a characteristic tertiary structure. The properties of each amino acid depend on the structure of its chain (size and electric charge). Eight of them (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) are considered essential from a nutritional perspective because their carbon skeletons cannot be synthesized in higher animals.

The nutritive value of a protein is determined not only by its amino acid composition, but also by its digestibility or proportion of ingested protein that is digested in the gut and absorbed as free amino acids. The main factors involved in protein digestibility in rabbits, as in other non-ruminant species, are chemical structure and properties (the insoluble proteins are more resistant to digestion) and accessibility to enzyme activity.

Plant proteins are divided into two major classes: seed and leaf proteins. The main seed proteins are a part of the reserve material that is necessary for the development of the embryo of the future plant. Thus, the cereal endosperm contains approximately 0.7 of total cereal protein; the remainder is in the germ and in the outer bran. The proportions of the different types of proteins (Table 3.1) differ between cereals: the soluble albumins and globulins derive from the cytoplasm of the cells, and the insoluble prolamins and glutelins are storage proteins. The bran includes the aleurone layer of endosperm (inner bran) and, because of this, has higher proportions of both crude protein (CP) and cell walls than the whole grain. The storage proteins are richer in non-essential amino acids (especially glutamic acid and proline) and lower in lysine and threonine than cytoplasmic proteins (Table 3.2). As a consequence, the amino acid composition of cereals depends on the relative proportions of the different types of proteins. Protein from cereals represents about 0.13 of the total protein of rabbit diets (EGRAN databank), whereas for cereal by-products, mainly wheat bran, it is about 0.2.

In general, the grains of legumes and oil seeds contain higher proportions of albumins and globulins than cereal grains (Table 3.1). Thus the proteins of legumes are richer in essential amino acids (especially lysine) and should be more digestible than those of cereals. However, the value of these seeds, when

Table 3.1. Proportions of the different types of proteins in total protein of cereal and legume grains (Boulter and Derbyshire, 1978; Larkins, 1981; Miflin and Shewry, 1981).

	Cytoplasmic or salt-soluble proteins		Storage or insoluble proteins	
	Albumins	Globulins	Prolamins	Glutelins
Wheat (*Triticum aestivum*)	0.03–0.05	0.10–0.15	0.50–0.65	0.10–0.20
Barley (*Hordeum vulgare*)	0.03–0.04	0.10–0.20	0.45–0.50	0.25–0.35
Oats (*Avena sativa*)	0.01	0.60–0.65	0.10–0.15	0.25–0.30
Maize (*Zea mays*)	Trace	0.05–0.06	0.65–0.75	0.15–0.20
Bean (*Vicia faba*)	0.04	0.67	–	0.29
Peas (*Pisum sativum*)	0.21	0.66	–	0.12
Soya (*Glycine max*)	0.10	0.90	–	0

Table 3.2. Amino acid composition (g 16 g^{-1} nitrogen) of wheat proteins (Bushuk and Wrigley, 1974).

	Albumins	Globulins	Prolamins	Glutelins
Methionine	1.8	1.7	1.0	1.3
Lysine	3.2	5.9	0.5	1.5
Threonine	3.1	3.3	1.5	2.4
Tryptophan	1.1	1.1	0.7	2.2
Glutamic acid	22.6	15.5	41.1	34.2

they are used unprocessed, is limited by the presence of various antinutritive factors (e.g. trypsin inhibitors, lectins or tannins). The protein concentrates used the most in rabbit diets in Europe are soybean and sunflower meals, with inclusion levels of 80–90 g kg^{-1}, which thus comprise from 0.35 to 0.4 of total dietary protein.

The proteins of forage plants are concentrated in the leaves (Table 3.3). Leaf proteins, unlike storage proteins in grains, are concerned with the growth and biochemical functions of the cells. Because of their enzymatic nature, the amino acid composition of plant leaf proteins varies within narrow limits (Makoni *et al.*, 1993). The major portion of leaf proteins are separated from the cell wall by a membrane, although a comparatively small fraction of insoluble protein remains tightly bound to the cellulose of the cell wall. The forage most extensively used in rabbit diets is lucerne hay (0.90 of diets; Villamide *et al.*, 2009) with inclusion levels from 200 to 400 g kg^{-1}. Therefore, lucerne protein represents at least 0.25 of the dietary protein. The protein content of lucerne hay is very variable, mainly depending on its maturation state and drying process. Thus, INRA (2002) tables classify dehydrated lucerne into four groups according to protein content (from <160 to 220–250 g kg^{-1}).

3.2 Protein and Amino Acid Balance

The capability of the different feedstuffs to meet the protein and amino acid require-

Table 3.3. Crude protein (g kg^{-1} dry matter) of leaves and stems of lucerne hay and the amino acid composition (as a proportion of total amino acids) of leaf protein from fresh lucerne.

Lucerne[a]	Crude protein	Amino acid	Cytoplasmic protein[b]	Chloroplast membrane[b]
Lucerne hay	191.9	Lysine	0.060	0.051
Leaves (0.82 hay)	277.5	Sulphur amino acids	0.016	0.013
Stems (0.18 hay)	125.0	Arginine	0.037	0.051

[a]Alvir *et al.* (1987).
[b]Makoni *et al.* (1993).
The ratio of cytoplasmic to chloroplast protein is 0.25:0.75 of total leaf protein.

ments of rabbits depends on the nitrogen unit used (Carabaño *et al.*, 2000). Figure 3.1 shows the relative value of sunflower meal, wheat, wheat shorts and lucerne hay with reference to soybean meal using different units (total CP or methionine, apparent digestible faecal or ileal, and true digestible ileal contents of both protein and methionine). Thus, lucerne hay could represent 0.21 of the soybean meal value if it is evaluated as apparent digestible faecal CP, and 0.42 if it is compared as a crude methionine source (Fig. 3.1). Similarly, the corresponding values for sunflower meal are 0.71 and in 1.46. Therefore, a proper definition of the nitrogen unit both in rabbit requirements and in feedstuff evaluation will allow an increase in the accuracy of diet formulation, reducing the risks of intestinal pathologies such as epizootic rabbit enteropathy (Carabaño *et al.*, 2008) and environmental pollution (Maertens *et al.*, 2005).

3.2.1 Crude protein and total amino acids

CP and amino acid contents are the most common units used to express nitrogen requirements and the nutritive value of feedstuffs. The main advantage of this is the considerable amount of available information, because feedstuff evaluation can be directly determined in the laboratory and extrapolated between animal species. However, in experiments designed to determine the amino acid requirements for rabbits, a different apparent faecal digestibility of amino acids was observed depending on whether they came from conventional feeds (from 0.64 to 0.80, on average) or had a synthetic origin (from 0.93 to 1.0) (Taboada *et al.*, 1994, 1996; de Blas *et al.*, 1998). These results emphasize the importance of using digestible instead of total units to express both protein and amino acid requirements for rabbits.

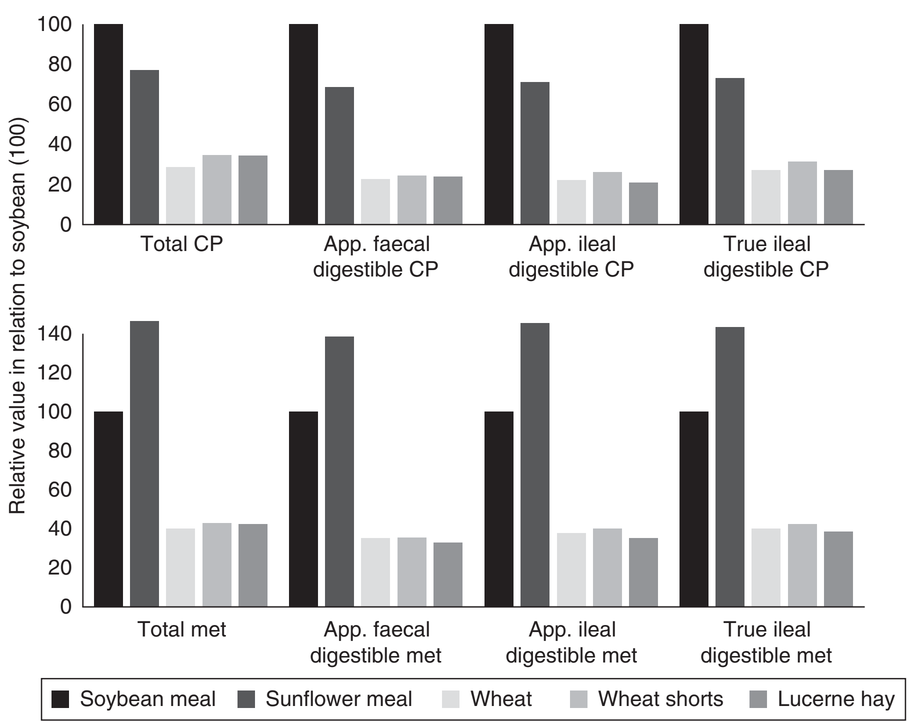

Fig. 3.1. Relative value of some protein sources in rabbit diets in relation to soybean meal (100) using different nitrogen units (total versus apparent (app.) faecal digestible versus app. ileal digestible versus true ileal digestible crude protein (CP) or methionine (met) contents). Adapted from data of Llorente *et al.* (2006, 2007a).

3.2.2 Faecal digestibility

Current nitrogen recommendations (see Chapter 6) are expressed in apparent faecal digestible protein (DP), being the DP: digestible energy (DE) ratio directly related to body nitrogen retention and excretion (Trocino *et al.*, 2000). Its use brings a better relationship between dietary supply and rabbit requirements as it considers the high variation of protein digestibility among raw materials (see Chapter 8, Table 8.5). The main features of protein digestibility are difficult to assess through the chemical analyses commonly used in feed evaluation. Only 0.5 and 0.16 of apparent faecal CP digestibility (CPd) variation of complete diets and ingredients, respectively, is explained by the chemical composition. In an interlaboratory study in which 164 experimental diets were evaluated, Villamide *et al.* (2009) found a negative correlation between CPd and acid detergent lignin (r = –0.7). When dietary CP is included in the equation with a positive sign, the validation error of the prediction decreases slightly (from 0.045 to 0.043). Protein digestibility seems to vary more according to type of feed ingredient than to the chemical composition (de Blas *et al.*, 1979, 1984). In the above-mentioned data, a negative relationship of lucerne hay content (r = –0.22) or of the proportion of dietary protein from lucerne hay (r = –0.27) with CPd was observed, whereas this relationship was positive for sunflower meal content (r = 0.22).

Villamide and Fraga (1998) proposed some equations to predict the DP of different groups of feed ingredients (Table 3.4). The best single predictor for all groups is the CP content, although with a different correlation for each group (from 0.904 to 0.967 for fibrous by-products and protein concentrates, respectively). An increase in the CP content of a feedstuff increases its CPd because the proportional contribution of endogenous nitrogen to faecal nitrogen decreases. In the same way, the structure of proteins of feedstuffs with a high CP content (e.g. legume feeds, lucerne leaves) is generally less resistant to digestion. The determination of the proportion of nitrogen bound to acid detergent fibre, which includes heat-damaged protein and nitrogen associated with lignin, permits the estimation of that portion of the nitrogen content of feeds that is indigestible. In fact, a high negative correlation between CPd and nitrogen linked to acid detergent fibre has been observed in both diets (r = –0.87, n = 8; Martinez and Fernández, 1980) and feedstuffs (r = –0.95, n = 11; Villamide and Fraga, 1998). However, as this analysis is not frequently undertaken in rabbit assays, it is still not possible to obtain more homogeneous and representative data that allow an accurate CPd prediction of the main ingredients included in rabbit diets.

Information regarding the digestibility of amino acids is even more limited. García *et al.* (1995b) observed that the type of lucerne hay affects both the content and the digestibility of most amino acids. A positive correlation between protein and amino acid digestibility is evident, but there is a difference of 0.07 between extreme CPd values, whereas a variation of 0.14 is obtained for lysine. However, when attempting to predict

Table 3.4. Prediction equations for apparent faecal digestible protein (DP, g kg^{-1} dry matter) from chemical composition (g kg^{-1} dry matter) for different groups of feed ingredients (Villamide and Fraga, 1998).

	n	Equation	R^2	RSD
Dry forages	26	DP = –38.4 + 0.831 CP	0.892	3.44
Fibrous by-products	11	DP = 8.73 + 0.716 CP – 0.184 ADL	0.964	3.16
Cereals and their co-products	27	DP = –2.34 + 0.751 CP	0.911	3.90
Protein concentrates	18	DP = –55.3 + 0.941 CP	0.936	7.14

ADL, acid detergent lignin; CP, crude protein; RSD, residual standard deviation.

the amino acid digestibility of feeds, another problem arises. Not all of the amino acids that disappear from the large intestine are used for protein synthesis, unless they have been reused through soft faeces.

3.2.3 Ileal digestibility

The ileum is the last segment of the digestive tract where the amino acids can be absorbed. Therefore, ileal digestibility is considered to give a more precise estimate of the real availability of amino acids for animal protein synthesis both in rabbits (Carabaño *et al.*, 2000) and in other non-ruminant species.

Ileal and faecal digesta contain important amounts of protein of endogenous origin (3.8 and 2.5 g 100 g^{-1} dry matter DM intake at the ileal and faecal level, respectively; García *et al.*, 2004; Llorente *et al.*, 2005) originating from digestive secretions, epithelial cells and mucins or microorganisms. This endogenous protein represents about 0.64 of the total nitrogen flow at both the ileal and the faecal levels. The relative importance of endogenous protein varies with the DM intake, but also varies with the type of diet and protein origin. Thus, in diets with the same intake and similar chemical composition based on peas or soybean hulls, the endogenous protein at the ileal level represents 0.65 and 0.55, respectively, of the total ileal flux.

The amino acid composition of endogenous protein at the ileal and faecal level is shown in Table 3.5. The endogenous protein at the ileal level contains higher concentrations of some non-essential (glutamic acid, glycine and serine) amino acids than faecal ones. These differences can be explained by the different composition of endogenous secretions when compared to those of microbial origin. Therefore, for a more reliable definition of digestible protein and amino acids of feedstuffs, a correction for endogenous losses must be performed. When this correction is done, a new unit arises and is referred to as 'true' (TID) instead of 'apparent' (AID) ileal digestibility.

Table 3.5 Amino acid composition (g 16 g^{-1} nitrogen) of endogenous flow at the ileal and faecal levels.

	García *et al.* (2004)	Llorente *et al.* (2006)	
	Ileum	Ileum	Faeces
Essential amino acids			
Cystine	3.1	2.7	3.3
Histidine	1.6	1.3	1.2
Isoleucine	3.7	3.8	3.2
Leucine	4.5	4.3	4.7
Lysine	3.2	3.6	3.3
Methionine	0.9	0.8	1.1
Phenylalanine	1.7	4.1	4.1
Threonine	4.9	5.6	5.3
Tyrosine	1.7	3.5	3.4
Valine	5.3	5.1	4.7
Total	30.6	34.8	33.2
Non-essential amino acids			
Alanine	3.1	3.4	3.7
Arginine	4.1	3.6	4.3
Aspartic acid	7.0	7.2	7.0
Glutamic acid	12.6	12.5	9.1
Glycine	6.1	8.0	4.1
Proline	4.8	4.7	3.3
Serine	6.6	5.8	4.4
Total	44.3	45.2	35.9

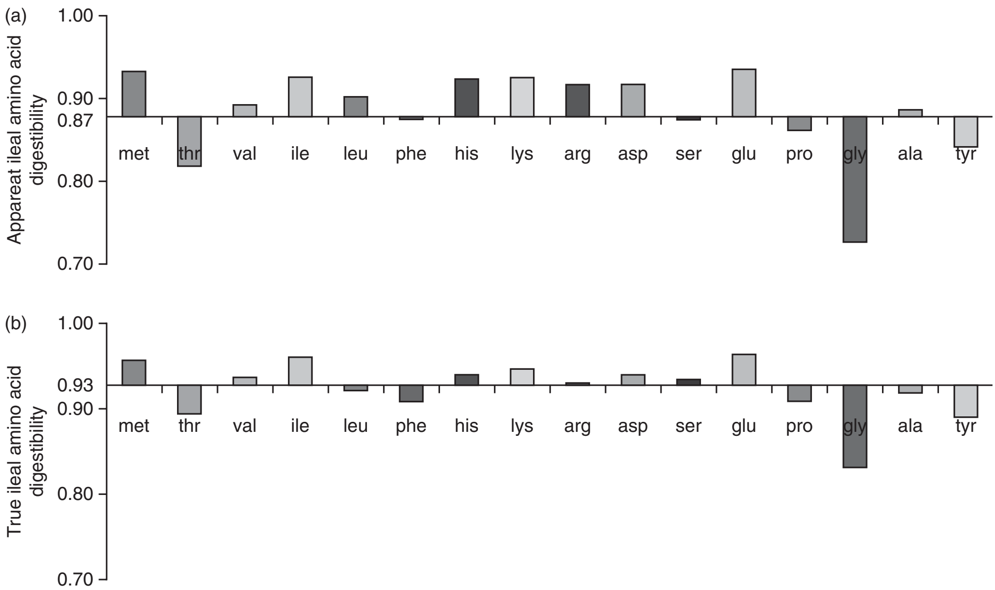

Fig. 3.2. Apparent (a) and true (b) ileal amino acid digestibility of soybean meal with respect to its crude protein digestibility (Llorente *et al.*, 2006).

The variation in each amino acid digestibility with respect to the CP ileal digestibility (AID or TID) for soybean meal is shown in Fig. 3.2. Some amino acids (e.g. glycine, threonine) are considerably less digestible than protein (0.14 and 0.06 respectively for AID and TID), whereas others (e.g. methionine, isoleucine) are more digestible (0.06 for AID and 0.05 for TID). Therefore, using the same digestibility value for all amino acids leads to major errors, mainly when apparent values are used, because the endogenous correction further leads to a decrease in the variation of amino acid digestibility with respect to protein digestibility.

Studies using cannulated does have aimed to evaluate the main protein sources of rabbit diets (García *et al.*, 2005; Llorente *et al.*, 2005, 2006, 2007a). Total and ileal (apparent and true) and faecal (apparent) digestible contents of CP and of most limiting amino acids of these raw materials are shown in Table 3.6. Total CP averages 205 g kg^{-1} DM, because feedstuffs with very low CP content have not been evaluated due to a low effect on dietary nitrogen and the considerable errors associated with its evaluation. Lysine, methionine and threonine represent 0.05, 0.015 and 0.036 on average of the total CP of these feed ingredients. TID of CP is relatively high (average 0.81), while the apparent ileal values (average 0.66) are 0.15 lower due to the great importance of endogenous losses at the ileal level. This effect is especially important for threonine and lysine digestibilities in the evaluation of cereals (0.38 and 0.22 as average higher TID than AID of threonine and lysine, respectively). Apparent faecal digestibility of CP shows intermediate values (average 0.76), indicating an important disappearance of protein in the large intestine (about 0.1), although at a different rate for each amino acid. Threonine seems to disappear to a larger extent (0.12 as average) than lysine (0.01) and methionine, which is apparently more digestible at the ileal than faecal level for 11 out of 15 feedstuffs. AID values are necessary to estimate the total ileal flux arriving in the caecum (indigestible plus endogenous nitrogen), which should be used for microbial growth. This micro-

Table 3.6. Total and digestible protein and amino acid content (g kg^{-1} dry matter) using different units for the most important sources of protein in rabbit diets (García *et al.*, 2005; Llorente *et al.*, 2005, 2006, 2007a).

	Total				Apparent faecal digestible				Apparent ileal digestible				True ileal digestible			
	CP	Lys	Met	Thr	CP	Lys	Met	Thr	CP	Lys	Met	Thr	CP	Llys	Met	Thr
Sunflower meal 1	306	12.8	7.7	12.3	254	11.1	7.0	9.8	232	11.3	7.0	9.1	266	12.5	7.3	11.0
Sunflower meal 2	393	14.2	8.6	14.3	335	11.3	7.9	11.0	317	12.0	8.1	10.6	338	13.0	8.3	10.6
Sunflower meal 3	417	15.6	9.7	15.9	354	13.3	8.9	12.8	333	13.3	9.0	12.2	367	14.5	9.3	14.0
Soybean meal	540	31.9	6.6	20.1	518	30.6	6.4	18.5	469	29.5	6.2	16.4	503	30.7	6.5	18.3
Full-fat soybean	442	26.5	5.6	17.0	405	25.0	5.2	15.0	364	23.9	5.1	13.0	398	25.2	5.3	14.9
Peas	283	17.4	2.9	9.4	231	15.4	2.5	6.8	213	15.7	2.6	6.0	249	16.9	2.9	7.9
Barley	121	4.2	2.0	4.1	83	2.5	1.4	2.2	75	2.6	1.6	1.9	96	3.3	1.8	2.9
Maize	119	4.2	2.3	4.1	79	2.7	1.8	1.7	59	2.7	1.8	0.7	93	3.9	2.1	2.5
Wheat	156	6.2	2.6	4.7	118	4.7	2.3	2.7	104	4.6	2.3	2.1	139	5.9	2.6	4.0
Wheat shorts	186	7.9	2.8	5.7	126	5.9	2.3	2.9	122	6.2	2.5	2.5	157	7.4	2.7	4.4
Wheat bran	160	6.9	3.2	5.9	90	3.3	1.9	3.1	85	3.2	2.2	2.6	112	4.5	2.5	4.4
Gluten feed	237	11.7	3.9	8.7	179	9.7	3.3	5.8	155	9.1	3.3	4.3	185	10.4	3.6	6.2
Soybean hulls	163	11.6	2.4	6.7	107	8.7	1.8	4.5	51	7.2	1.5	1.4	86	8.5	1.8	3.3
Lucerne hay 1	183	8.9	2.8	7.4	125	6.7	2.1	4.5	99	6.4	2.1	3.2	134	7.6	2.4	5.1
Lucerne hay 2	202	8.5	2.8	8.3	140	4.7	1.9	4.2	119	5.0	2.1	4.7	150	6.1	2.4	6.2

CP, crude protein.

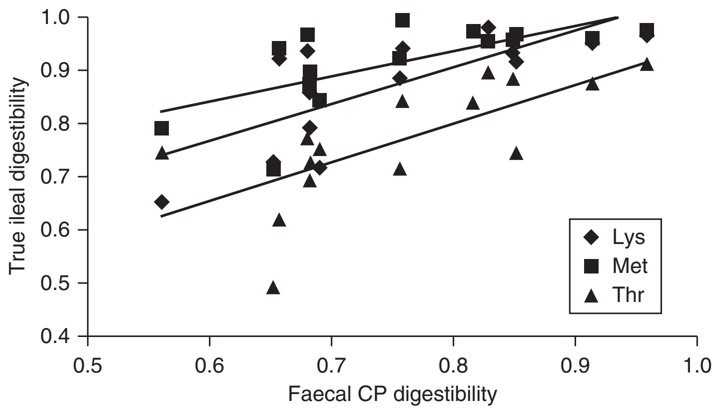

Fig. 3.3. Relationship between faecal crude protein (CP) digestibility and amino acid true ileal digestibility.

bial activity in the caecum produces considerable changes in the amino acid composition of digesta and, consequently, the faecal amino acid balance leads to a problematic interpretation (García *et al.*, 2005). Nevertheless, values for CP faecal digestibility of feed ingredients measured in cannulated does fit with average dietary values determined in growing rabbits (0.73, $n = 164$; Villamide *et al.*, 2009).

There are important differences among feedstuffs both in protein and in amino acid digestibility (from 0.4 to 0.9), being mainly related to the CP content ($r = 0.91$, 0.81 and 0.61 for CPd, AID and TID, respectively) and the type of protein (concentrates versus forages or fibrous by-products). The AIDs of CP and threonine for the different feedstuffs are lower in rabbits than in pigs (INRA, 2002), whereas lysine and methionine AID values are similar. However, both CP and limiting amino acid TID values are higher for almost all feedstuffs than the standard ileal digestibility as determined in pigs, due to the greater importance of endogenous protein in rabbits.

TID determination is time-consuming and expensive because of the use of semi-purified diets supplied to cannulated animals, endogenous determinations and amino acid analysis. Therefore, an attempt has been made to predict TIDs from easier and less costly methods. Encouraging results have been obtained using an *in vitro* method (Llorente *et al.*, 2007b) developed for pigs and adapted to rabbits (Ramos *et al.*, 1992). Although the *in vitro* CP digestibility is higher than the corresponding *in vivo* values (0.225, 0.119 and 0.58 on average for AID, CPd and TID, respectively), the precision of their estimation is high. In fact, the coefficients of variation for amino acid TID estimation are <0.057, even when the *in vitro* CP digestibility is used as predictor. The same process has been undertaken using *in vivo* CPd as predictor, in order to estimate ileal digestibility of different feed ingredients from the faecal figure. Figure 3.3 shows the estimated relationships for the three main amino acids. These equations have been used to estimate the values shown in Table 8.5 (Chapter 8).

The effect of using ileal digestible units in practical formulation is not exactly known, because rabbit requirements have not yet been determined. However, in a formulation study using ileal digestible threonine instead of total threonine, the cost of the diet decreased (by 3.3% and 2.8% for AID and TID, respectively) and the inclusion of concentrates and cereal by-products instead of forages was favoured (Carabaño *et al.*, 2009). The presence of many fibrous sources in small amounts in rabbit diets has a limited influence on protein formulation irrespective of the unit used because of the very low protein and amino acid content.

3.3 Nitrogen Metabolism in the Caecum

Residues of intestinal digestion and the urea recycled through the blood are potential substrates that allow caecal bacteria to obtain energy and nitrogen for growth. At the end of the ileum, fibre is the main component of the digesta (about 0.70 of total DM), while nitrogen is second in importance (about 0.15 of total DM). However, these figures may be poor indicators of the contribution of each component to microbial growth. Taking into account the low fermentability of the fibre (0.30 for neutral detergent fibre digestibility) and the high content of endogenous substances in nitrogen residues (about 0.64), both components may contribute equally to maintain the resident intestinal microbiota. There is very little information about the quantitative utilization of nitrogen by caecal microbiota. However, early qualitative studies suggest that the caecal microbiota is able to utilize the nitrogen that enters the caecum and transform it into other nitrogen-containing components such as microbial protein and ammonia. Several studies (Yoshida *et al.*, 1968, 1971, 1972; Rerat, 1978), where germ-free animals have been compared with conventional ones, have observed that the caecal content of germ-free rabbits is enriched in different nitrogenous compounds such as urea, free amino acids, peptides and other nitrogen sources of endogenous origin (mucoproteins, pancreatic enzymes or desquamated cells). On the other hand, in conventional animals the caecum contains more ammonia and true protein (enriched in essential amino acids) and lower quantities (up to ten fold) of endogenous components. Further studies focusing on the characterization of the caecal microbiota (Emaldi *et al.*, 1979; Forsythe and Parker, 1985a) have confirmed that the enzymatic capacity of bacteria might be able to hydrolyse digesta that reaches the caeca. These bacteria are in decreasing order of importance, ammonia-users, ureolytic species, proteolytic species, pectinolytic species, xylanolytic species and cellulolytic species. Similar to in the rumen, 0.57 of viable counts are ammonia-users, being *Bacillus* species, *Staphylococcus* species and *Bacteroides vulgates*, with *Clostridium clostridioforme* the main ureolytic bacteria. Furthermore, some of the most frequent isolated caecal bacteria, *Bacteroides* species, are the most active genera in mucin digestion (Hill, 1986; Sirotek *et al.*, 2003), one of the non-protein components of the endogenous nitrogen that enters the caecum.

Figure 3.4 shows a tentative scheme of caecal nitrogen metabolism. The proteolytic activity of caecal bacteria results in volatile

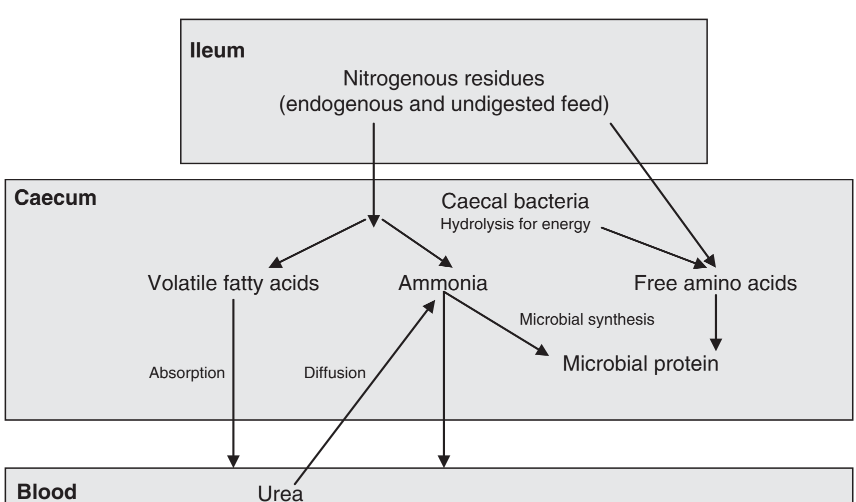

Fig. 3.4. Caecal nitrogen metabolism in the rabbit.

fatty acids (VFAs) as energy-yielding compounds and ammonia production for growth. Hoover and Heitmann (1975) observed that 0.95–0.98 of labelled C14-alanine infused into the caecum was located in VFAs, with a small proportion in other amino acids. These labelled VFAs appear rapidly in the blood, showing a maximum at 0.5 h post-infusion. However, the activity of labelled amino acids was negligible or undetected in the blood, suggesting that amino acids are only minimally absorbed, if at all, in the last segments of the intestinal tract. A similar conclusion has been obtained by using labelled lysine in animals that have been prevented from caecotrophy (Belenguer *et al.*, 2005).

Ammonia produced from protein and urea hydrolysis is partially used by caecal bacteria as the main substrate for protein synthesis (Hoover and Heitmann, 1975; Forsythe and Parker, 1985a,b) and another portion is absorbed in the caecal wall, contributing to urea production (0.27 of total urea; Forsythe and Parker, 1985c). The extent of these processes has not yet been totally quantified; however, the characteristics of the diet affect total ammonia caecal concentration and the incorporation of ammonia nitrogen into microbial protein.

An increment in caecal ammonia concentration has been related by different authors (Carabaño *et al.*, 1988, 1989, 1997; Fraga *et al.*, 1991; Motta-Ferreira *et al.*, 1996; García *et al.*, 1995a, 2000, 2002; Nicodemus *et al.*, 2002) to the dietary DE:DP ratio (Fig. 3.5). When the protein intake exceeds nutritional requirements, urea recycling from the blood to the caecum may be increased, leading to an elevation in the caecal ammonia concentration. According to Forsythe and Parker (1985c) the contribution of urea-nitrogen to the caecal ammonia pool is around 0.25 of caecal ammonia turnover.

Other dietary factors can affect the caecal ammonia concentration. The presence of condensed tannins or other phenolic components may decrease the proteolytic capacity of caecal microorganisms, as occurs in the rumen (Waghorn *et al.*, 1987). This may in part explain the low caecal ammonia values obtained in diets that contain grape pomace or olive leaves (Motta-Ferreira *et al.*, 1996; García *et al.*, 2000).

The efficacy of synthesis of microbial protein from ammonia seems to be more related to the characteristics of dietary carbohydrates than to nitrogenous composition. The caecal ammonia concentration in the rabbit fed a balanced diet is in the range of 4.5–6 mmol l^{-1} ammonia, which seems adequate for appropriate protein microbial synthesis when compared with the ammonia concentration of the rumen. However, the availability of energy in the caecum could be

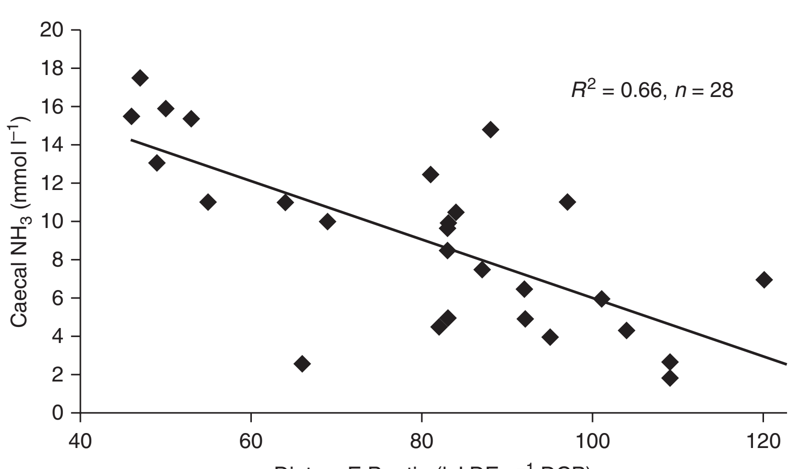

Fig. 3.5. Effect of the dietary energy (E) to protein (P) ratio on caecal ammonia concentration. DCP, digestible crude protein.

the limiting factor for bacterial growth. Inclusion in the diet of increasing levels of fibre or sources of fibre with high lignification decreases VFA production and the protein concentration in the caecum (strongly related to microbial protein; Carabaño *et al.*, 1988; Fraga *et al.*, 1991). On the other hand, the inclusion of more fermentable fibre (linked to pectins or hemicelluloses) or fibre with a high proportion of fine particles (<0.3 mm in diameter) improves both total and microbial nitrogen in the soft faeces (García *et al.*, 2000).

The final result of bacterial activity in the caecum is a substantial change in the amino acid composition of the protein that enters the caecum from the ileum. According to García *et al.* (2005) the bacterial activity leads to an enrichment in lysine (0.072 g day^{-1}; 0.63 of the ileal flow), methionine (0.026 g day^{-1}; 0.95 of the ileal flow) and threonine (0.059 g day^{-1}; 0.40 of the ileal flow) when comparing total excretion in hard and soft faeces with apparent ileal flow. Furthermore, the enrichment of these essential amino acids was higher in diets where the faeces contained a higher proportion of microbial protein.

3.4 Protein Digestion in Young Rabbits

The study of protein digestion in young rabbits has acquired more relevance in the last 10 years due to its influence on intestinal health (Carabaño *et al.*, 2009). Although information has increased with respect to the previous decade, important gaps in knowledge in some aspects make it difficult to interpret results. Methodological aspects related to markers, sample collection and analysis (Gallois *et al.*, 2008), diet design or endogenous losses may explain some conflicting results. The enzymatic capacity to digest protein at the ileal level seems to be higher than that for some nutrients, but, around weaning, the enzymatic capacity for protein digestion may be limited (see Chapter 1). However, the AID of CP in young rabbits (from 21 to 35 days) shows similar or even higher figures compared to that of older animals (42–45 days) (Table 3.7) (García-Ruiz *et al.*, 2006; Gallois *et al.*, 2008). The lack of stability in intake (feed and caecotrophy) and caecal contents or a lower importance of endogenous losses could explain these discrepancies. In fact, when the average values of AID are compared to faecal ones, only 0.71 of the total digested protein is digested at the ileum in young rabbits. This figure is lower than those reported in adult animals, which vary from 0.82 to 0.90 for forages or concentrates, respectively (Table 3.7).

Protein digestion in young rabbits is not only limited for forages. Gutierrez *et al.* (2003) also found differences in ileal digestibility among protein sources with the same faecal digestibility. The low protein digestion at the ileum and a greater limitation of

Table 3.7. Effect of age on apparent ileal crude protein (CP) digestibility and the distribution of protein apparently digested in the digestive tract of rabbits.

Age (days)	Apparent ileal CP digestibility (AID)	Apparent faecal CP digestibility (CPd)	Apparent caecal CP digestibility	Ratio AID:CPd
28[a]	0.691	0.791	0.100	0.872
42[a]	0.681	0.782	0.101	0.871
35[b]	0.701			
45[b]	0.678			
35[c]	0.643	0.789	0.146	0.712
Adult does[d]				
Concentrates	0.732	0.813	0.081	0.900
Forages	0.548	0.671	0.122	0.817

[a]Gallois *et al.* (2008).
[b]García-Ruiz *et al.* (2006), age effect $P = 0.003$.
[c]Average data from diets with a 0.6:0.4 ratio of concentrate to forage ($n = 9$) (Gutierrez *et al.*, 2003; Chamorro *et al.*, 2007; Gómez-Conde *et al.*, 2007).
[d]Average data from feed ingredients. Concentrates: oilseed and meals and cereals; forages: cereal by-products, lucerne and soybean hulls (Carabaño *et al.*, 2009).

energy in the caecum (lower proportion of VFAs) agrees with a higher caecal ammonia concentration observed in the youngest when compared with adult rabbits (García *et al.*, 2002; Gidenne and Fortun-Lamothe, 2002; Nicodemus *et al.*, 2002).

3.5 Soft Faeces and Protein Digestibility

The main effect of soft faeces ingestion is protein reutilization. There are many data on the chemical composition of soft faeces, suggesting that the composition is similar to that of the caecal contents. When comparing the protein concentration of soft faeces with that of the caecal contents of rabbits, employing the same methodology, the following equation was obtained (Fig. 3.6):

$$y = 100.88 + 0.689\ (\pm 0.8)\ x,\ R^2 = 0.712,\ P<0.001,\ n = 31,$$

where y = CP (g kg^{-1} DM) of soft faeces, and x = CP (g kg^{-1} DM) of caecal contents.

The CP concentration of caecal contents in these studies ranged from 190 to 340 g kg^{-1} and the corresponding CP concentration in soft faeces from 230 to 335 g kg^{-1}. This suggests that the nitrogen content of the mucosal envelope, which covers the caecal contents in the last sections of the large intestine to produce the final soft faeces, may be near to 55 g kg^{-1}. The amount of endogenous nitrogen secreted daily as the mucosal envelope is near to 0.05 g day^{-1} (assuming an average soft faeces production of 20 g DM day^{-1}, with 280 g CP kg^{-1} DM). In the same way, the relationship between the crude fibre content of the soft faeces and caecal contents is high (*r* = 0.90), although the crude fibre of soft faeces is 8% lower than that of the caecal contents (Carabaño *et al.*, 1988).

The amount of soft faeces excretion varies with age, physiological status, diet and faeces collection method. Using data from 36 diets supplied to rabbits weighing 2.0 kg live weight, in which a wooden collar was worn at 08.00 h and removed 24 h later, and with diets in which the neutral detergent fibre content varied from 230 to 550 g kg^{-1} (Carabaño *et al.*, 1988, 1989, 1997; García *et al.*, 1995a, 2000; Motta-Ferreira *et al.*, 1996; Nicodemus, 2000; Nicodemus *et al.*, 2006), the excretion of soft faeces ranged from 15 to 32 g DM day^{-1}, with a

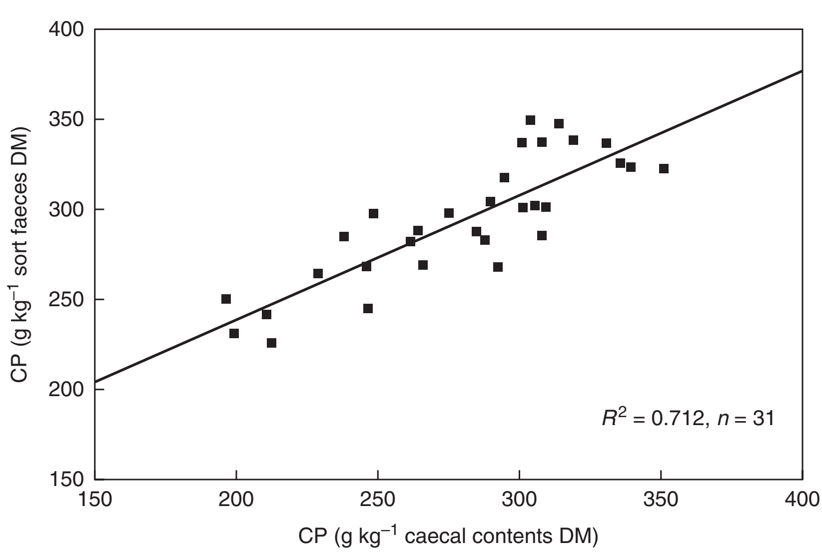

Fig. 3.6. Effect of the crude protein (CP) concentration of the caecal contents on the CP of soft faeces (Carabaño *et al.*, 1988, 1989, 1997; Fraga *et al.*, 1991; García *et al.*, 1995a; Motta-Ferreira *et al.*, 1996). DM, dry matter.

mean value of 21.3 g DM day^{-1} (i.e. around 10 g DM kg^{-1} live weight). On the other hand, Gidenne and Lebas (1987) reported a constant and positive relationship ($r = 0.64$) between the amount of hard and soft faeces excreted from 28 to 133 days of age.

The contribution of soft faeces to the total CP intake varies, according to the chemical composition of the diet and the composition of the feed ingredients within diets, from 0.104 to 0.286 (using the studies previously mentioned). The highest values are associated with low-digestible diets that increase the flow of indigestible protein to the caecum (Motta-Ferreira *et al.*, 1996), whereas the lowest values are related to diets that supply small amounts of protein to the caecum (García *et al.*, 2000). In practical diets, the protein supply from the soft faeces is, on average, 0.18 of the total CP intake.

The values for soft faeces production obtained in lactating does fed conventional diets are higher (around 35 g DM day^{-1}) than in growing rabbits (Lorente *et al.*, 1988; Nicodemus *et al.*, 1999), but the contribution of soft faeces to the total CP intake (around 0.16) is maintained in the same range because of the higher feed intake of the does. In this sense, the microbial lysine contribution to tissue lysine in lactating does estimated by using milk and liver lysine enrichments is 0.23 and 0.19, respectively (Abecia *et al.*, 2007). These values are similar to those obtained in growing rabbits using liver enrichment (0.23; Belenguer *et al.*, 2005).

As a result, the ingestion of soft faeces improves the diet's apparent faecal digestibility, especially protein digestibility. When coprophagy is prevented, DM digestibility decreases slightly by around 0–0.17, but CP digestibility decreases by 0.04 to 0.72. This decrease is higher when the dietary protein comes from forage than from mixed or non-forage diets (Fraga and de Blas, 1977; Fraga *et al.*, 1984; Raharjo *et al.*, 1990; Sakaguchi *et al.*, 1992; Merino, 1994). However, in these studies the CPd of rabbits not practising caecotrophy has been calculated including the sum of nitrogen of hard and soft faeces, as nitrogen excreted. As a consequence of the higher proportion of nitrogen excreted, the apparent protein digestibility was lower in animals not practising caecotrophy than in those practising it. When the soft faeces' nitrogen was removed from the balance, this difference disappeared (Merino, 1994) and coprophagy had no effect on either the ileal digestibility of DM (0.537 versus 0.572) or the ileal digestibility of CP (0.723 versus 0.721) in cannulated adult females (Merino and Carabaño, 2003).

On the other hand, the ingestion of soft faeces increases the ileal flow of DM (by 0.31), nitrogen (by 0.18) and the endogenous proportion of the most important limiting essential amino acids (arginine, lysine, phenylalanine and threonine) (García *et al.*, 2004). This increment in the endogenous ileal nitrogen flow is due to the increase in the DM intake, as both variables are closely related (García *et al.*, 2004).

As a consequence, the ingestion of soft faeces enables rabbits to use part of the amino acids that will not be absorbed beyond the ileum for microbial protein synthesis. In fact, caecotrophy contributes to recycling 0.36 of the total protein excreted (soft plus hard faeces), which is mainly of bacterial origin (around 0.67; García *et al.*, 2005). Moreover, this protein is a good source of the most frequently limiting amino acids (methionine, lysine and threonine), as has been reported by several authors (Proto, 1976; Nicodemus *et al.*, 1999; García *et al.*, 2004, 2005). However, the amino acid composition of soft faeces is influenced by its microbial content, differences in the digestibility of dietary amino acids and the contribution of nitrogen of endogenous sources (especially that of the mucosal envelope). The enrichment in these essential amino acids of soft faeces is higher when animals are fed on diets that increase the synthesis of microbial nitrogen (García *et al.*, 2005).

In lactating does fed on diets that meet all of the essential nutrient requirements, Nicodemus *et al.* (1999) observed that the contributions of some of the essential amino acids (methionine, lysine, threonine, isoleucine and valine) are higher than the CP contribution of soft faeces to nutrient intake (0.15; see Fig. 3.7). However, the difference

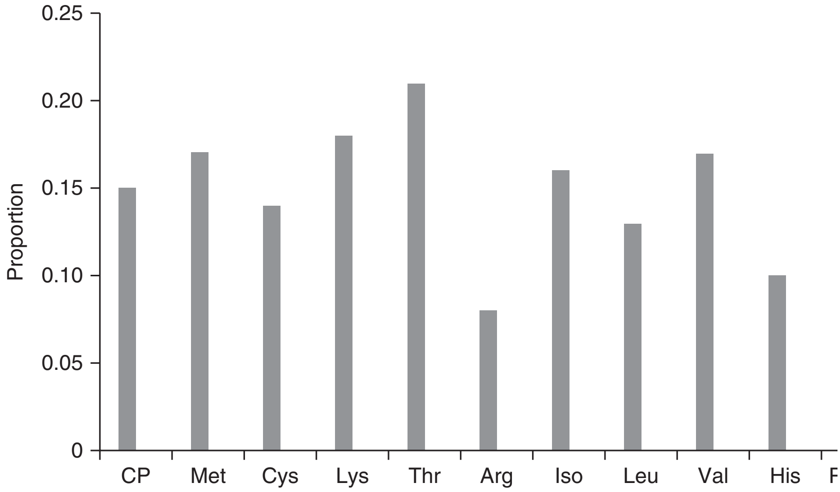

Fig. 3.7. Contribution of soft faeces to the total intake of crude protein and some amino acids (Nicodemus *et al.*, 1999).

was only significant in the case of threonine, which has been identified as the third most limiting amino acid in rabbits. The main results of digestive processes, which determine the amino acid composition of soft faeces with respect to diet composition, are the increase in the methionine to cystine ratio as a consequence of the relatively high value of this ratio in bacterial protein and the decrease in arginine, histidine and phenylalanine. Therefore, the amino acid supply from soft faeces from conventional diets does not seem to be enough to alter the dietary amino acid pattern in order to meet the essential amino acid requirements of rabbits.

Many advances have been achieved in understanding nitrogen digestion of rabbits, which has allowed the adaptation of rabbit nutrition to meet commercial changes, environmental laws and so on. However, many opportunities exist to further expand the knowledge of amino acid metabolism to meet specific requirements, mainly in young animals, for improving the health and welfare of rabbits.

References

Abecia, L., Balcells, J., Fondevila, M., Belenguer, A., Holtrop, G. and Lobley, G.E. (2007) Contribution of gut microbial lysine to liver and milk amino acids in lactating does. *British Journal of Nutrition* 100, 977–983.

Alvir, M.R., González, J. and Argamentería, A. (1987) Dégradabilité comparée dans le rumen des différentes fractions azotées des feuilles et des tiges du foin de luzerne. *Reproduction Nutrition Development* 27 (1B), 267–268.

Belenguer, A., Balcells, J., Guada, J.A., Decoux, M. and Milne, E. (2005) Protein recycling in growing rabbits: contribution of microbial lysine to amino acid metabolism. *British Journal of Nutrition* 94, 763–770.

Boulter, D. and Derbyshire, E. (1978) The general properties, classification and distribution of plant proteins. In: Norton, G. (ed.) *Plant Protein*. Butterworths, London, UK, pp. 3–24.

Bushuk, W. and Wrigley, C.W. (1974) Proteins: composition, structure and function. In: Inglett, G.E. (ed.) *Wheat Production and Utilization*. AVI Publishing Company, Wesport, Colorado, USA, pp. 119–145.

Carabaño, R., Fraga, M.J., Santomá, G. and de Blas, J.C. (1988) Effect of diet on composition of caecal contents and on excretion and composition of soft and fard feces of rabbits. *Journal of Animal Science* 66, 901–910.

Carabaño, R., Fraga, M.J. and de Blas, J.C. (1989) Effect of protein source in fibrous diets on performance and digestive parameters of fattening rabbits. *Journal of Applied Rabbit Research* 12, 201–204.

Carabaño, R., Motta-Ferreira, W., de Blas, J.C. and Fraga, M.J. (1997) Substitution of sugarbeet pulp for alfalfa hay in diets for growing rabbits. *Animal Feed Science and Technology* 65, 249–256.

Carabaño, and R., de Blas, C. and García, A.I. (2000) Recent advances in nitrogen nutrition in rabbits. *World Rabbit Science* 8, 14–28.

Carabaño, R., Badiola, I., Chamorro, S., García, J., García-Ruiz, A.I., García-Rebollar, P., Gomez-Conde, M. S., Gutiérrez, I., Nicodemus, N., Villamide, M.J. and de Blas, J.C. (2008) New trends in rabbit feeding: influence of nutrition on intestinal health. *Spanish Journal of Agriculture Research* 6 (Sp. Iss.), 15–25.

Carabaño, R, Villamide, M.J., García, J., Nicodemus, N., Llorente, A., Chamorro, S., Menoyo, D., García-Rebollar, P., García-Ruiz, A.I. and de Blas, J.C. (2009) New concepts and objectives for protein-amino acid nutrition in rabbits: a review. *World Rabbit Science* 17, 1–14.

Chamorro, S., Gomez Conde, M.S., Perez De Rozas, A.M., Badiola, I., Carabaño, R. and de Blas, J.C. (2007) Effect on digestion and performance of dietary protein content and increased substitution of lucerne hay with soya-bean protein concentrate in starter diets for young rabbits. *Animal* 1, 651–659.

de Blas, J.C., Merino, Y., Fraga, M.J. and Gálvez, J.F. (1979) A note on the use of sodium hydroxide treated straw pellets in diets for growing rabbits. *Animal Production* 29, 427–430.

de Blas, J.C., Fraga, M.J., Rodríguez, J.M. and Méndez, J. (1984) The nturitive value of feeds for growing fattening rabbits. 2. Protein evaluation. *Journal of Applied Rabbit Research* 7, 97–100.

de Blas, J.C., Taboada, E., Nicodemus, N., Campos, R., Piquer, J. and Mendez, J. (1998) Performance response of lactating and growing rabbits to dietary threonine content. *Animal Feed Science Technology* 70, 151–160.

Emaldi, O., Crociani, F. and Matteuzzi, D. (1979) Bacteria in the caecal content, soft and hard faeces of rabbit. *Journal of Applied Bacteriology* 46, 169–172.

Forsythe, S.J. and Parker, D.S. (1985a) Nitrogen metabolism by the microbial flora of the rabbit caecum. *Journal of Applied Bacteriology* 58, 363–369.

Forsythe, S.J. and Parker, D.S. (1985b) Urea turnover and transfer to the digestive tract in the rabbit. *British Journal of Nutrition* 53, 183–190.

Forsythe, S.J. and Parker, D.S. (1985c) Ammonia-nitrogen turnover in the rabbit caecum and exchange with plasma urea-N. *British Journal of Nutrition* 54, 285–292.

Fraga, M.J. and de Blas, J.C. (1977) Influencia de la coprofagia sobre la utilización digestiva de los alimentos por el conejo. *Anales INIA Serie Producción Animal* 8, 43–47.

Fraga, M.J., Barreno, C., Carabaño, R., Méndez, J. and de Blas, J.C. (1984) Efecto de los niveles de fibra y proteína del pienso sobre la velocidad de crecimiento y los parámetros digestivos de los conejos. *Anales INIA Serie Ganadera* 21, 91–110.

Fraga, M.J., Pérez, P., Carabaño, R. and de Blas, J.C. (1991) Effect of type of fiber on the rate of passage and on the contribution of soft feces to nutrient intake of finishing rabbits. *Journal of Animal Science* 69, 1566–1574.

Gallois, M., Fortun-Lamothe, L., Michelan, A. and Gidenne, T. (2008) Adaptability of the digestive function according to age at weaning in the rabbit: II. Effect on nutrient digestion in the small intestine and in the whole digestive tract. *Animal* 2, 536–547.

García, A.I., de Blas, J.C. and Carabaño, R. (2004) Effect of type of diet (casein-based or protein-free diet) and caecotrophy on ileal endogenous nitrogen and amino acid flow in rabbits. *Animal Science* 79, 231–240.

García, A.I., de Blas, J.C. and Carabaño, R. (2005) Comparison of different methods for nitrogen and amino acid evaluation in rabbit diets. *Animal Science* 80, 169–178.

García, J., de Blas, J.C., Carabaño, R. and García, P. (1995a) Effect of type of lucerne hay on caecal fermentation and nitrogen contribution through caecotrophy in rabbits. *Reproduction, Nutrition and Development* 35, 267–275.

García, J., Pérez, L., Alvarez, C., Rocha, R., Ramos, M. and de Blas, C. (1995b) Prediction of the nutritive value of lucerne hay in diets for growing rabbits. *Animal Feed Science and Technology* 54, 33–44.

García, J., Carabaño, R., Pérez Alba, L. and de Blas, J.C. (2000) Effect of fiber source on cecal fermentation and nitrogen recycled through cecotrophy in rabbits. *Journal of Animal Science* 78, 638–646.

García J., Nicodemus, N., Carabaño, R. and de Blas, J.C. (2002) Effect of inclusion of defatted grape seed meal in the diet on digestion and performance of growing rabbits. *Journal of Animal Science* 80, 162–170.

García-Ruiz, A.I., García-Palomares, J., García-Rebollar, P., Chamorro, S., Carabaño, R. and de Blas, J.C. (2006) Effect of protein source and enzyme supplementation on ileal protein digestibility and fattening performance in rabbits. *Spanish Journal of Agriculture Research* 4, 297–303

Gidenne, T. and Fortun-Lamothe, L. (2002) Feeding strategy for young rabbits around weaning: a review of digestive capacity and nutritional needs. *Animal Science* 75, 169–184.

Gidenne, T. and Lebas, F. (1987) Estimation quantitative de la caecotrophie chez le lapin en croissance: variations en fonction de l'âge. *Annales de Zootechnie* 36, 225–236.

Gómez-Conde, M.S., García J., Chamorro, S., Eiras P., García Rebollar, P.G., Perez De Rozas, A., Badiola, I., de Blas, C. and Carabaño, R. (2007) Neutral detergent-soluble fiber improves gut barrier function in 25 d old weaned rabbits. *Journal of Animal Science* 85, 3313–3321.

Gutiérrez I., Espinosa, A., García, J., Carabaño, R. and de Blas, J.C. (2003) Effect of source of protein on digestion and growth performance of early-weaned rabbits. *Animal Research* 52, 461–472.

Hill, R.R.H. (1986) Digestion of mucin polysaccharides *in vitro* by bacteria isolated from the rabbit cecum. *Current Microbiology* 14, 117–120.

Hoover, W.H. and Heitmann, R.N. (1975) Cecal nitrogen metabolism and amino acid absorption in the rabbit. *Journal of Nutrition* 105, 245–252.

INRA (2002) Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage. Sauvant, D., Perez, J.M. and Tran G. (eds). INRA Editions, Paris, France.

Larkins, B.A. (1981) Seed storage proteins: characterization and biosynthesis. In: Stumpf, P.K. and Conn, E.E. (eds) *The Biochemistry of Plants*, Vol. 6. Academic Press, London, UK, pp. 449–489.

Lorente, M., Fraga, M.J., Carabaño, R. and de Blas, C. (1988) Coprophagy in lactating does fed different diets. *Journal of Applied Rabbit Research* 11, 11–15.

Llorente, A., García, A.I., Nicodemus, N., Villamide, M.J. and Carabaño, R. (2005) Utilización de una nueva metodología para la determinación de la digestibilidad ileal aparente y real en la valoración nitrogenada de la harina de girasol en conejos. In: *Proceedings of XI Jornadas sobre Producción Animal ITEA*. Zaragoza, Spain, pp. 497–499.

Llorente, A., García, A.I., Nicodemus, N., Villamide, M.J. and Carabaño, R. (2006) Digestibilidad ileal aparente y verdadera de aminoácidos de harinas de girasol, productos de soja y guisante en conejos. In: *Proccedings of the XXXI Symposium de Cunicultura de ASESCU*. Lorca, Spain, pp. 117–124.

Llorente, A., Villamide, M.J., García, A.I. and Carabaño, R. (2007a) Digestibilidad de la proteína de los aminoácidos de cereales y sus subproductos en conejos. In: *Proceedings of XXXII Symposium de Cunicultura de ASESCU*. Vila-Real, Portugal, pp. 87–90.

Llorente, A., García, A.I., Villamide, M.J. and Carabaño, R. (2007b) Prédiction de la digestibilité iléale azotée pour méthodes *in vitro*. In: *Proceedings of the 12èmes Journées de la Recherche Cunicole*. Le Mans, France, pp. 93–96.

Maertens, L., Cavani, C. and Petracci, M. (2005) Nitrogen and phosphorus excretion on commercial rabbit farms: calculations based on the input-output balance. *World Rabbit Science* 13, 3–16.

Makoni, N.F., Von Keyserlingk, M.G.A., Shelford, J.A., Fisher, L.J. and Puchala, R. (1993) Fractionation of fresh, wilted and ensiled alfalfa proteins. *Animal Feed Science and Technology* 41, 1–13.

Martinez, J. and Fernández, J. (1980) Composition, digestibility, nutritive value and relations among them of several feeds for rabbits. In: Camps, J. (ed.) *Proceedings of the 2nd World Rabbit Congress*. Asociación Española de Cunicultura, Barcelona, pp. 214–223.

Merino, J.M. (1994) Puesta a punto de una técnica de canulación ileal en el conejo para el estudio del aprovechamiento de los nutrientes de la dieta. Doctoral thesis, Universidad Complutense de Madrid, Spain.

Merino, J.M. and Carabaño, R. (2003) Efecto de la coprofagía sobre la composición química de la digesta y sobre la digestibilidad ileal. *ITEA* 24, 657–659.

Miflin, B.J. and Shewry, P.R. (1981) Seed storage proteins: genetics, synthesis, accumulation and protein quality. In: Bewlwy, J.D. (ed.) *Nitrogen and Carbon Metabolism*. Martinus Nijhoff, The Hague, The Netherlands, pp. 195–248.

Motta-Ferreira, W., Fraga, M.J. and Carabaño, R. (1996) Inclusion of grape pomace, in substitution for lucerne hay, in diets for growing rabbits. *Animal Science* 63, 167–174.

Nicodemus, N. (2000) Recomendaciones sobre el nivel óptimo de inclusión de fibra: FND, fibra larga y lignina, en piensos de conejos de alta productividad. Doctoral thesis, Universidad Polotécnica de Madrid, Spain.

Nicodemus, N., Mateos, J., de Blas, J.C., Carabaño, R. and Fraga, M.J. (1999) Effect of diet on amino acid composition of soft faeces and the contribution of soft faeces to total amino acid intake, through caecotrophy in lactating doe rabbits. *Animal Science* 69, 167–170.

Nicodemus, N., García, J., Carabaño, R. and de Blas, J.C. (2002) Effect of inclusion of sunflower hulls in the diet on performance, disaccharidase activity in the small intestine and caecal traits of growing rabbits. *Animal Science* 75, 237–243.

Nicodemus, N., García, J., Carabaño, R. and de Blas, J.C. (2006) Effect of a reduction of dietary particle size by substituting a mixture of fibrous by-products for lucerne hay on performance and digestion of growing rabbits and lactating does. *Livestock Science* 100, 242–250.

Proto, V. (1976) Fisiologia della nutrizione del coniglio con particolare riguardo alla ciecotrofia. *Rivista di Conigliocoltura* 7, 15–33.

Raharjo, Y.C., Cheeke, P.R. and Patton, N.M. (1990) Evaluation of tropical forages and by-products feeds for rabbits production. 1. Nutrient digestibility and effect of heat treatment. *Journal of Applied Rabbit Research* 13, 56–61.

Ramos, M.A., Carabaño, R. and Boisen, S. (1992) An *in vitro* method for estimating digestibility in rabbits. *Journal of Applied Rabbit Research* 15, 938–946.

Rerat, A. (1978) Digestion and absorption of carbohydrates and nitrogenous matters in the hindgut of the omnivorous non ruminant animal. *Journal of Animal Science* 46, 1808–1837.

Sakaguchi, E., Kaizu, K. and Nakamichi, M. (1992) Fibre digestion and digesta retention from different physical forms of the feed in the rabbit. *Comparative Biochemistry and Physiology* 102A, 559–563.

Sirotek, K., Santos, E., Benda, V. and Marounek, M. (2003) Isolation, identification and characterization of rabbit caecal mucinolytic bacteria. *Acta Veterinaria Brunensis* 72, 365–370.

Taboada, E., Méndez, J., Mateos, G.G. and de Blas, J.C. (1994) The response of highly productive rabbits to dietary lysine content. *Livestock Production Science* 40, 329–337.

Taboada, E., Méndez, J. and Blas, C. (1996) The response of highly productive rabbits to dietary sulphur amino acid content for reproduction and growth. *Reproduction, Nutrition and Development* 36, 191–203.

Trocino, A., Xiccato, G., Queaque, P.I. and Sartori, A. (2000) Feeding plans at different protein levels: effects on growth performance, meat quality and nitrogen excretion in rabbits. In: Blasco, A. (ed.) *Proceedings of the 7th World Rabbit Congress, Valencia*, Vol. C. Valencia University Publications, Valencia, Spain, pp. 467–474.

Villamide, M.J. and Fraga, M.J. (1998) Prediction of the digestible crude protein and protein digestibility of feed ingredients for rabbits from chemical analysis. *Animal Feed Science and Technology* 70, 211–224.

Villamide, M.J., Carabaño, R., Maertens, L., Pascual, J., Gidenne, T., Falcao-E-Cunha, L. and Xiccato, G. (2009) Prediction of the nutritional value of European compound feeds for rabbits by chemical components and *in vitro* analysis. *Animal Feed Science and Technology* 150, 283–294.

Waghorn, G.C., Ulyatt, M.J., John, A. and Fisher, M.T. (1987) The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep on *Lotus corniculatus* L. *British Journal of Nutrition* 57, 115–126.

Yoshida, T., Pleasants, J.R., Reddy, B.S. and Wostmann, B.S. (1968) Efficiency of digestion in germfree and conventional rabbits. *British Journal of Nutrition* 22, 723–737.

Yoshida, T., Pleasants, J.R., Reddy, B.S. and Wostmann, B.S. (1971) Amino acid composition of cecal contents and feces in germfree and conventional rabbits. *Journal of Nutrition* 101, 1423–1430.

Yoshida, T., Pleasants, J.R., Reddy, B.S. and Wostmann, B.S. (1972) The pH values and nitrogen fractionations of cecal contents and feces of germfree and conventional rabbits. *Japanese Journal of Zootechnical Science* 43, 284–289.

4 Fat Digestion

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4.1 Chemical Structure and Physical Properties of Fats

The word 'fat' is commonly misused to indicate all lipids, a complex group of organic substances composed of carbon, hydrogen and oxygen, and characterized by solubility in organic apolar solvents. Lipids can be divided into simple lipids, which do not contain fatty acids (FAs), and complex lipids, which are esterified with FAs (Fig. 4.1).

Triglycerides can be considered 'true' fats because they represent the most typical form of energy accumulation in animal and vegetable organisms. Therefore, only these lipids have real nutritional importance. Triglycerides are the highest energy-yielding component of feeds, yielding an average of 2.25 times more energy than other components (i.e. protein and starch). Triglycerides are formed by one glycerol molecule, a trihydric alcohol, to which three FAs are esterified (Fig. 4.2). The physical, chemical and nutritive properties of triglycerides depend on the characteristics of their FAs (Fig. 4.3) – in other words, the number of carbon atoms and the number and position of unsaturated bonds (double bonds).

The number of carbon atoms in triglyceride FAs is usually even due to the addition or subtraction of a pair of carbon atoms during FA synthesis or oxidation in higher animals and plants. In comparison, microorganisms are capable of producing FAs with odd numbers of carbon atoms. Short-chain FAs are formed of two (C2) to eight (C8) carbon atoms, medium-chain FAs have 10–16 carbon atoms and long-chain FAs have ≥18 carbon atoms (up to 22–24).

The number of double bonds is the second distinctive property of FAs: saturated FAs (SFAs) contain only single (saturated) bonds between carbon atoms, while unsaturated FAs (UFAs) present one or more double (unsaturated) bonds. UFAs can be divided into monounsaturated FAs with only one double bond (e.g. oleic acid, C18:1) and polyunsaturated FAs (PUFAs) with two (e.g. linoleic acid, C18:2) or more (up to six) double bonds. The position of the double bonds in the carbon chain determines the ability of PUFAs to act as precursors of other essential compounds, such as hormones. Mammals and other higher animals are able to elongate the carbon chain (e.g. from C18 to C22), but are unable to insert double bonds between the carbon atoms in position 1 (n-1 or ω-1) of the chain (starting from the terminal methyl group, CH_3-) and the carbon in position 9 (n-9 or ω-9). For this reason, animals need an adequate quantity of

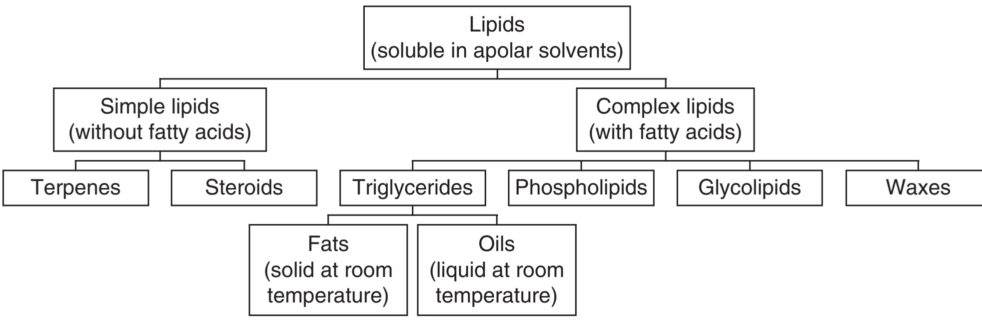

Fig. 4.1. Classification of lipids.

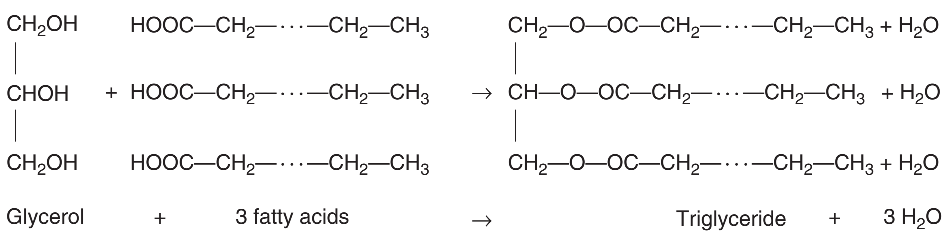

Fig. 4.2. Synthesis of triglycerides by esterification of glycerol and fatty acids.

Fatty acid	Designation	Chemical structure
Myristic	C14:0	$CH_3–(CH_2)_{12}–COOH$
Palmitic	C16:0	$CH_3–(CH_2)_{14}–COOH$
Palmitoleic	C16:1, n-7	$CH_3–(CH_2)_5–CH=CH–(CH_2)_7–COOH$
Stearic	C18:0	$CH_3–(CH_2)_{16}–COOH$
Oleic	C18:1, n-9	$CH_3–(CH_2)_7–CH=CH–(CH_2)_7–COOH$
Linoleic	C18:2, n-6	$CH_3–(CH_2)_4–CH=CH–CH_2–CH=CH–(CH_2)_7–COOH$
Linolenic	C18:3, n-3	$CH_3–CH_2–CH=CH–CH_2–CH=CH–CH_2–CH=CH–(CH_2)_7–COOH$

Fig. 4.3. Chemical structures of major fatty acids in fats and oils.

essential FAs (EFAs) in their diet, namely n-3 FAs (with their first double bond in position 3) and n-6 FAs (with their first double bond in position 6). Dietary EFAs are primarily represented by linoleic acid (C18:2, n-6) and linolenic acid (C18:3, n-3). The former is essential for the synthesis of arachidonic acid (C20:4, n-6), the precursor of prostaglandins and prostacyclins (reproductive function) or thromboxanes (haemostasis function); the latter is essential for the synthesis of eicosapentaenoic acid (C20:5, n-3), the precursor of several compounds essential for heart, retina and brain functions, and the immune system (Enser, 1984; Sanders, 1988). A low n-6 to n-3 FA ratio in foods is beneficial in reducing the incidence of cardiovascular and thrombotic diseases in humans.

The melting point of fats and oils is influenced by FA chemical structure and falls with a decrease in the number of carbon atoms and an increase in the number of unsaturated bonds (Table 4.1). For this

Table 4.1. Physical and chemical properties of various fats and oils (Cheeke, 1987).

Type of fat	Iodine number	Melting point (°C)	Fatty acids (g 100 g^{-1} of total oil) 16:0	18:0	18:1	18:2	18:3
Vegetable oils							
Coconut oil	8–10	20–35	8.0	2.8	5.6	1.6	–
Maize oil	115–127	<20	12.0	2.7	30.1	54.7	1.4
Olive oil	79–90	20	14.0	2.6	74.0	8.1	–
Safflower oil	145	<20	12.3	1.8	11.2	74.3	–
Soybean oil	130–138	<20	11.5	4.3	27.3	49.7	6.9
Animal fats							
Butter	26–38	28–36	27.0	12.5	35.0	3.0	0.8
Beef tallow	35–45	36–45	26.2	22.4	45.3	1.6	–
Lard	50–65	35–45	25.7	5.6	49.2	9.6	1.1
Poultry fat	80	<30	21.4	5.9	39.5	23.5	1.0

reason, triglycerides of vegetable origin are liquid at room temperature (oils) being richer in unsaturated bonds, while triglycerides of animal origin are solid (fats).

The degree of unsaturation also affects fat stability because double bonds are easily oxidized, thereby forming hydroperoxides that are rapidly broken down into short-chain compounds, which give fat and feed their typically rancid odour. The rate of oxidation rises as the number of unsaturated bonds increases. As an example, linolenic acid (C18:3) is oxidized ten times more rapidly than linoleic acid (C18:2), which is oxidized ten times more rapidly than oleic acid (C18:1) (Enser, 1984).

A chemical index of the degree of unsaturation is the iodine number: the weight (in g) of iodine capable of reacting with 100 g of triglyceride. In fact, two iodine atoms can react with each double bond. In animal and vegetable lipids, the iodine number represents the average degree of unsaturation of the entire pool of FAs composing the triglycerides (Table 4.1).

4.2 Fats in Rabbit Feeds

The triglycerides usually present in rabbit feed and pure vegetable and animal fats contain primarily medium- or long-chain FAs (C14–C20), with C16 and C18 FAs being most common (Table 4.1). Rabbits have no specific fat requirements apart from a small amount of EFAs (INRA, 1989). This need is easily met by the lipids contained in the conventional raw materials used in the formulation of compound feeds. Traditionally, rabbit feeding is based on low- or moderate-energy diets. Pure fats or oils are therefore not added and the dietary crude fat content does not exceed 30–35 g kg^{-1}, on average. Only a part of this chemical constituent is composed of triglycerides, given that the larger part is composed of other compounds such as glycolipids, phospholipids, waxes, carotenoids and saponins (Fig. 4.1) (Van Soest, 1982; Cheeke, 1987). All of these substances are soluble in ethyl ether or petroleum ether, the solvents utilized to determine the crude fat or ether extract (EE) content using the Weende feed analysis method. These lipids possess rather low digestibility and metabolic utilization and are therefore considered scarcely relevant from a nutritional point of view.

Currently, the addition of limited amounts of fats (10–30 g kg^{-1}) to rabbit diets is rather common under intensive rearing systems (Maertens, 1998). In breeding does, this increases dietary energy concentrations and stimulates energy intake by the female, who experiences a severe energy deficit during lactation (Xiccato, 1996; Pascual *et al.*, 2003, 2006). In weaning rabbits, the dietary addition of fat may improve body condition, stimulate development of the immune system and improve health (Xiccato *et al*., 2003; Maertens *et al*., 2005). In growing and fattening rabbits, fat supplementation may favourably change the FA profile and the nutritional value of rabbit meat (Hernández, 2008).

4.3 Triglyceride Digestion and Utilization

Triglycerides ingested by rabbits in the diet are submitted to rather complex processes of digestion and absorption but, on the whole, these processes are similar to those observed in other non-ruminants (Brindley, 1984; Freeman, 1984; Cheeke, 1987). Triglycerides are emulsified and then hydrolysed by lipolytic enzymes before being finally absorbed in the small intestine.

As observed in different species (human, pig, rat, cattle), the digestive process in suckling animals begins in the stomach, where pre-duodenal lipases (oral and sometimes gastric) hydrolyse the naturally emulsified fat in milk. In suckling rabbits, gastric lipases account for most of the lipolytic activity (Marounek *et al*., 1995; Zita *et al*., 2008).

After weaning, triglycerides from solid feed require emulsification, and therefore fat digestion occurs only in the small intestine (schematically shown in Fig. 4.4). Fat emulsification is promoted by bile salts secreted by the liver. Bile salts mix with fat droplets, breaking them down into minute globules that can be easily hydrolysed by pancreatic lipase and other lipolytic enzymes (colipase, sterol ester hydrolase and phospholipase). The enzymatic hydrolysis of triglycerides leads to the separation of glycerol, free FAs and monoglycerides, which remain emulsified with bile, forming microscopic micelles. These micelles move to the microvilli of the

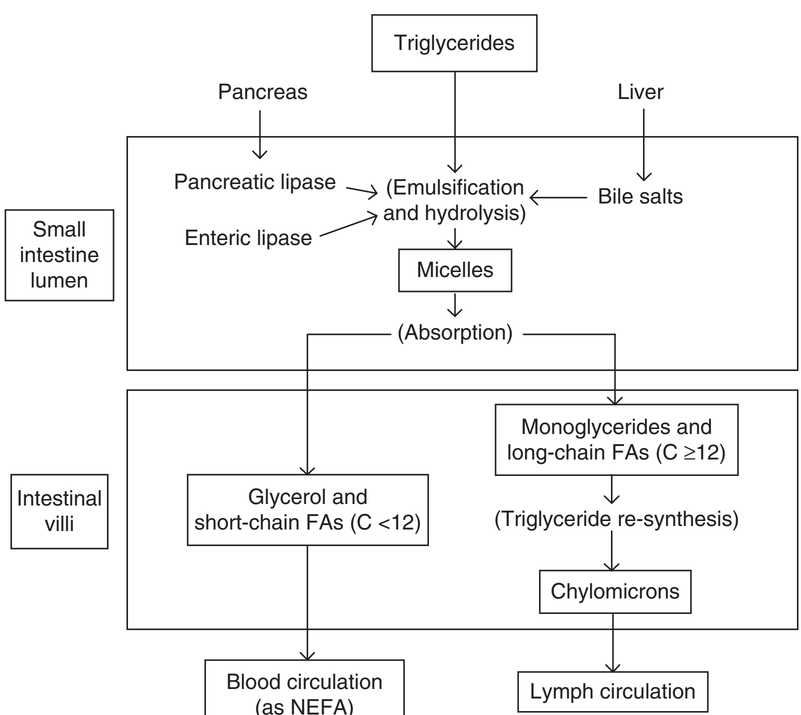

Fig. 4.4. Digestion and absorption of triglycerides in rabbits and other non-ruminants. NEFA, non-esterified fatty acids.

duodenum and jejunum, which absorb the glycerol, free FAs and monoglycerides. Bile salts are left in the intestinal lumen, and are then absorbed lower down the tract (distal ileum). Fat absorption is passive (i.e. a non-energy-consuming process). When absorbed into enterocytes, glycerol and short-chain FAs (C <12) go directly into the blood, where they circulate as non-esterified FAs. Monoglycerides and medium- and long-chain FAs (C >12) are re-synthesized as triglycerides. Droplets of synthesized triglycerides are then covered by a lipoproteic membrane, forming chylomicrons that pass to the lymph circulation system.

Long-chain FAs that are esterified in the triglycerides of chylomicrons can be metabolized as energy sources or either incorporated directly into fat tissue or transferred unchanged to the milk. For this reason, the composition of the dietary fat can significantly influence fat characteristics in the rabbit carcass (Hernández, 2008) or the FA composition of the milk fat (Pascual *et al.*, 2003).

FAs that are not digested can pass through the lowest part of gut and be excreted in the faeces as soaps or enter the caecum, where UFAs are hydrogenated by the caecal microflora. A bacterial *de novo* FA synthesis also occurs in caecotrophs: SFAs are the most abundant, followed by monounsaturated FAs and PUFAs; a rather high proportion of branched-chain FAs is also observed (0.07–0.13 of FAs) (Leiber *et al.*, 2008). In addition, an increase of FAs with odd numbers of carbon atoms (C15 and C17) has been observed (Fernández *et al.*, 1994).

4.4 Effect of the Analytical Method on Digestibility Determination

The precise determination of EE digestibility (EEd) is essential for a correct energy evaluation of complete diets and raw materials for rabbits. The digestible energy content of rabbit feeds can be calculated with good precision (1% residual standard deviation) whenever the digestible EE and other digestible components (crude protein, crude fibre CF and nitrogen-free extract) are known (Jentsch *et al.*, 1963; Maertens *et al.*, 1988; Villamide *et al.*, 2009). Despite the considerable amount of experimental data available on fat digestion efficiency in rabbits, however, the results often conflict, with EEd ranging from 0.40 to 0.95.

Parigi Bini *et al.* (1974) demonstrated the presence of soaps (salts of FAs and calcium ions) in rabbit faeces, which was favoured by a high level of dietary calcium (mostly present in lucerne meal). Such soaps are only partially detected by ether extraction analysis, therefore providing an underestimation of the undigested lipids (faecal lipids) and consequently an overestimation of EEd (Table 4.2). These authors suggested submitting the faecal samples to acid hydrolysis treatment (in 5N hydrochloric acid) before extraction with ether in order to remove the FAs from the soap bonds. Using this method, Maertens *et al.* (1986) were able to recalculate the EEd values previously found for different types of fat (0.87–0.88) and obtain the correct values of 0.64 (beef tallow), 0.74 (lard), 0.75 (mixed

Table 4.2. Digestibility coefficients of ether extract (EEd) before and after acid hydrolysis of faeces.

			EEd of diets		
Type of fat	Added fat in the diet (g kg^{-1})	EE in the diet (g kg^{-1})	Before HCl treatment	After HCl treatment	Reference
Basal diet	0	37	0.72	0.52	Parigi Bini *et al.* (1974)
Beef tallow	50	92	0.85	0.67	
	100	137	0.89	0.69	
Basal diet	0	22	0.69	0.57	Maertens *et al.* (1986)
Beef tallow	60	81	0.88	0.64	
Lard	60	84	0.87	0.74	
Mixed fat	60	84	0.88	0.75	
Soybean oil	60	81	0.87	0.83	

fat) and 0.83 (soybean oil) after acid hydrolysis pre-treatment (Table 4.2).

The European Group on Rabbit Nutrition (EGRAN) has performed various ring-tests on the chemical analyses of rabbit diets and faeces (Xiccato *et al.*, 1996; EGRAN, 2001). It demonstrated poor reproducibility among laboratories for EE values of feed and faeces; the EEd calculated in different laboratories ranged from 0.57 to 0.73 for the same diet.

4.5 Effect of the Level and Source of Fat

Lipid digestibility depends primarily on the level and source of added fats. The EEd of a non-added-fat diet, which contains 25–30 g structural lipids kg^{-1} is rather low (0.45–0.65), while the EEd of added-fat diets is higher because of the higher digestibility (0.85–0.95) of pure fats (Tables 4.3 and 4.4). In three diets with 0 (control), 30 and 60 g added fat kg^{-1}, Santomá *et al.* (1987) observed a significant increase in the EEd as the fat level increased (0.64, 0.75 and 0.79, respectively), without any significant difference among fat sources (see Tables 4.3 and 4.4 for more information).

The increase in EEd with higher levels of dietary fat could also be ascribed to a reduction in dry matter (DM) intake. This usually occurs when feed of a higher dietary energy value is given, as a consequence of the chemostatic regulation of appetite (Forbes, 1995; Xiccato, 1996). The decrease of DM intake is associated with a lower transit of digesta and consequently leads to increased digestion efficiency (Falcão e Cunha *et al.*, 2004).

On the other hand, when the inclusion of fat is high (e.g. >60 g kg^{-1}) EEd may decrease (Table 4.3), probably because both digestive efficiency and microflora activity in the caecum are negatively affected by the excessive fat (Maertens *et al.*, 1986; Falcão e Cunha *et al.*, 1996). Falcão e Cunha *et al.* (2004) observed reduced cellulolytic and pectinolytic activity in the caecum and caecotrophes of rabbits fed high-fat diets. Similar negative effects of high dietary fat supplementation have been reported in ruminants (Van Soest, 1982; Hess *et al.*,

Table 4.3. Effect of the inclusion of added fat on the digestibility of ether extract (EEd).

Type of full-fat seed	Inclusion level (g kg^{-1})	EE in the diet (g kg^{-1})	EEd of diets	Reference
Basal diet	0	22	0.57	Maertens *et al.* (1986)
Beef tallow	60	81	0.64	
	120	133	0.52	
Lard	60	84	0.74	
	120	136	0.71	
Basal diet	0	46	0.82	Falcão e Cunha *et al.* (1996)
Beef tallow	40	83	0.86	
	80	113	0.84	

Table 4.4. Effect of the inclusion of full-fat oilseed on the digestibility of ether extract (EEd).

Type of fat	Inclusion level (g kg^{-1})	EE in the diet (g kg^{-1})	EEd of diets	Reference
Basal diet	0	28	0.70	Cavani *et al.* (1996)
Soybean	30	32	0.82	
	60	38	0.85	
Basal diet	0	29	0.71	Maertens *et al.* (1996)
Oilseed rape	300	156	0.89	
Basal diet	0	48	0.83	Peiretti and Meineri (2008)
Golden flax seed	80	66	0.87	
	160	89	0.91	

2008), poultry (Wiseman, 1984) and horses (Jansen *et al.*, 2007).

The differences observed in EEd among various sources of fats are mostly attributed to their molecular structure and chemical bonds. The fat contained in conventional raw materials is linked to plant structures and is therefore poorly digested. Pure added fats are much more easily digestible, and this is also true for the fat contained in heated (or extruded) full-fat oil seeds, such as full-fat soybean or golden flax seed (Table 4.4). The digestibility coefficients (calculated by difference) of pure fats are 0.86 (beef tallow), 0.90 (oleins) and 0.98 (soybean oil) (Fernández *et al.*, 1994). These values are similar to those listed by Maertens *et al.* (1990) for animal fat (0.90) and soybean oil (0.95). The EEd of full-fat soybean has been found to be very high (0.97), and only slightly higher than that of full-fat rapeseed (0.93) (Maertens *et al.*, 1996).

It is unclear whether EEd variation depends on the proportion of the different FAs in the various sources of fat. As in other species, a negative relationship has been reported between the degree of saturation and fat digestibility in rabbits: more saturated fats (e.g. beef tallow, lard) are less digestible than unsaturated fats (e.g. sunflower or soybean oils), probably because the latter are more easily emulsified and therefore digested in the gut (Maertens *et al.*, 1986; Santomá *et al.*, 1987).

Fernández *et al.* (1994) found that the digestibility of different FAs depends more on the source of fat than the degree of saturation. In two diets – diet T containing 30 g beef tallow kg^{-1} and diet S containing 30 g soybean oil kg^{-1} – the digestibility coefficients of the two principal saturated FAs (i.e. C16:0, C18:0) were higher (0.67 and 0.71) in the T diet than in the S diet (0.57 and 0.31). On the other hand, the PUFAs (i.e. C18:2, C18:3) were more digestible in the more unsaturated diet (0.69 and 0.80 in the T diet versus 0.84 and 0.84 in the S diet). The authors concluded that UFA:SFA ratio in dietary fats may not be the most appropriate predictor of fat digestibility.

The digestibility evaluation of specific FAs in rabbits is probably affected by a systematic bias due to the lipid metabolism of caecal microflora. This changes the composition of faecal fat and modifies the ratio between digestible SFAs and UFAs (Fernández *et al.*, 1994; Fernández-Carmona *et al.*, 2000; Leiber *et al.*, 2008).

4.6 Effect of Age, Physiological State and Nutritive Level

The digestion efficiency for fat, as well as for other nutrients, varies during the life of a rabbit. Rabbit milk contains a high quantity of lipids (100–150 g kg^{-1}, depending on lactation period) that are easily digested and absorbed by suckling rabbits, which show high gastric lipase activity (Marounek *et al.*, 1995; Dojana *et al.*, 1998; Maertens *et al.*, 2006). Parigi Bini *et al.* (1991b) estimated the digestibility of milk and solid feed during weaning (from 21 to 26 days of age) by multiple regression: the EEd of milk was found to be practically complete (0.97), while the EEd of pelleted food was much lower (0.74).

When kits begin to consume solid feed, fat digestion occurs in the small intestine. Lipase activity in the total proteic extract of the gastric mucosa decreases from 15 to 43 days of age and is quite low or nearly absent in older rabbits (Marounek *et al.*, 1995; Dojana *et al.*, 1998). In contrast, the lipase activity of the pancreas, intestinal mucosa and small intestinal contents increases from 25 to 32 and 42 days of age (Debray *et al.*, 2003; Gidenne *et al.*, 2007; Gallois *et al.*, 2008). Similarly, lipase activity significantly increases in the colon of rabbits from 28 to 90 days of age (Marounek *et al.*, 1995), but remains unchanged in the caecum of kits from 15 to 35 days of age (Zita *et al.*, 2008).

Several studies have compared digestibility efficiency in growing and adult rabbits. Digestibility coefficients of different components tend either to decrease or remain constant with age, but EEd seems to follow an opposite trend. Evans and Jebelian (1982) observed increasing EEd from 0.78 at 5 weeks to 0.82 at 8 weeks. Xiccato and

Cinetto (1988) confirmed these results, with EEd rising from 0.72 at 7 weeks to 0.79 at 12 weeks. Conversely, other authors have observed higher EEd values in recently weaned rabbits (4–5 weeks) than in older rabbits (7–10 weeks) (Fernández *et al.*, 1994; Debray *et al.*, 2003; Gidenne *et al.*, 2007).

Comparing digestibility efficiency in growing rabbits and adult does fed a non-added-fat diet, Xiccato *et al.* (1992) observed a significantly lower EEd in young rabbits than in adult rabbits (0.58 versus 0.64), but no differences between the sexes in growing rabbits or between physiological states (pregnant or not pregnant) in adult does. The latter results confirm an absence of any effect attributable to reproductive status, as was previously observed by Parigi Bini *et al.* (1991a) in does during late pregnancy, early lactation and late lactation.

The influence of age and physiological state on EEd may be attributed to variations in feed intake, as suggested by Fernández *et al.* (1994). However, this hypothesis does not agree with the results achieved by other studies involving growing rabbits (Xiccato and Cinetto, 1988; Xiccato *et al.*, 1992) and lactating does (Parigi Bini *et al.*, 1992) on either *ad libitum* or restricted feeding diets.

Parigi Bini (1971) found a very significant negative correlation between the dietary CF level and both DM digestibility (DMd) and EEd:

$$\mathrm{DMd} = 0.812 - 1.17 \times 10^{-3}\,\mathrm{CF}\ (\mathrm{g\ kg^{-1}\ DM}),\quad r = -0.929.$$

$$\mathrm{EEd} = 0.993 - 2.08 \times 10^{-3}\,\mathrm{CF}\ (\mathrm{g\ kg^{-1}\ DM}),\quad r = -0.936.$$

The decrease in both DMd and EEd is linked to the poorer diet quality, higher feed intake and faster transit rate that occur when higher fibre levels are given. However, even if this research was unable to distinguish between the simultaneous effects of the crude fibre level on DMd and EEd, it appears that the decrease of EEd with increasing CF levels is probably accentuated by a negative interaction between fibre and crude fat. The latter is strictly associated with cell walls in non-added-fat diets.

References

Brindley, D.N. (1984) Digestion, absorption and transport of fats: general principles. In: Wiseman, J. (ed.) *Fats in Animal Nutrition*. Butterworths, London, UK, pp. 85–103.

Cavani, C., Zucchi, P., Minelli, G., Tolomelli, B., Cabrini, L. and Bergami, R. (1996) Effects of whole soybeans on growth performance and body fat composition in rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 127–133.

Cheeke, P.R. (1987) *Rabbit Feeding and Nutrition*. Academic Press Inc., Orlando, Florida.

Debray, L., Le Huëron-Luron, I., Gidenne, T. and Fortun-Lamothe, L. (2003) Digestive tract development in rabbit according to the dietary energetic source: correlation between the whole tract digestion, pancreatic and intestinal enzymatic activities. *Comparative Biochemistry Physiology* 135, 43–455.

Dojana, M., Costache, M. and Dinischiotu, A. (1998) The activity of some digestive enzymes in domestic rabbits before and after weaning. *Animal Science* 66, 501–507.

EGRAN (European Group on Rabbit Nutrition); Gidenne, T., Perez, J.M., Xiccato, G., Trocino, A., Carabaño, R., Villamide, M.J., Blas, E., Cervera, C., Falcão e Cunha, L. and Maertens, L. (2001) Technical note: attempts to harmonize chemical analyses of feeds and faeces for rabbit feed evaluation. *World Rabbit Science* 9, 57–64.

Enser, M. (1984) The chemistry, biochemistry and nutritional importance of animal fats. In: Wiseman, J. (ed.) *Fats in Animal Nutrition*. Butterworths, London, UK, pp. 23–51.

Evans, E. and Jebelian, V. (1982) Effects of age upon nutrient digestibility by fryers rabbits. *Journal of Applied Rabbit Research* 5, 8–9.

Falcão e Cunha, L., Bengala Freire, J.P. and Gonçalves, A. (1996) Effect of fat level and fiber nature on performances, digestibility, nitrogen balance and digestive organs in growing rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 157–162.

Falcão e Cunha, L., Peres, H., Freire, J.P.B. and Castro-Solla, L. (2004) Effects of alfalfa, wheat bran or beet pulp, with or without sunflower oil, on caecal fermentation and on digestibility in the rabbit. *Animal Feed Science and Technology* 117, 131–149.
Fernández, C., Cobos, A. and Fraga, M.J. (1994) The effect of fat inclusion on diet digestibility in growing rabbits. *Journal of Animal Science* 72, 1508–1515.
Fernández-Carmona, C., Pascual, J.J. and Cervera, C. (2000) The use of fat in rabbit diets. *World Rabbit Science* 8 (Suppl. 1, Vol. C), 29–59.
Forbes, J.M. (1995) *Voluntary Feed Intake and Diet Selection in Farm Animals*. CAB International, Wallingford, UK.
Freeman, C.P. (1984) The digestion, absorption and transport of fats – non-ruminants. In: Wiseman, J. (ed.) *Fats in Animal Nutrition*. Butterworths, London, UK, pp. 105–122.
Gallois, M., Le Huërou-Luron, I., Fortun-Lamothe, L., Lallès, J.P. and Gidenne, T. (2008) Adaptability of the digestive function according to age at weaning in the rabbit: I. Effect on feed intake and digestive functionality. *Animal* 2, 525–535.
Gidenne, T., Debray, L., Fortun-Lamothe, L. and Le Huërou-Luron, I. (2007) Maturation of the intestinal digestion and of microbial activity in the young rabbit: impact of the dietary fibre:starch ratio. *Comparative Biochemistry and Physiology Part A* 148, 834–844.
Hernández, P. (2008) Enhancement of nutritional quality and safety in rabbit meat. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1287–1299.
Hess, B.W., Moss, G.E. and Rule, D.C. (2008) A decade of developments in the area of fat supplementation research with beef cattle and sheep. *Journal of Animal Science* 86, E188–E204.
INRA (Institut Nationale de la Recherche Agronomique) (1989) *L'alimentation des Animaux Monogastriques: Porc, Lapin, Volailles*. INRA, Versailles, France.
Jansen, W.L., Cone, J.W., Geelen, S.N.J., Sloet van Oldrruitenborgh-Oosterbaan, M.M., Van Gelder, A.H., Oude Elferink, S.J.W.H. and Beynen, A.C. (2007) High fat intake by ponies reduces both apparent digestibility of dietary cellulose and cellulose fermentation by faeces and isolated caecal and colonic contents. *Animal Feed Science and Technology* 133, 298–308.
Jentsch, W., Schiemann, L., Hofmann, L. and Nehering, K. (1963) Die energetische Verwertung der Futterstoffe. 2. Die energetische Verwertung der Kraftfutterstoffe durch Kaninchen. *Archiv fur Tierernährung* 13, 133–145.
Leiber, F., Meier, J.S., Burger, B., Wettstein, H.R., Kreuzer, M., Hatt, J.M. and Clauss, M. (2008) Significance of coprophagy for the fatty acids profile in body tissues of rabbits fed different diets. *Lipids* 43, 853–865.
Maertens, L. (1998) Fat in rabbit nutrition: a review. *World Rabbit Science* 6, 341–348.
Maertens, L., Huyghebaert, G. and De Groote, G. (1986) Digestibility and digestible energy content of various fats for growing rabbits. *Cuni-Science* 3, 7–14.
Maertens, L., Moermans, R. and De Groote, G. (1988) Prediction of the apparent digestible energy content of commercial pelleted feeds for rabbits. *Journal Applied Rabbit Research* 11, 60–67.
Maertens, L., Janssen, W.M.M., Steenland, E., Wolfers, D.F., Branje, H.E.B. and Jager, F. (1990) Tables de composition, de digestibilité et de valeur énergétique des matières premières pour lapins. In: *Proceedings of the 5èmes Journées de la Recherche Cunicole*, Vol. 2. Comm. no. 57. INRA-ITAVI, Paris, France, pp. 1–9.
Maertens, L., Luzi, F. and Huybrechts, I. (1996) Digestibility of non-transgenic and transgenic oilseed rape in rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 231–236.
Maertens, L., Aerts, J.M. and De Brabander, D.L. (2005) Effet d'un aliment riche en acides gras omega–3 sur les performances et la composition du lait des lapines et la viabilité de leur descendance. In: *Proceedings of the 11èmes Journées de la Recherche Cunicole*. INRA-ITAVI, Paris, France, pp. 205–208.
Maertens, L., Lebas, F. and Szendrö, Zs. (2006) Rabbit milk: a review of quantity, quality and non dietary affecting factors. *World Rabbit Science* 14, 205–230.
Marounek, M., Vovk, S.J. and Skrivanová, V. (1995) Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *British Journal of Nutrition* 73, 463–469.
Parigi Bini, R. (1971) Ricerche sulla digeribilità ed il valore energetico dei concentrati nel coniglio. *Alimentazione Animale* 15, 17–27.
Parigi Bini, R., Chiericato, G.M. and Lanari, D. (1974) I mangimi grassati nel coniglio in accrescimento. Digeribilità e utilizzazione energetica. *Rivista di Zootecnia e Veterinaria* 2, 193–202.
Parigi Bini, R., Xiccato, G. and Cinetto, M. (1991a) Utilizzazione e ripartizione dell'energia e della proteina digeribile in coniglie non gravide durante la prima lattazione. *Zootecnica e Nutrizione Animale* 17, 107–120.

Parigi Bini, R., Xiccato, G. and Cinetto, M. (1991b) Efficienza digestiva e ritenzione energetica e proteica dei coniglietti durante l'allattamento e lo svezzamento. *Zootecnica e Nutrizione Animale* 17, 167–180.

Parigi Bini, R., Xiccato, G., Cinetto, M. and Dalle Zotte, A. (1992) Energy and protein utilization and partition in rabbit does concurrently pregnant and lactating. *Animal Production* 55, 153–162.

Pascual, J.J., Cervera, C., Blas, E. and Fernández-Carmona, J. (2003) High energy diets for reproductive does: effect of energy source. *Nutrition Abstracts and Reviews. Series B: Livestock Feeds and Feeding* 73, 27R–39R.

Pascual, J.J., Xiccato, G. and Fortun-Lamothe, L. (2006) Strategies for does corporal condition improvement – relationship with litter viability and career length. In: Maertens, L. and Coudert, P. (ed.) *COST 848 Action Advances in Rabbit Science*. ILVO, Melle, Belgium, pp. 247–258.

Peiretti, P.G. and Meineri, G. (2008) Effects of golden flaxseed supplementation on the performance and feed digestibility of rabbits. *Journal of Animal and Veterinary Advances* 7, 56–60.

Sanders, T.A.B. (1988) Essential and trans-fatty acids in nutrition. *Nutrition Research Reviews* 1, 57–78.

Santomá, G., de Blas, J.C., Carabaño, R. and Fraga, M.J. (1987) The effects of different fats and their inclusion level in diets for growing rabbits. *Animal Production* 45, 291–300.

Van Soest, P.J. (1982) *Nutritional Ecology of the Ruminant*. O&B Books, Corvallis, Oregon, USA.

Villamide, M., Carabaño, R., Maertens, R., Pascual, J., Gidenne, T., Falcão e Cunha, L. and Xiccato, G. (2009) Prediction of the nutritional value of European compound feeds for rabbits by chemical components and *in vitro* analysis. *Animal Feed Science and Technology* 150, 283–294.

Wiseman, J. (1984) Assessment of the digestible and metabolisable energy of fats for non-ruminants. In: Wiseman, J. (ed.) *Fats in Animal Nutrition*. Butterworths, London, UK, pp. 277–297.

Xiccato, G. (1996) Nutrition of lactating does. In: Lebas, F. (ed.) *Proceeding of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 29–50.

Xiccato, G. and Cinetto, M. (1988) Effect of nutritive level and of age on feed digestibility and nitrogen balance in rabbit. In: *Proceedings of the 4th World Rabbit Congress*, *Budapest*, Vol. 3. Sandor Holdas Hercegalom, Budapest, Hungary, pp. 96–103.

Xiccato, G., Cinetto, M. and Dalle Zotte, A. (1992) Effetto del livello nutritivo e della categoria di conigli sull'efficienza digestiva e sul bilancio azotato. *Zootecnica e Nutrizione Animale* 18, 35–43.

Xiccato, G., Carazzolo, A., Cervera, C., Falcão e Cunha, L., Gidenne, T., Maertens, L., Perez, J.M. and Villamide, M.J. (1996) European ring-test on the chemical analyses of feed and faeces: influence on the calculation of nutrient digestibility in rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 293–297.

Xiccato, G., Trocino, A., Sartori, A. and Queaque, P.I. (2003) Effect of weaning diet and weaning age on growth, body composition and caecal fermentation of young rabbits. *Animal Science* 77, 101–111.

Zita, L., Fučíková, A., Marounek, M., Tůmová, E. and Skřivanová, V. (2008) Lipase activity till 35 days of age in broiler rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 869–872.

5 Fibre Digestion

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5.1 Introduction

Dietary fibre (DF) the major fraction of rabbit diets, where it accounts for 0.40–0.50 of the total diet (Table 5.1). The importance of fibre is due to its influence on the rate of passage of digesta and mucosa functionality, and its role as a substrate for microbiota. All of these factors are related to rabbit health (see Chapter 10) and performance. The concept of fibre, its quantification and its characterization as either the total fraction or different constituents have been discussed extensively. The difficulty in reaching agreement on the concept of DF is based on its complex physical structure and the chemical composition of cell walls, the considerable diversity of cells types (and, accordingly, of cell walls) that constitute different plant tissues and the wide and different physiological effects of the different constituents. This all implies that the quantitative analysis of the whole components of this fraction cannot be obtained by any analytical method or combination of methods.

This chapter initially considers the definition and structure of the different classes of fibre and of cell wall constituents, followed by a description of some analytical methods employed for animal or human feeds. Second, the effects of fibre on rabbit digestion are described.

5.2 Dietary Fibre in Animal Feeds: Definition, Physicochemical Properties and Analysis

5.2.1 Plant cell wall and dietary fibre: definition

The terms 'cell wall' and 'dietary fibre' are often imprecisely used because they refer to a common plant structure. However, they do not describe the same chemical components and therefore do not have the same meaning. Accordingly, it is useful to define separately these two terms. The term 'plant cell walls' (PCWs) must be employed when describing the structure of the plant cell, which is extremely complex. PCWs are not uniform: the type, size and shape of the walls are closely linked to the function of the cell within the plant (e.g. skeletal tissue, seeds). In general, PCWs consist of a series of polysaccharides often associated and/or substituted with glycoproteins (extensin), phenolic compounds and acetic acid, together with, in some cells, the phenolic polymer lignin. Cutin and silica are also found in the walls and/or in the middle lamella. A growing plant cell is gradually enveloped by a primary wall that contains a few cellulosic microfibrils and some non-cellulosic components such as pectic substances. During plant

Table 5.1. Levels of fibre (g kg^{-1} dry matter) in complete experimental feeds used for the growing rabbit (n = 111) (Villamide *et al.*, 2009).

Residue analysed	Mean	Range
Neutral detergent fibre (aNDFom)	368	248–443
Acid detergent fibre (ADFom)	196	135–284
Acid detergent lignin	56	27–195
Hemicelluloses	172	59–251
Cellulose	140	42–220
Crude fibre	166	122–244
Soluble fibre[a]	109	61–188
Total dietary fibre[b]	478	352–560
Other feed constituents		
Starch	176	82–324
Sugars[c]	53	31–163
Crude protein	176	134–232
Ether extract	32	10–71

ADFom, acid detergent fibre expressed exclusive of residual ash; aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; CP: crude protein; EE, ether extract; OM, organic matter.
[a]Calculated as: OM – CP – EE – aNDFom – starch – sugars.
[b]Calculated as: aNDFom + soluble fibre.
[c]Estimated according to ingredient composition (FEDNA, 2003).

ageing, some cells develop a thick secondary cell wall consisting of cellulose embedded in a polysaccharide and lignin matrix (Selvendran *et al.*, 1987; McDougall *et al.*, 1996). Thus, in brief, the PCW is formed of cellulose microfibrils (the backbone) embedded in a matrix of lignins, hemicelluloses, pectins and proteins (Fig. 5.1).

The concept of DF used in human nutrition, and extended to all mammals, is defined as the feed components resistant to mammalian endogenous enzyme digestion and absorption, and that can be partially or totally fermented in the gut. This empirical 'catch-all' definition describes mainly PCW constituents, but also other substances including resistant starch, oligosaccharides, fructans, protein linked to the cell wall (De Vries and Rader, 2005). Another approximation is the DF for herbivores defined by Mertens (2003) as the indigestible or slowly digested organic matter of feeds that occupies space in the gastrointestinal tract, mainly insoluble fibre. It excludes rapidly fermenting and soluble carbohydrates (e.g. oligosaccharides, fructans).

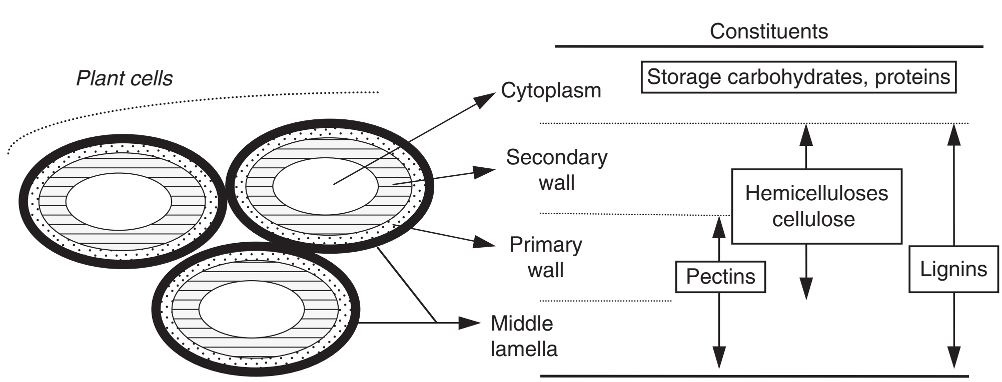

Fig. 5.1. Schematic representation of plant cell walls and their main constituents.

5.2.2 Biochemical characteristics of dietary fibre

The chemical features of DF are highly variable, depending on many factors such as molecular weight, the nature of the monomers and types of linkages. Accordingly, the chemical features of DF are one of the main factors responsible for variations in digestibility; thus, it is important to describe them. With the exception of lignin, cell wall constituents are predominantly polysaccharides composed of neutral and/or acidic sugars. They can be determined using sophisticated extraction techniques, and examples of their concentration in some feedstuffs are given in the Table 5.2.

There are two main groups of DF components according to their location, chemical structure and properties (Fig. 5.2):

- Cell wall components:
  - water-soluble non-starch polysaccharides (e.g., part of β-glucans, arabinoxylans and pectic substances).
  - water-insoluble polymers: lignin, cellulose, hemicelluloses and pectic substances.
- Cytoplasm components:
  - oligosaccharides, fructans, resistant starch and mannans.

Water-soluble polysaccharides and oligosaccharides include several classes of molecules with a degree of polymerization ranging from about 15 to >2000 (β-glucans). Most of them are insoluble in ethanol (80% v/v). Examples include soluble hemicelluloses such as arabinoxylans (in wheat, oats and barley, approximately 20–40 g kg^{-1} dry matter (DM)) and β-glucans (in barley or oat, approximately 10–30 g kg^{-1} DM), oligosaccharides such as α-galactosides (in lupin, pea or soya seeds, 50–80 g kg^{-1} DM) and soluble pectic substances (fruit or beet pulp, from 100 to 400 g kg^{-1} DM). Because of their highly variable structures, no satisfactory method is at present available to determine precisely the amount of these compounds in animal feeds.

Table 5.2. Proximate composition of cell wall constituents (g kg^{-1} DM) in some raw materials used in rabbit feeds, according to several methods of analysis.

Ingredients	Wheat straw	Wheat bran	Dehydrated lucerne	Sugarbeet pulp	Sunflower meal	Soybean hulls	Grape pomace
aNDFom	800	450	450	460	420	620	640
ADFom	540	110	340	220	310	440	540
ADL	160	30	80	20	100	20	340
NDSF	–	30	180	300	–	220	–
Crude fibre[a]	400	100	270	190	260	360	260
WICW	840	450	470	500	380	720	690
INSP	550	360	330	640	260	550	360
Rhamnose	<10	<10	<10	110	<10	110	<10
Arabinose	20	80	20	180	30	40	<10
Xylose	180	160	60	20	50	70	80
Mannose	<10	<10	<10	10	10	60	20
Galactose	<10	<10	<10	40	10	20	20
Glucose	330	90	190	190	110	290	190
Uronic acids	20	20	70	180	50	60	50
SNSP	10	30	30	100	10	20	10
Crude protein	30	150	160	90	340	110	130

ADFom, acid detergent fibre expressed free of ash; ADL, acid detergent lignin (Van Soest *et al.*, 1991); aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed free of ash; INSP, insoluble non-starch polysaccharides (not including the lignin, determined by direct monomeric analysis of cell wall polysaccharides) (Englyst, 1989; Barry *et al.*, 1990); NDSF, neutral detergent soluble fibre (Hall *et al.*, 1997, 1999); SNSP, water-soluble non-starch polysaccharides (Brillouet *et al.*, 1988; Englyst, 1989); WICW, water-insoluble cell wall (including lignin) (Carré and Brillouet, 1989).
[a]According to the Weende method (AOAC, 2000: official method 962.10).

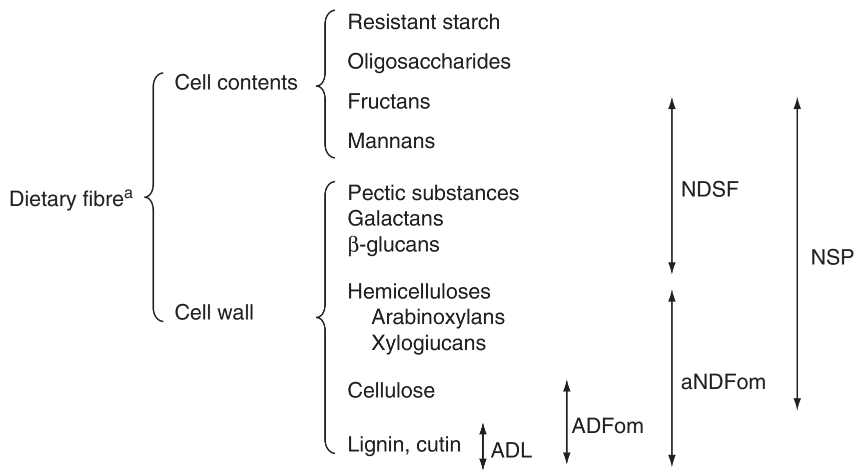

Fig. 5.2. Dietary fibre constituents and their quantification by different methods (adapted from Hall, 2003). ADFom, acid detergent fibre expressed free of ash; ADL, acid detergent lignin; aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed free of ash; NDSF, neutral detergent soluble fibre; NSP, non-starch polysaccharides. [a]Includes other associated compounds: proteins, tannins, waxes, saponins, suberin, phytates, phytosterols.

Pectic substances are a group of polysaccharides present in the middle lamellae and closely associated with the primary cell wall, especially in the primary cells (young tissues) of dicotyledonous plants, such as in legume seeds (40–140 g kg^{-1} DM in soybean, pea, faba bean and white lupin), and also in fruit and pulp. Pectic substances correspond to several classes of polymers, including pectins (rhamnogalacturonan backbone and side chains of arabinose and galactose) and neutral polysaccharides (arabinans, galactans, arabinogalactans) frequently associated with pectins. Their extraction requires the use of a chelating agent such as ammonium oxalate or ethylene diamine tetra-acetic acid (EDTA) (present in the solution for determining neutral detergent fibre (NDF) so pectins are not completely recovered in NDF analysis, as described below). Pectins of the middle lamellae serve as an adhesive in plant tissue, cementing plant cells together.

Cellulose is the major structural polysaccharide of the PCW and the most widespread polymer on earth. It is a homopolymer (in contrast to hemicelluloses and pectins), formed from linear chains of β[1→4] linked D-glucopyranosyl units (whereas starch is formed of α[1→4] linked D-glucopyranosyl chains). The degree of polymerization is usually around 8000–10,000. Individual glucan chains aggregate (hydrogen bonding) to form microfibrils and may serve as the backbone of the plant. Thus, cellulose is only soluble in strong acid solutions (i.e. 72% sulphuric acid), where it is hydrolysed. Quantitatively, cellulose represents 400–500 g kg^{-1} DM in the hulls of legume and oilseeds, 100–300 g kg^{-1} DM in forages and beet pulp and 30–150 g kg^{-1} DM in oilseeds or legume seeds. Most cereal grains contain small quantities of cellulose (10–50 g kg^{-1} DM), except for oats (100 g kg^{-1} DM).

The hemicelluloses are a group of several polysaccharides, with a lower degree of polymerization than cellulose. They have a β[1→4] linked backbone of xylose, mannose or glucose residues that can form extensive hydrogen bonds with cellulose. Xyloglucans are the major hemicelluloses of the primary cell wall in dicotyledonous plants (legumes, seeds), whereas mixed linked glucans (β[1→3,4]) and arabinoxylans are the predominant hemicelluloses in cereal seeds (the latter two include partly water-soluble and water-insoluble polymers, described above). Hemicelluloses include other branched heteropolymers (units linked β[1→3], β[1→6], α[1→4] α[1→3]) such as highly branched arabinogalactans (in soybean), galactomannans (seeds of legumes) and glucomannans. Polymers formed of linear chains of pentose (linked β[1→4]) such as xylans (in secondary walls) or hexose such as mannans (in palm kernel meal) are also considered as hemicelluloses. Pentosans such as xylans and arabinoxylans are soluble in weak basic solutions (5–10%) or in hot dilute acids (5% sulphuric acid). Hexosans such as mannans, glucomannans or galactans can only be dissolved in strong basic solutions (17–24%). Quantitatively, hemicelluloses constitute of 100–250 g kg^{-1} DM in forages and agro-industrial by-products (brans, oilseeds and legume seeds, hulls and pulp) and about 20–120 g kg^{-1} DM in grains and roots.

Lignin is a non-carbohydrate constituent of the cell wall. It can be described as a highly branched and complex three-dimensional network (with a high molecular weight), built up from three phenylpropane units (conyferulic, coumarilic and sinapinic acid). Lignin networks tend to fix the other polymers in place, exclude water and make the cell wall more rigid and resistant to various agents, such as bacterial enzymes. Most concentrate feeds and young forages contain less than 50 g lignin kg^{-1}. The degree of lignification of the PCW may reach 120 g kg^{-1} with ageing in forages or up to 590 g kg^{-1} in grapeseed meal.

Other constituents are also present in PCWs, but in smaller quantities. Minerals, such as silica, are essentially found in graminaceous leaves. Phenolic acids are chemically linked to hemicelluloses and lignin in graminaceous plants. Some proteins are linked to cell walls through intermolecular bonds from amino acids such as tyrosine, and thus resist standard extractions. In addition, plant epidermal cells may be covered by a complex lipid (cutin for aerial parts, suberin for underground structures), which can encrust and embed the cell walls, making them impermeable to water.

Other phenolic compounds can also be mentioned – for example, condensed tannins, which may exist in higher plants. They form cross-linkages with protein and other molecules. They may be included in the sum of indigestible polysaccharides plus lignin. However, condensed tannins, lignins and indigestible proteins are closely related because indigestible complexes of these substances are common in plants (Van Soest, 1994).

Methods for estimating the dietary fibre content of animal feeds

According to the DF definition, DF can only be truly measured by the digestive process of the animal. Currently no method is totally adequate to quantify or fractionate DF due to its complexity. Although many methods have been developed to estimate the DF content in animal feeds, none of them corresponds to a precise DF fraction. Detailed

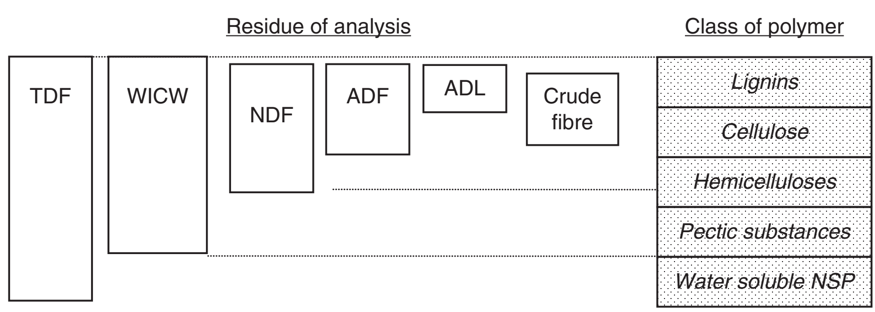

Fig. 5.3. Gravimetric methods for the determination of dietary fibre and identification of the residue of analysis. ADF, acid detergent fibre; ADL, acid detergent lignin (Van-Soest *et al.*, 1991); NDF, neutral detergent fibre; NSP, non-starch polysaccharides; TDF, total dietary fibre (Lee *et al.*, 1992; Li, 1995); WICW, water-insoluble cell wall (Carré and Brillouet, 1989). [a]According to the Weende technique (AOAC, 2000: official method 962.10).

reviews have been published on this subject (Hall, 2003; Mertens, 2003; De Vries and Rader, 2005). Accordingly, only those techniques currently used in rabbit feeding are described here (Fig. 5.3).

Initially, the crude fibre method (AOAC, 2000: official method 962.10) must be mentioned because it is highly reproducible, quick, simple, cheap and frequently used all over the world. This technique extracts a fibrous residue after acidic followed by basic hydrolysis. The main drawback of this method lies in the high variability in the chemical composition of its residue as, depending on the feed, it can dissolve up to 60% cellulose, 80% pentosans and 95% lignin. As a consequence, crude fibre digestibility was higher than that of nitrogen-free extract in 25% of the feeds studied by Morrison (1956). For these reasons, this method is not very useful in explaining the effects exerted by fibre on the animal. However, it has been demonstrated to be very useful in predicting dietary energy value (Wiseman *et al.*, 1992) or within a raw material to verify the fibre content compared to tables.

The main alternative to the crude fibre method is the sequential procedure of Van Soest, developed in 1967 and successively updated (Mertens, 2003). The NDF method was designed to isolate insoluble DF components in PCWs by using a hot neutral detergent solution – cellulose, hemicelluloses and lignin (Mertens, 2002) – as the majority of pectin substances are partially solubilized. This method has been criticized due to its variability among laboratories, especially when the results are compared with those obtained with other feed constituents (Xiccato *et al.*, 1996). This variability is partially due to the different procedures that can be used to perform the method (with heat-stable amylase and/or sodium sulfite or not, ash free or not), but usually described with the same reference (Uden *et al.*, 2005).

The acid detergent fibre (ADF) (AOAC, 2000: official method 973.18) method isolates cellulose and lignin, the worst digested fibrous fractions, using a hot acid detergent solution. It is designed to be performed after NDF analysis, as pectins are retained when it is performed directly. As with the crude fibre method, the ADF method is very useful in predicting dietary energy value (Wiseman *et al.*, 1992). Finally, the lignin fraction is isolated from the ADF residue after removal of the cellulose by using a strong acid solution at room temperature (Robertson and Van Soest, 1981).

The main advantage of this sequential methodology is that is possible to obtain an approximate estimation of lignin (ADL), cellulose (ADF-ADL) and hemicellulose (NDF-ADF) content. In addition, it is relatively quick, simple and economical, and has an acceptable reproducibility when used in a standardized methodology (EGRAN, 2001). Furthermore, it improves the fractionation of the cell wall. These methods have been complemented by the estimation of the fibre dissolved by the neutral detergent solution (NDSF: neutral detergent soluble fibre) (Hall *et al.*, 1997), which mainly includes fructans, galactans, β-glucans and pectic substances. However, the determination of NDSF is too difficult to be used as routine analysis, as occurs with other methods used to estimate soluble fibre.

Another approach is to estimate DF as the sum of non-starch polysaccharides (NSP) and lignin. Several methods are available to estimate total, soluble and insoluble NSP (Bach Knudsen, 2001; De Vries and Rader, 2005), where the non-fibrous components are extracted by solubilization, enzymatic hydrolysis or a combination of both procedures. Once isolated, the fibre residue can be quantified gravimetrically (by weighing the residue) or chemically (by hydrolysing the residue and determining its single constituents: sugars and lignin). According to these procedures, there are three types of methodologies: chemical-gravimetric, enzymatic-gravimetric and enzymatic-chemical. In this way the total DF can be quantified (NSP and lignin) and separated into insoluble and soluble fibre (in aqueous solution), and the monosaccharide composition obtained. The combination of the monosaccharide composition of fibre with additional chemical information may allow a better description of fibre structure that influence its physicochemical properties and, accordingly, the effect exerted on an animal's digestive physiology and digestibility. However, these methodologies are complex, expensive and have relatively low reproducibility (especially for monomers determination). They are difficult to implement as routine analysis.

Dietary insoluble fibre can also be estimated by using near infra-red (NIR) technology. NIR technology has demonstrated usefulness in predicting dietary DM, protein, fat, starch and even digestible energy value. However, ADF is the only fibre fraction that can be adequately estimated by this technique, whereas both NDF and ADL are estimated with lower precision (Xiccato *et al.*, 2003).

In conclusion, the determination of the fibre content of a compound feed (Table 5.1) or raw material (Table 5.2) is highly variable, depending on the analytical method of estimation. The choice of which definition is to be used by the nutritionist thus depends on the type of information required (to relate to digestive processes, predict the nutritive value).

5.2.3 Physicochemical properties of fibre related to digestion: particle size, water-holding capacity and buffering capacity

As described below, part of the fibre requirements of rabbits are related to the effect of the large-size fibre particles on the passage rate of digesta through the gut. Particle size can be measured by dry or wet sieving and varies largely depending on the fibre source. Table 5.3 shows the distribution of particle size of several commercial sources of fibre, once pelleted. Part of this variation can be explained by differences in chemical composition, hydration capacity or previous processing. Dietary particle size is modified considerably during feed manufacturing (see Chapter 11) and it is essential to determine the particle size profile on pellets (Lebas and Lamboley, 1999) instead of the meal. Particle size is reduced by pelleting and by successive millings, even using sieves of the same diameter. In this way Morisse (1982), by milling a feed one or three times with the same sieve size (4 mm), observed an increase in the proportion of fine particles (<0.25 mm) from 0.31 to 0.74. On the other hand, fibre composition is not homogeneous among particles of different size within the same feed: the proportion of

Table 5.3. Particle size determined by wet sieving, hydration and buffering capacity of some commercial sources of fibre after pelleting (García *et al.*, 1999, 2002a).

	Paprika meal	Olive leaves	Lucerne hay	Soybean hulls	NaOH-barley straw	Sunflower husk	Grape-seed meal
Particle size, mm							
<0.160	0.84	0.54	0.54	0.24	0.23	0.02	0.31
>0.315	0.07	0.38	0.29	0.53	0.54	0.74	0.45
>1.250	0.00	0.09	0.02	0.04	0.11	0.04	0.02
Water-holding capacity, %	389	408	581	600	652	654	192
Acid-buffering capacity[a]							
Whole sample	210	93	151	88	57	64	–
NDF	13	32	24	0	22	19	–
Base-buffering capacity[a]							
Whole sample	121	47	54	27	0	27	–
NDF	12	24	17	33	13	20	–

NDF, neutral detergent fibre.

[a]Microequivalents of HCl/NaOH required to lower/increase the pH of 0.5 g dry matter suspended in 50 ml water to pH 4/9, divided by total pH change.

lignin tends to increase with particle size, because the force required to shear the fibrous particles increases with lignification (Van Soest, 1994).

Some PCW constituents, such as β-glucans, pentosans and pectins, are hydrophilic, tending to form gels in solution, whereas lignin is more hydrophobic. These gel-forming substances may increase digesta viscosity, as observed with sugarbeet pulp (Volek *et al.*, 2005), but their consequences have not been studied in rabbits. On the other hand, hydration is negatively related to the size of the fibre particles (Van Soest, 1994).

The cation-exchange capacity of fibre is dependent on its concentration of carboxyl, amino and hydroxyl groups (Van Soest *et al.*, 1991). Accordingly, buffering capacity is high in feeds containing pectins (e.g. 70 mEq 100 g^{-1} for beet pulp) and is also significantly higher in legumes than in grasses (50 versus 13 mEq 100 g^{-1}). This is in agreement with the lower buffering capacity of the insoluble fibre fraction (NDF) compared to the complete raw material (Table 5.3). The type of fibre would interact with the acidity of caecal contents, as the base-buffering capacity of fibrous sources is positively related to that of dry caecal content, which is also related to the pH of dry caecal content (García *et al.*, 2000a).

5.3 Degradation of Dietary Fibre in the Rabbit Digestive Tract

5.3.1 Precaecal digestion of fibre

Traditionally, fermentation of DF has been considered to be a post-ileal activity of the endogenous microbiota. However, there is evidence that some components of structural carbohydrates are degraded prior to entering the caecum of rabbits. This has also been observed in other non-ruminant species such as pigs and poultry.

The extent of precaecal fibre digestion in rabbits varies from 0.07 to 0.19 for crude fibre (Yu *et al.*, 1987), from 0.05 to 0.43 for NDF (Gidenne and Ruckebusch, 1989; Merino and Carabaño, 1992) and from 0 to 0.37 for NSP (Gidenne, 1992; Carabaño *et al.*, 2001). It must be pointed out that the values obtained using NDF and crude fibre with respect to those obtained with NSP might be overestimated due to solubilization and filtration of cell wall components that would be considered digested. When NSPs have been analysed, arabinose and uronic acids, typical monomers of pectic substances, have been found to be largely digested before the ileum (from 0.2 to 0.5). On the other hand, glucose and xylose, the major monomers in most fibre sources, showed a much lower ileal digestibility (0–0.2). These results imply that around 0.4 (from 0.2 to 0.8) of total digestible fibre (including water-soluble NSP) is degraded before the caecum, which is similar to that observed in pigs (Bach Knudsen, 2001). This could be explained by the ‘remaining’ fibrolytic activity from microbiota (Gómez-Conde *et al.*, 2007, 2009) supplied by soft faeces, and observed in the stomach and small intestine (Marounek *et al.*, 1995) by the established microbiota.

5.3.2 Caecal digestion of fibre

Fibre degradation is ultimately determined by microbial activity, digesta retention time in the caecum and fibre chemical composition and structure.

Microbial activity

Most of the effects exerted by fibre on the rabbit digestive physiology depend on its hydrolysis and fermentation by the digestive microbiota. However, it is difficult to study the influence of any dietary component on the microbiota, as traditional cultivation techniques only recover around one-quarter of the intestinal microbiota. For this reason, other indirect techniques have been used, such as the volatile fatty acid (VFA) concentration, microbial nitrogen synthesized or fibrolytic activity. The caecal microbial population secretes enzymes capable of hydrolysing the main components of DF. Greater enzymatic activity for degrading pectins and hemicelluloses than for degrading cellulose has been detected in several studies (Marounek *et al.*, 1995; Jehl and Gidenne, 1996; Falcão e Cunha *et al.*, 2004). These results are parallel to the faecal digestibility of the corresponding DF constituents in rabbits (Table 5.4), and are also consistent with the smaller counts of cellulolytic bacteria in the rabbit caecum compared with xylanolytic or pectinolytic bacteria (Boulahrouf *et al.*, 1991).

The dietary factors affecting the variability of fibrolytic activity have not been extensively studied, but it seems that low-fibre diets might reduce or have no effect on fibrinolytic activity at the caecum (Gidenne *et al.*, 2000, 2002). The type of fibre influences fibrolytic activity, with sugarbeet pulp increasing caecal pectinolytic and cellulolytic activity, and wheat-bran-based diets increasing xylanolytic activity (Falcão e Cunha *et al.*, 2004).

In many situations, indirect methods do not seem to reflect adequately the changes produced in the microbiota population (Abecia *et al.*, 2007). The development of new molecular tools to characterize intestinal microbiota will improve our knowledge of nutrition and digestive microbiota functions (Gómez-Conde *et al.*, 2007, 2009; Michelland *et al.*, 2010). Table 5.5 presents a summary of some recent results obtained with restriction fragment length polymorphism.

Table 5.4. Mean apparent faecal digestibility coefficient of dietary fibre in experimental diets.[a]

Class of dietary fibre	n[b]	Mean	Range
Neutral detergent fibre (aNDFom)	127	0.34	0.03–0.71
Uronic acids	7	0.58	0.30–0.85
Hemicelluloses (aNDFom – ADF)	127	0.46	0.00–0.82
Cellulose (ADFom – ADL)	52	0.27	0.01–0.59
Lignin (ADL)	34	0.11	–0.08 to 0.25

ADFom, acid detergent fibre expressed exclusive of residual ash; ADL, acid detergent lignin; aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed free of ash.
[a]Falcão e Cunha and Lebas (1986), Gidenne (1987, 1992), Fraga *et al.* (1991), Gidenne *et al.* (1991b, 1998, 2000, 2001, 2002, 2004a, b), García *et al.* (1992, 1993, 1999, 2000b, 2002b), Gidenne and Perez (1993, 1994, 2000), Perez (1994), Bellier and Gidenne (1996), Gidenne and Jehl (1996), Motta-Ferreira *et al.* (1996), Carabaño *et al.* (1997), Falcão e Cunha *et al.* (1998, 2000, 2004), Gidenne and Bellier (2000), Volek *et al.* (2005, 2007).
[b]n = number of diets.

Table 5.5. Effect of type of diet on biodiversity, frequency of detection of some bacteria and mortality in rabbits 10 days after weaning (Nicodemus *et al.*, 2004; Gómez-Conde *et al.*, 2007, 2009).

	Site	Decrease in fibre level (300 versus 250 g NDF kg^{-1})	Increase in particle size (normal versus large)	Increase in soluble fibre
Biodiversity	Ileum	Increase	Decrease	No effect
	Caecum	Decrease	Decrease with 250 g NDF kg^{-1}	No effect
Clostridium perfringens	Ileum	No effect	No effect	No effect
	Caecum	No effect	No effect	Decrease
Other bacteria	Ileum	Increase *Bacteroides*	Decrease *Escherichia coli*, *Helicobacter*, *Yersinia* Increase *Ruminococcus*	Decrease *Campylobacter*
	Caecum	Decrease *Bacteroides*, *Ruminococcus*	Decrease *Helicobacter*, *Propionibacterium*	Decrease *Campylobacter*
Mortality	–	Increase	No effect	Decrease

NDF, neutral detergent fibre.

Fermentation time

The retention time of digesta in the caeco-colic segments can be estimated from the difference in ileo-rectal mean retention time (i-r MRT, h) and minimal transit time (TTm, h) obtained using ileally cannulated animals. The latter value is relatively constant with a range from 3.5 to 4.5 h, averaging 3.7 h (Gidenne, 1994; García *et al.*, 1999). Several studies (Gidenne *et al.*, 1991b; Gidenne and Perez, 1993; Gidenne, 1994; García *et al.*, 1999) have measured the i-r MRT for diets based on lucerne hay, wheat bran and fibrous by-products. This trait was linear and negatively correlated with dietary NDF content, which varied from 220 to 470 g kg^{-1} (DM basis). The regression equation obtained was:

$$\text{i–r MRT} = 26.5(\pm 4.9) - 0.0368(\pm 0.015)\ \text{NDF (DM)},\ R^2 = 0.35,\ n = 13,\ P = 0.03.$$

According to this equation, the i-r MRT of an average rabbit diet containing 360 g NDF kg^{-1} diet DM would be 13.2 h. The time of fermentation (i.e. retention in the caecum and proximal colon) could be estimated as 9.5 h (13.2–3.7 h).

Ileo-rectal MRT is also related to the weight of the caecal contents (CCW as a proportion of body weight; $P = 0.04$), according to studies where both traits have been determined (Gidenne, 1992; García *et al.*, 1999, 2000a). CCW is easier to determine than retention time, and the amount of information available in the literature is much larger. Some results are shown in Fig. 5.4, where CCW has been related to dietary NDF content, but the type of fibre (particle size and lignin content) also influences CCW (Nicodemus *et al.*, 1999, 2006). Sampling time significantly affects this trait (see Chapter 1), so that only results obtained using the same methodology have been chosen (García *et al.*, 2002b). The response shown in this figure indicates that, when a wide range of level and sources of fibre is considered, the dietary NDF content exerts a quadratic effect on CCW. From this equation, CCW is minimal for a dietary NDF content of 393 g kg^{-1} DM. The additional effect of the degree of lignification of NDF on CCW indicates an additional influence of source of fibre. Diets containing low-lignified beet pulp or high-lignified sunflower husk tended to give, respectively, higher and lower CCW values at the same NDF level.

Another factor related to fermentation time is particle size. As was observed by Björnhag (1972), the particle size of fibre influences the entry of digesta in the caecum. Also, Gidenne (1993) observed that particles <0.3 mm were retained for longer (≥10 h, on average) than particles >0.3 mm.

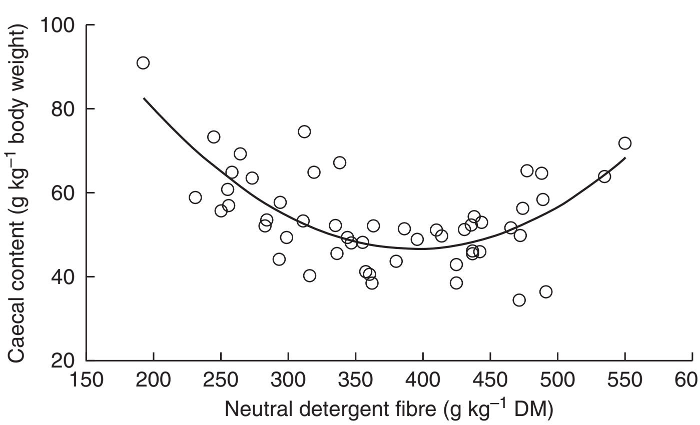

Fig. 5.4. Effect of dietary neutral detergent fibre (NDF) content on the weight of caecal contents (CCW) (reviewed by García *et al.*, 2002b). (CCW = 19.1 [±2.0] – 0.070 NDF[±0.11] + 0.000089[±0.0015] NDF^2 – 0.0031 [±0.0091] ADL, $n = 52$, $R^2 = 0.57$, $P < 0.001$). ADL, acid detergent lignin; DM, dry matter.

Digestion rate

The rate of fermentation of PCW constituents is a primary factor influencing their digestion efficiency in rabbits, because of the relatively short mean retention time (about 10 h) within the fermentative region. The caecal microbial NDF degradation rate may be derived from *in situ* rumen measurements (García *et al.*, 1995; Escalona *et al.*, 1999). From these data, it can be concluded that the relative value of fibre is highly dependent on the time of fermentation. For instance, paprika meal has a relatively high degradation rate at 10 h and a low degradation rate at 72 h, whereas the opposite occurs for NaOH-treated wheat straw.

The PCW composition is the main factor affecting the degradation rate. Hemicelluloses show a higher digestibility than cellulose (Table 5.4) and accordingly these diets contained higher average levels of digestible hemicelluloses than cellulose (68 versus 47 g kg^{-1}, respectively). However, it must be stated that around one-third of the diets contained higher digestible cellulose content than digestible hemicelluloses. Lignin and cutin are considered almost totally undegradable, although positive values for lignin digestibility have been obtained that might indicate a solubilization rather than digestion. Lignin is covalently linked to hemicelluloses (Van Soest, 1994) and, consequently, the degree of lignification of NDF negatively affects the level of digestible hemicelluloses ($n = 127$; $r = -0.57$; $P < 0.001$), but not that of cellulose. The two raw materials that increase the digestible hemicellulose level in the diet are sugar-beet pulp (low lignified and with a high hemicellulose to cellulose ratio of 1.1 compared to lucerne at 0.4) and wheat bran (with the highest hemicellulose to cellulose ratio at 3.2). Uronic acids, an important constituent of pectins (and, depending on the source of fibre, also of hemicelluloses) and more soluble than other cell wall components, are the substrate more easily fermented (Table 5.4). This would suggest that other components of soluble fibre (e.g. pentosans, mannans, galactans) might have a similar or even higher degradability than uronic acids.

The faecal NDF digestibility of several fibrous feedstuffs is presented in Table 5.6. The highest value (0.845) was obtained for beet pulp. Fibre in this feed is poorly lignified and would have a lengthy fermentation time in the caecum. The lowest NDF digestibilities (0.086 and 0.100) were found for grape-seed meal and sunflower husk. Both of these are highly lignified (590 and 210 g kg^{-1}), and the latter has a low proportion (0.262) of fine particles (<0.3 mm). Similar NDF digestibility was observed for paprika meal and soybean hulls. The lower lignin content in soybean hulls (150 versus 210 g kg^{-1}, respectively) was compensated for by its lower proportion of fine particles (0.932 versus 0.469, respectively) and a shorter fermentation time.

Fibre digestibility is frequently not affected by the DF concentration. In fact, it may be concluded that the quantity of fibre entering the caecum is not a limiting factor for the fermentation processes as the digesta retention time in the caecum is relatively short, allowing, predominantly, degradation

Table 5.6. Neutral detergent fibre digestibility (NDFd) of several feedstuffs in rabbits.

Feedstuff	NDFd	Reference
Dehydrated lucerne	0.15–0.18	Gidenne *et al.* (1991a)
Dehydrated lucerne ($n = 12$)	0.255–0.407	Perez (1994)
Lucerne hay ($n = 6$)	0.175–0.276	García *et al.* (1995, 1999)
Beet pulp	0.845	Gidenne (1987)
Paprika meal	0.351	García *et al.* (1999)
Soybean hulls	0.282	García *et al.* (1999)
Sunflower husk	0.100	García *et al.* (1999)
Barley straw, NaOH-treated	0.167	García *et al.* (1999)
Grape-seed meal	0.086	García *et al.* (2002a)

of the more easily digestible fibre fractions such as pectins and hemicelluloses.

Fermentation pattern

VOLATILE FATTY ACIDS. VFAs are the main products of carbohydrate microbial fermentation. Their concentration in the caecum has been used as an indirect estimation of microbial activity, although great dietary changes are required to significantly modify VFAs due to their high variability. VFAs are rapidly absorbed in the hindgut and provide a regular source of energy for the rabbit. Butyrate seems to be a preferential source of energy for the hindgut, whereas acetate is mainly metabolized in the liver for lipogenesis and cholesterogenesis (Vernay, 1987). Furthermore, VFAs have been suggested to enhance colon mucosal growth (Chiou *et al.*, 1994). Although VFAs have been proposed as a protective factor against pathogen microbiota (*Escherichia coli*) infections *in vitro* (Prohaszka, 1980; Wallace *et al.*, 1989), no clear effects have been observed in rabbits *in vivo* (Gidenne and Licois, 2005).

Carbohydrate uptake by the gut microbiota includes most of the cell wall constituents, in addition to starch not digested in the small intestine and endogenous mucopolysaccharides. Additionally, protein residues from the ileal digesta (undigested dietary protein, mucosal-cell protein, enzymes) can be utilized (after deamination) as an energy source for the microbial population. In this way, Vernay and Raynaud (1975) observed a caecal VFA concentration of 17.8 mmol l^{-1} in fasted rabbits, suggesting that a significant amount of endogenous materials can be fermented in the caecum. In fact, a decrease of ileal protein digestibility increased caecal acidity, suggesting an important role of protein in caecal fermentation (Gidenne and García, 2006). However, the relative contribution of these sources to total caecal VFA production is unknown.

The caecal VFA concentration determined in 78 experimental diets averaged 57.4 mmol l^{-1} and ranged from 18.1 to 99.8 mmol l^{-1}. It increased with dietary uronic acid and NDF levels and decreased with the degree of lignification of NDF (García *et al.*, 2002b). Chiou *et al.* (1994), using isolated components of DF (cellulose, pectins and lignin) and lucerne hay, also observed a negative effect of lignin on caecal VFA concentration.

The caecal VFA profile is specific to the rabbit, with a predominance of acetate (77 mmol 100 ml^{-1} on average, range 65–87 mmol 100 ml^{-1}), followed by butyrate (17 mmol 100 ml^{-1} on average, range 6–28 mmol 100 ml^{-1}) and then propionate (6 mmol 100 ml^{-1} on average, range 3–11 mmol 100 ml^{-1}). These molar proportions are affected by fibre level. For instance, the proportion of acetate increases and that of butyrate generally decreases significantly when the fibre level increases, whereas the propionic acid proportion is only positively correlated to dietary uronic acids concentration (García *et al.*, 2002b).

CAECAL PH. Caecal pH is frequently determined in digestion studies because it gives an estimation of the extent of the fermentation. Caecal pH is negatively related to both dietary uronic acid and digestible NDF concentrations (Fig. 5.5) (García *et al.*, 2002b), as it decreases with the inclusion of ingredients such as sugarbeet pulp, soy hulls and lucerne in the diet; the opposite occurs with cereal straw and grape-seed meal.

From a chemical point of view, caecal pH is expected to be related to the main sources of hydrogen ions, hydroxide ions, VFAs and ammoniacal nitrogen. However, a poor or absent relationship has been found between these variables, which only account for 12% of the variability observed in caecal pH (García *et al.*, 2002b). This may due to the presence of buffer substances in the caecum from endogenous or feed origin, which may also explain the stability of caecal pH among animals fed different diets. In fact, 68% of the variability of caecal pH is explained by the pH of the dry caecal contents (free from VFA and ammoniacal nitrogen), which is negatively related to the base-buffering capacity of the dry caecal contents (García *et al.*, 2000a).

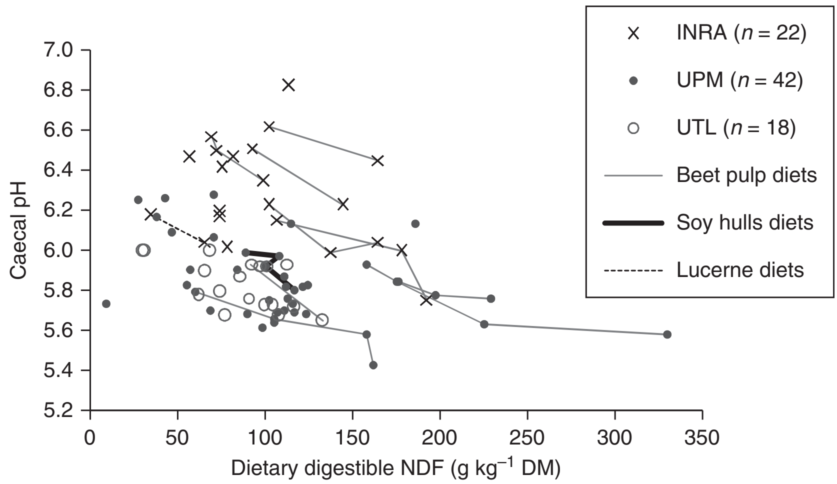

Fig. 5.5. Effect of dietary digestible neutral detergent fibre (NDF) and of increasing levels of sources of fibre rich in soluble fibre on caecal pH (reviewed by García *et al.*, 2002b). DM, dry matter; INRA, Institut National de la Recherche Agronomique; UPM, Universidad Politécnica de Madrid; UTL, Universidade Técnica de Lisboa.

References

Abecia, L., Fondevila, F., Balcells, J., Edwards, J.E., Newbold, C.J. and McEwan, N.R. (2007) Effect of antibiotics on the bacterial population of the rabbit caecum. *FEMS Microbiology Letters* 272, 144–153.

AOAC (Association of Official Analytical Chemists) (2000) *Official Methods of Analysis*, 17th edn. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Bach Knudsen, K.E. (2001) The nutritional significance of 'dietary fibre' analysis. *Animal Feed Science and Technology* 90, 3–20.

Barry, J.L., Hoebler, C., David, A., Kozlowski, F. and Gueneau, S. (1990) Cell wall polysaccharides determination: comparison of detergent method and direct monomeric analysis. *Sciences des Aliments* 10, 275–282.

Bellier, R. and Gidenne, T. (1996) Consequences of reduced fibre intake on digestion, rate of passage and caecal microbial activity in the young rabbit. *British Journal of Nutrition* 75, 353–363.

Björnhag, G. (1972) Separation and delay of contents in the rabbit colon. *Swedish Journal of Agricultural Research* 7, 105–114.

Boulahrouf, A., Fonty, G. and Gouet, P. (1991) Establishment, counts and identification of the fibrolytic bacteria in the digestive tract of rabbit. Influence of feed cellulose content. *Current Microbiology* 22, 1–25.

Brillouet, J.M., Rouau, X., Hoebler, C., Barry, J.L., Carré, B. and Lorta, E. (1988) A new method for determination of insoluble cell-walls and soluble non-starchy polysaccharides from plant materials. *Journal of Agricultural and Food Chemistry* 36, 969–979.

Carabaño, R., Motta-Ferreira, W., de Blas, J.C. and Fraga, M.J. (1997) Substitution of sugarbeet pulp for alfalfa hay in diets for growing rabbits. *Animal Feed Science and Technology* 65, 249–256.

Carabaño, R., García, J. and de Blas, C. (2001) Effect of fibre source on ileal apparent digestibility of non-starch polysaccharides in rabbits. *Animal Science* 72, 343–350.

Carré, B. and Brillouet, J.M. (1989) Determination of water-insoluble cell-walls in feeds: interlaboratory study. *Journal of the Association of Official Analytical Chemists* 72, 463–467.

Chiou, P.W.S., Yu, B. and Lin, C. (1994) Effect of different components of dietary fiber on the intestinal morphology of domestic rabbits. *Comparative Biochemistry and Physiology* 108A, 629–638.

De Vries, J.W. and Rader, J.I. (2005) Historical perspective as a guide for identifying and developing applicable methods for dietary fibre. *Journal of the AOAC International* 88, 1349–1366.

EGRAN (European Group on Rabbit Nutrition); Gidenne, T., Perez, J.M., Xiccato, G., Trocino, A., Carabaño, R., Villamide, M.J., Blas, E., Cervera, C., Falcão e Cunha, L. and Maertens, L. (2001) Technical note: attempts to harmonize chemical analyses of feeds and faeces for rabbit feed evaluation. *World Rabbit Science* 9, 57–64.

Englyst, H. (1989) Classification and measurement of plant polysaccharides. *Animal Feed Science and Technology* 23, 27–42.

Escalona, B., Rocha, R., García, J., Carabaño, R. and de Blas, C. (1999) Characterization of *in situ* fibre digestion of several fibrous foods. *Animal Science* 68, 217–221.

Falcão e Cunha, L. and Lebas, F. (1986) Influence chez le lapin adulte de l'origine et du taux de lignine alimentaire sur la digestibilite de la ration et l'importance de la caecotrophie. *4 emes Journees de la Reserche Cunicole*, Communication no. 8.

Falcão e Cunha, L., Ferreira, P. and Bengala Freire, J.P. (1998) Étude de l'effet de l'interaction fibre x lipides dans l'alimentation du lapin: croissance, digestibilité et paramètres fermentaires. *7emes Journees de la Recherche Cunicole,* 155–158.

Falcão e Cunha, L., Jorge, J., Freire, J.P. and Pérez, H. (2000) Fat addition to feeds for growing rabbits, differing in fiber level and nature: effects on growth rate, digestibility and caecal fermentation patterns. *World Rabbit Science* 8 (Suppl. 1, Vol. C), 191–198.

Falcão e Cunha, L., Peres, H., Freire, J.P.B. and Castro-Solla, L. (2004) Effects of alfalfa, wheat bran or beet pulp, with or without sunflower oil, on caecal fermentation and on digestibility in the rabbit. *Animal Feed Science and Technology* 117, 131–149.

FEDNA (2003) Tablas FEDNA de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos, 2ª ed. In: de Blas, J.C., Mateos, G.G. and Rebollar, P.G. (eds) *Fundación Española para el Desarrollo de la Nutrición Animal*. FEDNA, Madrid, Spain.

Fraga, M.J., Pérez, P., Carabaño, R. and de Blas, J.C. (1991) Effect of type of fiber on the rate of passage and on the contribution of soft feces to nutrient intake of finishing rabbits. *Journal of Animal Science* 69, 1566–1574.

García, G., Gálvez, J.F. and de Blas, J.C. (1992) Substitution of barley grain by sugar-beet pulp in diets for finishing rabbits. 2. Effect on growth performance. *Journal of Applied Rabbit Research* 15, 1017–1024.

García, G., Gálvez, J.F. and de Blas, J.C. (1993) Effect of substitution of sugarbeet pulp for barley in diets for finishing rabbits on growth performance and on energy and nitrogen efficiency. *Journal of Animal Science* 71, 1823–1830.

García, J., Pérez-Alba, L., Alvarez, C., Rocha, R., Ramos, M. and de Blas, C. (1995) Prediction of the nutritive value of lucerne hay in diets for growing rabbits. *Animal Feed Science and Technology* 54, 33–44.

García, J., Carabaño, R. and de Blas, J.C. (1999) Effect of fiber source on cell wall digestibility and rate of passage in rabbits. *Journal of Animal Science* 77, 898–905.

García, J., Carabaño, R., Pérez-Alba, L. and de Blas, C. (2000a) Effect of fiber source on cecal fermentation and nitrogen recycled through cecotrophy in rabbits. *Journal of Animal Science* 78, 638–646.

García, J., Nicodemus, N., Pérez-Alba, L., Carabaño, R. and de Blas, J.C. (2000b) Characterization of fibre digestion of grape seed meal and sunflower hulls in rabbits. I. Fibre digestibility and rate of passage. *World Rabbit Science* 8 (Suppl. 1, Vol. C), 225–231.

García, J., Nicodemus, N., Carabaño, R. and de Blas, J.C. (2002a) Effect of inclusion of defatted seed meal in the diet on digestion and performance of growing rabbits. *Journal of Animal Science* 80, 162–170.

García, J., Gidenne, T., Falcão e Cunha, L. and de Blas, C. (2002b) Identification of the main factors that influence caecal fermentation traits in growing rabbits. *Animal Research* 51, 165–173.

Gidenne, T. (1987) Effet de l'addition d'un concentré en fibres dans une ration à base de foin, distribuée à deux niveaux alimentaires chez la lapine adulte. 2. Mesures de digestiblité. *Reproduction Nutrition Development* 27, 801–810.

Gidenne, T. (1992) Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. *British Journal of Nutrition* 67, 133–146.

Gidenne, T. (1993) Measurement of the rate of passage in restricted-fed rabbits: effect of dietary cell wall level on the transit of fibre particles of different sizes. *Animal Feed Science and Technology* 42, 151–163.

Gidenne, T. (1994) Effets d'une reduction de la tener en fibres alimentaires sur le transit digestif du lapin. Comparaison et validation de modéles d'ajustement des cinétiques d'excrétion fécale des marqueurs. *Reproduction Nutrition Development* 34, 295–307.

Gidenne, T. and Bellier, R. (2000) Use of digestible fibre in replacement to available carbohydrates – effect on digestion, rate of passage and caecal fermentation pattern during the growth of the rabbit. *Livestock Production Science* 63, 141–152.

Gidenne, T. and García, J. (2006) Nutritional strategies improving the digestive health of the weaned rabbit. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Sciences*. ILVO, Belgium, pp. 229–238.

Gidenne, T. and Jehl, N. (1996) Replacement of starch by digestible fibre in the feed for the growing rabbit. 1. Consequences for digestibility and rate of passage. *Animal Feed Science and Technology* 61, 183–192.
Gidenne, T. and Licois, D. (2005) Effect of a high fibre intake on the resistance of the growing rabbit to an experimental inoculation with an enteropathogenic strain of *Escherichia coli*. *Animal Science* 80, 281–288.
Gidenne, T. and Perez, J.M. (1993) Effect of dietary starch origin on digestion in the rabbit. 2. Starch hydrolysis in the small intestine, cell wall degradation and rate of passage measurements. *Animal Feed Science and Technology* 42, 249–257.
Gidenne, T. and Perez, J.M. (1994) Apports de lignines et alimentation du lapin en croissance. I. Conséquences sur la digestion et le transit. *Annales de Zootechnie* 43, 313–322.
Gidenne, T. and Perez, J.M. (2000) Replacement of digestible fibre by starch in the diet of the growing rabbit. I. Effects on digestion, rate of passage and retention of nutrients. *Annales de Zootechnie* 49, 357–368.
Gidenne, T. and Ruckebusch, Y. (1989) Flow and passage rate studies at the ileal level in the rabbit. *Reproduction Nutrition Development* 29, 403–412.
Gidenne, T., Scalabrini, F. and Marchais, C. (1991a) Adaptation digestive du lapin à la teneur en constituants pariétaux du régime. *Annales de Zootechnie* 40, 73–84.
Gidenne, T., Carre, B., Segura, M., Lapanouse, A. and Gomez, J. (1991b) Fibre digestion and rate of passage in the rabbit: effect of particle size and level of lucerne meal. *Animal Feed Science and Technology* 32, 215–221.
Gidenne, T., Bellier, R. and van Eys, J. (1998) Effect of the dietary fibre origin on the digestion and on the caecal fermentation pattern of the growing rabbit. *Animal Science* 66, 509–517.
Gidenne, T., Pinheiro, V. and Falcão e Cunha, L. (2000) A comprehensive approach of the rabbit digestión: consequences of a reduction in dietary fibre supply. *Livestock Production Science* 64, 225–237.
Gidenne, T., Arveux, P. and Madec, O. (2001) The effect of the quality of dietary lignocelluloses on digestion, zootechnical performance and health of the growing rabbit. *Animal Science* 73, 97–104.
Gidenne, T., Jehl, N., Segura, M. and Michalet-Doreau, B. (2002) Microbial activity in the caecum of the rabbit around weaning: impact of a dietary fibre deficiency and of intake level. *Animal Feed Science and Technology* 99, 107–118.
Gidenne, T., Jehl, N., Lapanouse, A. and Segura, M. (2004a) Inter-relationship of microbial activity, digestion and gut health in the rabbit: effect of substituting fibre by starch in diets having a high proportion of rapidly fermentable polysaccharides. *British Journal of Nutrition* 92, 95–104.
Gidenne, T., Mirabito, L., Jehl, N., Perez, J.M., Arveux, P., Bourdillon, A., Briens, C., Duperray, J. and Corrent, E. (2004b) Impact of replacing starch by digestible fibre, at two levels of lignocellulose, on digestion, growth and digestive health of the rabbit. *Animal Science* 78, 389–398.
Gómez-Conde, M.S., García, J., Chamorro, S., Eiras, P., Rebollar, P.G., Pérez de Rozas, A., Badiola, I., de Blas, C. and Carabaño, R. (2007) Neutral detergent-soluble fibre improves gut barrier function in 25 d old weaned rabbits. *Journal of Animal Science* 85, 3313–3321.
Gómez-Conde, M.S., Pérez de Rozas, A., Badiola, I., Pérez-Alba, L., de Blas, J.C., Carabaño, R. and García, J. (2009) Effect of neutral detergent soluble fibre on digestion, intestinal microbiota and performance in twenty five day old weaned rabbits. *Livestock Science* 125, 192–198.
Hall, M.B. (2003) Challenges with nonfiber carbohydrate methods. *Journal of Animal Science* 81, 3226–3232.
Hall, M.B., Lewis, B.A., Van Soest, P.J. and Chase, L.E. (1997) A simple method for estimation of neutral detergent-soluble fibre. *Journal of the Science of Food and Agriculture* 74, 441–449.
Hall, M.B., Hoover, W.H., Jennings, J.P. and Webster, T.K.M. (1999) A method for partitioning neutral detergent-soluble carbohydrates. *Journal of the Science of Food and Agriculture* 79, 2079–2086.
Jehl, N. and Gidenne, T. (1996) Replacement of starch by digestible fibre in the feed for the growing rabbit. 2. Consequences for microbial activity in the caecum and on incidence of digestive disorders. *Animal Feed Science and Technology* 61, 193–204.
Lebas, F. and Lamboley, B. (1999) Liquid phase sifting determination of the size of particles contained in pelleted rabbits feeds. *World Rabbit Science* 7, 229–235.
Lee, S.C., Prosky, L. and De Vries, J.W. (1992) Determination of total, soluble and insoluble dietary fiber in foods-enzymatic-gravimetric method, MES-TRIS buffer: collaborative study. *Journal of the Association of Official Analytical Chemist* 72, 395–416.
Li, B.W. (1995) Determination of total dietary fiber in foods and food products by using a single-enzyme, enzymatic-gravimetric method: interlaboratory study. *Journal of the AOAC International* 78, 1440–1444.

Marounek, M., Vovk, S.J. and Skrinova, V. (1995) Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *British Journal of Nutrition* 73, 463–469.
McDougall, G.J., Morrison, I.M., Stewart, D. and Hillman, J.R. (1996) Plant cell walls as dietary fibre: range, structure, processing and function. *Journal of the Science of Food and Agriculture* 70, 133–150.
Merino, J. and Carabaño, R. (1992) Effect of type of fibre on ileal and faecal digestibilities. *Journal of Applied Rabbit Research* 15, 931–937.
Mertens, D.R. (2002) Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of the AOAC International* 85, 1217–1240.
Mertens D.R. (2003) Challenges in measuring insoluble dietary fibre. *Journal of Animal Science* 81, 3233–3249.
Michelland, R.J., Combes, S., Monteils, V., Cauquil, L., Gidenne, T. and Fortun-Lamothe, L. (2010) Molecular analysis of the bacterial community in digestive tract of rabbit. *Anaerobe* 16, 61–65.
Morisse, J.P. (1982) Taille des particules de l'aliment utilisé chez le lapin. *Revue Médecine Vétérinaire* 133, 635–642.
Morrison, F.B. (1956) *Feeds and Feeding*, 22nd edn. Morrison Publishing Co., Ithaca, New York, USA.
Motta-Ferreira, W., Fraga, M.J. and Carabaño, R. (1996) Inclusion of grape pomace, in substitution for lucerne hay, in diets for growing rabbits. *Animal Science* 63, 167–174.
Nicodemus, N., García, J., Carabaño, R. and de Blas, C. (1999) Performance response of lactating and growing rabbits to dietary lignin content. *Animal Feed Science and Technology* 80, 43–54.
Nicodemus, N., Pérez Alba, L., Carabaño, R., de Blas, C., Badiola, I., Pérez de Rozas, A. and Garcia, J. (2004) Effect of level of fibre and level of ground of fibre sources on digestion and ileal and caecal characterization of microbiota of early weaned rabbits. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, volume 4, pp. 928–929.
Nicodemus, N., García, J., Carabaño, R. and de Blas, C. (2006) Effect of a reduction of dietary particle size by substituting a mixture of fibrous by-products for lucerne hay on performance and digestión of growing rabbits and lactating does. *Livestock Science* 100, 242–250.
Perez, J.M. (1994) Digestiblité et valeur energetique des luzernes deshydratées pour le lapin: influence de leur composition chimique et de leur technologie de preparation. In: *Vièmes Journèes de la Recherche Cunicole, La Rochelle*, Vol. 2, pp. 355–364.
Prohaszka, L. (1980) Antibacterial effect of volatile fatty acids in enteric *E. coli* infections of rabbits. *Zentrabl Veterinaermed Reihe* B 27, 631–639.
Robertson, J.B. and Van Soest, P.J. (1981) The detergent system of analysis. In: James, W.P.T. and Theander, O. (eds) *The Analysis of Dietary Fibre in Food*. Marcel Dekker, New York, USA, pp. 123–158.
Selvendran, R.R., Stevens, B.J.H. and Du Pont, M.S. (1987) Dietary fiber: chemistry, analysis and properties. In: Chixhester, C.O. (ed.) *Advances in Food Research*. Academic Press, New York, USA, pp. 117–212.
Uden, P., Robinson, P.H. and Wiseman, J. (2005) Use of detergent system terminology and criteria for submission of manuscripts on new, or revised, analytical methods as well as descriptive information on feed analysis and/or variability. *Animal Feed Science and Technology* 118, 181–186.
Van Soest, P.J. (1994) *Nutritional Ecology of the Ruminant*, 2nd edn. Cornell University Press, Ithaca, New York, USA.
Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991) Methods for dietary fiber, neutral detergent fiber and on-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597.
Vernay, M. (1987) Origin and utilization of volatile fatty acids and lactate in the rabbit: influence of the faecal excretion pattern. *British Journal of Nutrition* 57, 371–381.
Vernay, M. and Raynaud, P. (1975) Répartition des acides gras volatils dans le tube digestif du lain domestique. II. Lapin soumis au jeûne. *Annales Recherches Vétérinaries* 6, 369–377.
Villamide, M.J., Carabaño, R., Maertens, L., Pascual, J., Gidenne, Falcão e Cunha, L. and Xiccato, G. (2009) Prediction of the nutritional value of European compound feeds for rabbits by chemical components and *in vitro* analysis. *Animal Feed Science and Technology* 150, 283–294.
Volek, Z., Marounek, M and Skrivanová, V. (2005) Replacing starch by pectin and inulin in diet of early-weaned rabbits: effect on performance, health and nutrient digestibility. *Journal of Animal and Feed Sciences* 14, 327–337.
Volek, Z., Marounek, M. and Skrivanová,V. (2007) Effect of a starter diet supplementation with mannan-oligosaccharide or inulin on health status, caecal metabolism, digestibility of nutrients and growth of early weaned rabbits. *Animal* 1, 523–530.

Wallace, R.J., Falconer, M.L. and Bhargava, P.K. (1989) Toxicity of volatile fatty acids at rumen pH prevents enrichment of *Escherichia coli* by sorbitol in rumen contents. *Current Microbiology* 19, 277–281.

Wiseman, J., Villamide, M.J., de Blas, C., Carabaño, M.J. and Carabaño, R.M. (1992) Prediction of the dietary energy value of feeds for rabbits. *Animal Feed Science and Technololgy* 39, 27–38.

Xiccato, G., Carazzolo, A., Cervera, C., Falcão e Cunha, L., Gidenne, T., Maertens, L., Perez, J.M. and Villamide, M.J. (1996) European ring-test on the chemical analyses of feed and faeces: influence on the calculation of nutrient digestibility in rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse,* Vol., 1. Association Française de Cuniculture, Lempdes, France, pp. 293–297.

Xiccato, G., Trocino, A., De Boever, J.L., Maertens, L., Carabaño, R., Pascual, J.J., Perez, J.M., Gidenne, T. and Falcão e Cunha, L. (2003) Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). *Animal Feed Science and Technology* 104, 153–168.

Yu, B., Chiou, P.W.S., Young, Ch.L. and Huang, H.H. (1987) A study of ratty T-type cannule and its ileal digestibilities. *Journal of the Chinese Society of Animal Science* 16, 73–81.

6 Energy and Protein Metabolism and Requirements

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6.1 Energy Units and their Measurement

Energy is the potential ability to produce work and the Joule (J), with its multiples, is the international unit used to measure all forms of energy, including feed energy. The standard calorie (cal), equivalent to the energy cost of increasing 1 g of distilled water by 1°C, is commonly used in practice to measure energy and may be converted into J by multiplying by 4.184.

In the nutrition and feeding of rabbits, as in other species, the following energy parameters are used to express energy requirements and the nutritive value of feeds: gross energy (GE), digestible energy (DE), metabolizable energy (ME) and net energy (NE) (Fig. 6.1).

6.1.1 Gross energy

GE is the quantity of chemical energy lost as heat when organic matter is completely oxidized, forming water and carbon dioxide as the main products. In feed, the GE content depends on the chemical composition of the organic matter: the caloric values of the individual components are approximately 22–24 kJ g^{-1} for crude protein, 38–39 kJ g^{-1} for ether extract and 16–17 kJ g^{-1} for carbohydrates. The GE concentration in complete diets or raw materials does not provide any useful information on the availability and utilization of dietary energy by the animal. For this reason, it is not a relevant unit in the energy evaluation of feeds or of animal energy requirements.

6.1.2 Digestible energy

DE can be measured *in vivo* by subtracting the quantity of energy recovered in the faeces from GE; in other words, the energy of undigested nutrients. In compound feeds for rabbits, the DE usually varies from 0.50 to 0.80 of the GE and offers a sufficiently precise estimation of the energy value of feeds.

6.1.3 Metabolizable energy

ME is calculated from DE by subtracting the energy loss associated with urine (UE) and intestinal fermentation gases (GasE), primarily methane. In ruminants, GasE accounts for an important part of DE. In rabbits, however, it is practically negligible, as is the heat loss from caecal fermentation. On the other hand, the energy loss in urine is substantial and depends on feed protein concentration (Maertens *et al.*, 2002; Xiccato *et al.*, 2007). Nitrogen losses (and consequently the energy

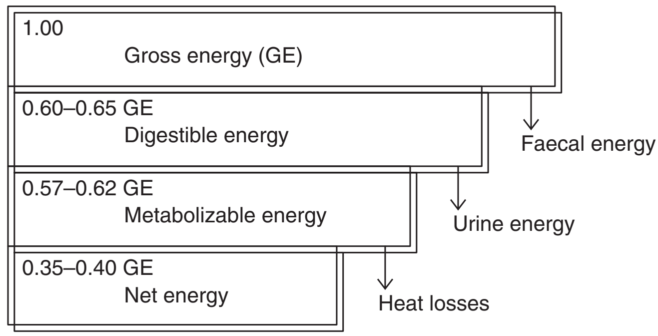

Fig. 6.1. Energy utilization of feeds by rabbits.

associated with urea and other nitrogen catabolites) increase as dietary protein increases. The UE can be calculated from the quantity of nitrogen excreted daily in the urine (UN) (Parigi Bini and Cesselli, 1977):

$$\text{UE (kJ day}^{-1}) = 51.76 \text{ UN (g day}^{-1}) - 3.01 \quad \text{residual standard deviation (RSD)} = 3.93,\ r = 0.90$$

6.1.4 Net energy

NE is the fraction of GE that is actually utilized by the animal for maintenance and productive purposes and is, therefore, the most precise estimate of feed energy value and animal energy requirements. The actual feed value within an NE unit is related to the specific energy utilization: NE for maintenance (NE_m), growth (NE_g), milk production (NE_l) and so on.

The experimental development of an NE system for rabbits (which proved extremely complicated and expensive) was abandoned some time ago (Parigi Bini *et al.*, 1974, 1978), but the choice between the DE and ME systems is still under discussion. ME is preferred for poultry, because birds excrete urine and faeces together, but the collection and measurement of urine energy values in rabbits is difficult and expensive. Although ME is more precise than DE, urinary energy losses are closely linked to the total intake of digestible protein (DP). Therefore, in common compound diets with 120–150 g DP kg^{-1}, DE and ME are closely correlated and ME can be easily estimated as 0.95 DE (Parigi Bini and Cesselli, 1977; Partridge *et al.*, 1986b; Ortiz *et al.*, 1989; Santomá *et al.*, 1989). For a more precise estimation of the ME concentration of diets (or raw materials), the following equations can be utilized to calculate the ME content for nitrogen equilibrium (Maertens *et al.*, 2002):

$$\text{ME (MJ kg}^{-1}) = \text{DE (MJ kg}^{-1}) \times \text{ME/DE}$$

where:

$$\text{ME/DE} = 0.995 - 0.0048 \times \text{DP (g kg}^{-1})/\text{DE (MJ kg}^{-1})$$

For the reasons outlined above, DE values are still commonly used both in studies on energy metabolism and in practical rabbit feeding. Since GE digestibility is closely correlated with dry matter (DM) digestibility, an international standardized method (Perez *et al.*, 1995) capable of offering repeatable and reproducible measurements of *in vivo* DM digestibility (and consequently energy digestibility) has been adopted by the scientific community.

6.2 Methods for Estimating Energy Requirements

The principal methods used for the measurement of energy requirements (for further details see Blaxter, 1989; Webster, 1989; Close, 1990) are as follows:

1. Long-term feeding experiments, carried out to establish the energy needed to maintain constant live weight or, on the other hand, to measure the variations in live weight (or milk production or fetal growth) induced by a certain quantity of energy. These experiments permit the breeding of high numbers of animals for long periods under conditions similar to those on farms. However, they do not provide any useful information on the body composition changes that affect rabbits during growth, lactation or pregnancy.

2. Calorimetric methods, which measure the heat lost by animals. Measurement is direct (direct calorimetry) when calorimeters are used and indirect (indirect calorimetry) when based on the gaseous exchanges measured in respiratory chambers of various types (open or closed circuit). These methods allow the direct measurement of ME intake (MEI) and energy lost as heat (HE). The retained energy (RE) in either the body or products (e.g. milk, fetal body, wool) is calculated by the difference (RE = ME – HE). Calorimetric methods require very complex and expensive equipment and can be utilized on only a few animals and in short-term experiments. Measurements are highly accurate and repeatable on the same animal in subsequent moments, but scarcely comparable to those obtained under practical rearing conditions. In addition, as in feeding experiments, calorimetric methods do not permit the identification of the origin of heat lost from different physiological functions (e.g. whether from feed digestion or body tissue utilization) or the partition of RE (e.g. energy retained in maternal or fetal tissues).

3. Comparative slaughter technique, which measures the variation of the energy contained in the body. This method constitutes the basis of the California Net Energy System developed for beef cattle (Lofgreen and Garrett, 1968) and has been largely applied to rabbits (Parigi Bini *et al.*, 1974, 1978, 1990a, 1992; Parigi Bini and Xiccato, 1986; Partridge *et al.*, 1989; Xiccato *et al.*, 1992b, 1995, 2004, 2005b; Fortun *et al.*, 1993; Nizza *et al.*, 1995; Pascual *et al.*, 2000b). This technique allows the direct measurement of MEI and RE, while HE is calculated by difference (HE = ME – RE). Body energy change is measured by first analysing the empty body (EB = live body – gut contents) of a reference group of animals (initial slaughter group). A second group of animals is fed a diet in which the ME (or DE) content is measured experimentally and their EBs are then analysed (final slaughter group). In growing rabbits, the RE is calculated by subtracting the body energy found in the final group from the body energy in the initial group – that is, the RE for growth (RE_g). Similarly, the energy excreted in the milk of lactating does (E_{milk}) and/or retained in the fetuses in pregnant does (E_{fetus}) can be measured during the entire lactation or pregnancy. The comparative slaughter technique is based on the assumption that the body composition of the initial slaughter group is very similar to the body composition of the final slaughter group at the beginning of the experiment. The difference in body composition at the beginning and the end of the experiment is a good estimate of RE when the animals in the initial and the final groups are homogeneous, their number is relatively high and the length of experiment is long enough (e.g. a complete growing period or an entire pregnancy or lactation) (Close, 1990). Comparative slaughter thus permits the partition of RE between the energy retained (or lost) as protein (RE_p) and fat (RE_f).

4. Non-destructive methods for body composition measurement, which measure the variation of body composition and then body RE without slaughter. Several methods have been proposed for rabbits, including dilution methods, nuclear magnetic resonance, computerized tomography and total body electrical conductivity (as reviewed by Fekete, 1992; Pascual *et al.*, 2006). These methods often need very expensive equipment and their efficacy is not completely proven. More recently, ultrasound scanning of perirenal fat thickness has been used to measure body-fat changes in the same animal during the reproductive period (Pascual *et al.*, 2000a, 2002b, 2004; Castellini *et al.*, 2003, 2006; Cardinali *et al.*, 2008), although with varying success depending on the doe's physiological state.

6.3 Energy Metabolism and Requirements

Several factors influence the energy metabolism and consequently the energy requirements in rabbits. The most important are: (i) body size, which depends on breed, age and sex; (ii) vital and productive functions, such as maintenance, growth, lactation and pregnancy; and (iii) environment (i.e. temperature, humidity, air speed).

Only those aspects of energy metabolism related to vital and productive functions are discussed here.

6.3.1 Voluntary feed and energy intake

Appetite in rabbits is mostly regulated by a chemostatic mechanism. Because of this, the total quantity of energy ingested daily tends to be constant. Growing rabbits in good sanitary conditions naturally consume sufficient feed to meet their energy requirements. However, reproducing does have high energy requirements for pregnancy, lactation and concurrent pregnancy and lactation that are often not covered by an adequate voluntary intake.

Voluntary energy intake is proportional to metabolic live weight ($LW^{0.75}$). In growing rabbits, voluntary intake is about 900–1000 kJ DE day^{-1} kg^{-1} $LW^{0.75}$ and chemostatic regulation appears only with a DE concentration in the diet of >9 MJ kg^{-1} (Lebas *et al.*, 1984; Partridge, 1986; Cheeke, 1987; Parigi Bini, 1988; Lebas, 1989; Santomá *et al.*, 1989). Below this level a physical-type regulation is prevalent, which is linked to the filling of the gut with dietary material (Fig. 6.2). Reproducing females can ingest on average 1100–1300 kJ DE day^{-1} kg^{-1} $LW^{0.75}$ during lactation, with the lowest value recorded by primiparous females (Maertens and De Groote, 1988; Lebas, 1989; Parigi Bini *et al.*, 1990b, 1992; Xiccato *et al.*, 1992b, 1995, 2004; Pascual *et al.*, 1998), and have a different energetic limit of chemostatic regulation compared to growing rabbits. An increase in DE concentration >9–9.5 MJ kg^{-1} permits a further increase in the daily energy intake of lactating females (Maertens and De Groote, 1988; Fraga *et al.*, 1989; Castellini and Battaglini, 1991; Xiccato *et al.*, 1995; Pascual *et al.*, 1998, 2000b). In these animals, the regulation limit probably varies by around 10.5–11 MJ kg^{-1} and also depends on the dietary energy source, tending to be higher in added-fat diets than in high-starch diets (Fraga *et al.*,

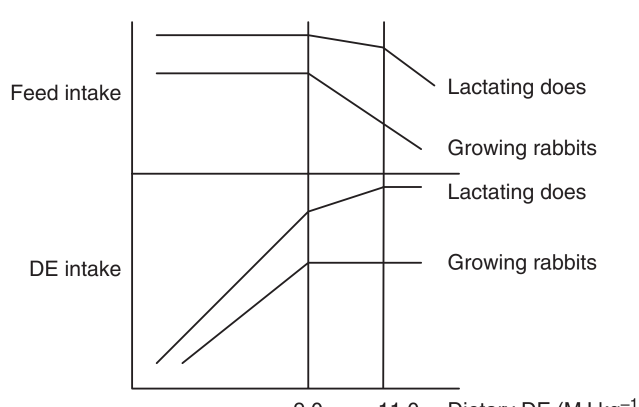

Fig. 6.2. Influence of dietary digestible energy (DE) concentration on voluntary feed and energy intake in lactating does and growing rabbits.

1989; Castellini and Battaglini, 1991; Xiccato *et al.*, 1995; Fortun-Lamothe and Lebas, 1996; Pascual *et al.*, 1998, 1999a, b, 2000b).

6.3.2 Energy for maintenance and efficiency of energy utilization

By varying the quantity of DE or MEI, it is possible to modify both the quantity of RE in the bodies of growing rabbits (RE_g) and the quantity of energy excreted in the milk (E_{milk}) or retained in the fetal tissues (E_{fetus}).

When the energy system is based on ME units, ME requirement for maintenance (ME_m) is the MEI that permits the maintenance of energy equilibrium in the body ($RE_g = 0$).

Once the needs for maintenance have been covered, the efficiency of utilization (k) of MEI is different for energy retained in the body tissues ($k_g = RE_g/MEI$), milk ($k_l = E_{milk}/MEI$) or fetal tissues ($k_{fetus} = E_{fetus}/MEI$).

When the energy system is based on DE, as with rabbits, energy retained in the body, milk or fetal tissue can be related to DE intake (DEI) instead of MEI. In different studies on rabbit energy metabolism, the efficiencies of utilization of DEI for maintenance, growth, lactation or pregnancy (RE/DEI) have been estimated instead of the k coefficients (de Blas *et al.*, 1985; Parigi Bini and Xiccato, 1986; Partridge *et al.*, 1989; Xiccato *et al.*, 1995). Assuming a constant ratio of ME = 0.95 DE, the efficiency of DEI utilization can be easily transformed into k values by dividing by 0.95. For example, using data from de Blas *et al.* (1985):

$$k_g = RE_g/DEI/0.95 = 0.53/0.95 = 0.56$$

Table 6.1 summarizes average values from the literature for energy requirements for the maintenance and efficiency of energy utilization for different categories of rabbits.

6.3.3 Energy requirements for maintenance

In all animals, energy losses for maintenance (basal metabolism and voluntary activity) are related to metabolic weight and physiological state.

Different estimates of DE requirements for the maintenance (DE_m) of growing rabbits have been found (see previous reviews of Parigi Bini, 1988 and Lebas, 1989), varying

Table 6.1. Digestible energy requirements for the maintenance (DE_m) and efficiency of utilization of DE intake (DEI) and body energy reserves.

	Young rabbits	Pregnant does	Lactating does	Pregnant and lactating does	Non-reproducing does and bucks
DE_m (kJ day^{-1} kg^{-1} LW$^{0.75}$)	430	430	430	470	400
Efficiency of energy utilization:					
Body retained energy (RE_g/DEI)	0.50	0.50	–	–	0.50
RE as protein (RE_p/DEI)	0.40	–	–	–	–
RE as fat (RE_f/DEI)	0.65	–	–	–	–
RE as fetuses (E_{fetus}/DEI)	–	0.30	–	0.30	–
Milk energy from DEI (E_{milk}/DEI)	–	–	0.65	0.65	–
Milk energy from doe body reserves (E_{milk}/RE_r)	–	–	0.80	0.80	–

LW, live weight; RE, retained energy.

Table 6.2. Energy requirements (kJ day^{-1} kg^{-1} LW$^{0.75}$) for maintenance of energy equilibrium (retained energy = 0) in New Zealand White or hybrid growing rabbits.

Authors	DE_m	ME_m[a]
Isar (1981)	470	446
Scheele *et al.* (1985)	413	392
Parigi Bini and Xiccato (1986)[b]	425–454	404–431
Partridge *et al.* (1989)	381	362
Nizza *et al.* (1995)	441–454	419–432

DE_m, digestible energy requirement for maintenance; ME_m, metabolizable energy requirement for maintenance.
[a]Calculated from DE_m by assuming ME = 0.95 DE.
[b]Recalculated values from original data expressed on metabolic empty body weight (EBW$^{0.75}$).

from 381 kJ day^{-1} kg^{-1} LW$^{0.75}$ in New Zealand White rabbits (Partridge *et al.*, 1989) to 552 kJ day^{-1} kg^{-1} LW$^{0.75}$ in Giant Spanish growing rabbits (de Blas *et al.*, 1985). These estimates vary depending on both the breeds, characterized by very different daily gain and body composition, and the measurement methods used (calorimetric methods usually give a lower DE_m than comparative slaughter). On the basis of a review of homogeneous studies on growing New Zealand White pure-breed or derived rabbits (Table 6.2), an average DE_m of 430 kJ day^{-1} kg^{-1} LW$^{0.75}$ and an average ME_m of 410 kJ day^{-1} kg^{-1} LW$^{0.75}$ might be proposed.

Similarly to in growing rabbits, experimental estimates of DE_m in reproducing does are often inconsistent (Table 6.3). Based on available data, DE_m may be proposed as 400 kJ day^{-1} kg^{-1} LW$^{0.75}$ for non-reproducing does, 430 kJ day^{-1} kg^{-1} LW$^{0.75}$ for pregnant or lactating does and 470 kJ day^{-1} kg^{-1} LW$^{0.75}$ for concurrently pregnant and lactating does.

6.3.4 Energy requirements for growth

Figure 6.3 shows the response in terms of daily gain and energy intake to an increase in feed DE density when the DP to DE ratio is held constant and protein contains the major amino acids in satisfactory equilibrium (Partridge *et al.*, 1989). This typical growth-response curve shows that the maximum average daily growth is achieved when the dietary DE concentration is about 10–10.5 MJ kg^{-1}.

An increase in the level of dietary energy intake also affects body gain composition and the partition of energy retained as protein and fat. The body composition changes are not linearly correlated

Table 6.3. Digestible energy requirements for maintenance in rabbit does (kJ day^{-1} kg^{-1} LW$^{0.75}$).

	Non-lactating does		Lactating does	
Author	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Partridge *et al.* (1983)	–	–	413–446	–
Partridge *et al.* (1986b)	326	352		500
Fraga *et al.* (1989)	–	452		473
Parigi Bini *et al.* (1990a)	398	431	–	–
Parigi Bini *et al.* (1991a)	–	–	432	468
Xiccato *et al.* (1992b)	–	–	–	470
Lebas (1989)		400		460
Maertens (1992)		420		460
Toschi *et al.* (2003)	439	–	–	–
Toschi *et al.* (2004)	–	458	–	–

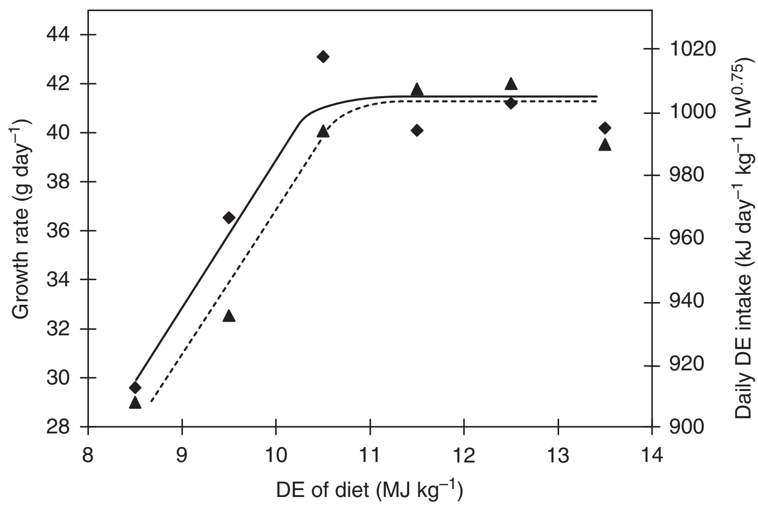

Fig. 6.3. Effect of dietary energy density on growth rate (◆) and total digestible energy (DE) intake (▲) (from Partridge *et al.*, 1989).

with DEI, because some constituents (i.e. fat) tend to increase more than proportionally. The following quadratic regression equations estimate the daily gains (g day^{-1} kg^{-1} LW$^{0.75}$) of EB (EBG), water (WG), protein (PG), fat (FG) and ash (AG) from DEI (MJ day^{-1} kg^{-1} LW$^{0.75}$; Parigi Bini and Xiccato, 1986):

$$\mathrm{EBG} = -12.61 + 48.50\ \mathrm{DEI} - 8.15\ \mathrm{DEI}^2,\quad \mathrm{RSD} = 1.64,\ r = 0.93$$

$$\mathrm{WG} = -6.52 + 33.59\ \mathrm{DEI} - 10.69\ \mathrm{DEI}^2,\quad \mathrm{RSD} = 0.61,\ r = 0.96$$

$$\mathrm{PG} = -2.42 + 10.01\ \mathrm{DEI} - 1.70\ \mathrm{DEI}^2,\quad \mathrm{RSD} = 0.38,\ r = 0.91$$

$$\mathrm{FG} = -2.76 + 1.69\ \mathrm{DEI} + 5.75\ \mathrm{DEI}^2,\quad \mathrm{RSD} = 0.45,\ r = 0.94$$

$$\mathrm{AG} = -0.91 + 3.21\ \mathrm{DEI} - 1.51\ \mathrm{DEI}^2,\quad \mathrm{RSD} = 0.12,\ r = 0.67$$

The same equations may be transformed to formulate other equations that estimate retained energy as protein (RE_p), fat (RE_f) and total RE (RE_g) as a function of DEI (all data are expressed as MJ day^{-1} kg^{-1} LW$^{0.75}$):

$$\mathrm{RE_p} = -0.057 + 0.234\ \mathrm{DEI} - 0.040\ \mathrm{DEI}^2,\quad \mathrm{RSD} = 0.009,\ r = 0.91$$

$$\mathrm{RE_f} = -0.098 + 0.060\ \mathrm{DEI} + 0.204\ \mathrm{DEI}^2,\quad \mathrm{RSD} = 0.016,\ r = 0.94$$

$$\mathrm{RE_g} = -0.155 + 0.294\ \mathrm{DEI} + 0.164\ \mathrm{DEI}^2,\quad \mathrm{RSD} = 0.012,\ r = 0.96$$

In fact, body protein and fat have specific caloric values, namely 23.2 and 35.6 MJ kg^{-1}, respectively, as determined by bomb calorimeter (Parigi Bini and Dalle Rive, 1978). Nizza *et al.* (1995) found similar caloric values (23.1 and 35.7 MJ kg^{-1}), thereby confirming that the caloric value of rabbit fat is lower than that of other animal fats (average 38–40 MJ kg^{-1}) (Close, 1990). The caloric value of fat was found to be higher in reproducing does (36–37 MJ kg^{-1}) than in growing animals, probably related to the lower content of phospholipids in older and fatter animals (Cambero *et al.*, 1991; Hulot *et al.*, 1992).

These equations can be used to calculate the daily gain composition throughout the entire growing period (e.g. from 0.8 to 2.4 kg LW). During this interval, the average LW$^{0.75}$ is about 1.42 kg. An increase in EBG and a strong modification of the chemical composition of EBG (and consequently its energy value) occurs as DEI increases (Table 6.4).

When DEI = 0, substantial losses of body weight and body tissues occur and

Table 6.4. Empty body gain (EBG) composition and retained energy (RE) partition as influenced by digestible energy intake (DEI) (all data are in kJ or g day^{-1} kg^{-1} LW$^{0.75}$) (recalculated from Parigi Bini and Xiccato, 1986).

		EBG composition						
DEI (kJ)	EBG (g)	WG (g)	PG (g)	FG (g)	AG (g)	RE_p (kJ)	RE_f (kJ)	RE_g (kJ)
0	−12.6	−6.5	−2.4	−2.8	−0.9	−57	−98	−155
273	0.0	1.8	0.2	−1.9	−0.1	4	−66	−62
425	6.5	5.8	1.5	−1.0	0.2	36	−36	0
900	24.4	15.0	5.2	3.4	0.8	122	122	244
1000	27.8	16.4	5.9	4.7	0.8	137	166	303

AG, FG, PG, WG, daily gains of ash, fat, protein and water, respectively; RE_p, RE_f, RE_g, retained energy as protein, fat and total RE, respectively.

the body loses 155 kJ day^{-1} kg^{-1} LW$^{0.75}$. This loss is called ‘fasting metabolism’, which means the loss of body energy at fasting. The requirements for the maintenance of energy equilibrium (RE_g = 0) and the maintenance of live body weight (live weight gain, LWG = 0) are different: DE_m has been measured at 425 kJ day^{-1} kg^{-1} LW$^{0.75}$ at RE_g = 0 and only 273 kJ day^{-1} kg^{-1} LW$^{0.75}$ when LWG = 0. When LWG = 0, the rabbit exhibits an energy deficit as a consequence of the loss of fat primarily compensated by water gain.

When DEI is 273 kJ day^{-1} kg^{-1} LW$^{0.75}$, the EB weight is maintained (EBG = 0), but losses of fat (–1.9 g day^{-1} kg^{-1} LW$^{0.75}$) and energy (–62 kJ day^{-1} kg^{-1} LW$^{0.75}$) are observed. When DEI = DE_m (425 kJ day^{-1} kg^{-1} LW$^{0.75}$), the energy equilibrium is reached as a consequence of a gain in energy (36 kJ day^{-1} kg^{-1} LW$^{0.75}$) as protein (1.5 g day^{-1} kg^{-1} LW$^{0.75}$) and an equivalent loss of energy as fat (–1.0 g day^{-1} kg^{-1} LW$^{0.75}$). At the same time, EBG is positive (6.5 g day^{-1} kg^{-1} LW$^{0.75}$), primarily due to water retention.

With increasing DEI, protein gain (in weight) always remains higher than fat gain, but at DEI = 900 kJ day^{-1} kg^{-1} LW$^{0.75}$, RE as protein and fat become equal (RE_p = RE_f = 122 kJ day^{-1} kg^{-1} LW$^{0.75}$).

With non-restricted feeding (DEI = 1000 kJ day^{-1} kg^{-1} LW$^{0.75}$), EBG is 39.5 g day^{-1} (27.8 g day^{-1} kg^{-1} LW$^{0.75}$ × 1.42) and LWG is 45.2 g day^{-1} (assuming EB weight = 0.87 LW). Daily EBG is then composed of 23.3 g (590 g kg^{-1}) water, 8.4 g (212 g kg^{-1}) protein, 6.7 g (169 g kg^{-1}) fat and 1.1 g (29 g kg^{-1}) ash. At the voluntary intake, $RE_f > RE_p$ and total RE reaches the maximum level (RE_g = 303 kJ day^{-1} kg^{-1} LW$^{0.75}$). Daily RE_g is 430 kJ day^{-1} and the energy concentration of growth is 10.9 kJ g^{-1} EBG and 9.5 kJ g^{-1} LWG. These chemical composition and energy partitions of daily growth are typical of a young, rapidly growing rabbit.

The above-listed regression equations of RE from DEI are not linear. From the same data set, the following linear regression equation can be estimated (data are expressed in kJ day^{-1} kg^{-1} LW$^{0.75}$):

$$RE_g = -235 + 0.527\ \mathrm{DEI},$$
$$\mathrm{RSD} = 22,\ r = 0.97$$

This indicates that DE_m = 446 kJ day^{-1} kg^{-1} LW$^{0.75}$ and gives an efficiency of utilization of DE for growth of 0.53, which is close to the values found by de Blas *et al.* (1985), Partridge *et al.* (1989) and others (Table 6.5).

The efficiency of energy utilization for growth is clearly influenced by the composition of growth, because energy is retained as protein less efficiently than energy retained as fat (Blaxter, 1989; Close, 1990). On the basis of the literature, we can utilize average values of efficiencies of DE utilization for total energy retention, energy retained as protein and energy retained as fat of 0.50, 0.40 and 0.65, respectively.

Using the above-mentioned coefficients of energy utilization and DE_m values, the DE requirement and energy and material balance in growing rabbits can be estimated. An example regarding rabbits from 0.8 to 2.4 kg is provided in Box 6.1.

Table 6.5. Efficiency of utilization of digestible energy for energy retained for growth (RE_g) and RE as protein (RE_p) and fat (RE_f).

Authors	RE_g/DEI	RE_p/DEI	RE_f/DEI
de Blas *et al*. (1985)	0.52	0.38	0.65
Parigi Bini and Xiccato (1986)	0.53	0.44	0.70
Partridge *et al*. (1989)	0.47	0.39	0.60
Nizza *et al*. (1995)	0.51[a]	0.39–0.41	0.64–0.66

DEI, digestible energy intake.
[a]Calculated value.

Box 6.1. Estimate of digestible energy (DE) requirements and body energy partition in growing rabbits.

Reference data
- Age at weaning = 32 days
- Age at slaughter = 72 days
- Live weight (LW) at weaning = 0.8 kg
- LW at slaughter = 2.4 kg
- Empty body gain (EBG) = 0.87 LW gain (LWG)
- EBG composition = water 610 g kg^{-1}; protein 210 g kg^{-1}; fat 150 g kg^{-1}; ash 30 g kg^{-1}
- Caloric value of body protein = 23.2 MJ kg^{-1} = 23.2 kJ g^{-1}
- Caloric value of body fat = 35.6 MJ kg^{-1} = 35.6 kJ g^{-1}
- DE required for maintenance (DE_m) = 430 kJ day^{-1} kg^{-1} $LW^{0.75}$
- Efficiency of DE utilization for retained energy (RE) as protein = 0.40
- Efficiency of DE utilization for RE as fat = 0.65
- Dietary DE concentration = 10.0 MJ kg^{-1} = 10.0 kJ g^{-1}

Calculated data
- Growing period = 40 days
- LWG = 1.6 kg
- Daily LWG = 40 g day^{-1}
- EBG = 0.87 × 40 g day^{-1} = 34.8 g day^{-1}
- Protein gain = 34.8 g day^{-1} × 0.21 = 7.3 g day^{-1}
- Fat gain = 34.8 g day^{-1} × 0.15 = 5.2 g day^{-1}
- Average metabolic LW = $([0.8 + 2.4] / 2)^{0.75}$ = 1.42 kg $LW^{0.75}$
- RE as protein (RE_p) = 7.3 g day^{-1} × 23.2 kJ g^{-1} = 169 kJ day^{-1}
- RE as fat (RE_f) = 5.2 g day^{-1} × 35.6 kJ g^{-1} = 185 kJ day^{-1}
- Total RE (RE_g) = 169 + 185 = 354 kJ day^{-1}
- Caloric value of EBG = 354 kJ day^{-1} / 34.8 g day^{-1} = 10.2 kJ g^{-1}
- Caloric value of LWG = 354 kJ day^{-1} / 40 g day^{-1} = 8.9 kJ g^{-1}

DE requirement and efficiency for growth
- DE_m requirement = 430 kJ day^{-1} kg^{-1} $LW^{0.75}$ × 1.42 kg $LW^{0.75}$ = 611 kJ day^{-1}
- DE requirement for RE_p = 169 kJ day^{-1} / 0.40 = 423 kJ day^{-1}
- DE requirement for RE_f = 185 kJ day^{-1} / 0.65 = 285 kJ day^{-1}
- DE requirement for growth (DE_g) = 423 + 285 = 708 kJ day^{-1}
- Efficiency of DE utilization for growth (DE_g) = 354 kJ day^{-1} / 708 kJ day^{-1} = 0.50
- Total DE requirement = DE_m + DE_g = 611 + 708 = 1319 kJ day^{-1} (929 kJ day^{-1} kg^{-1} $LW^{0.75}$)
- Required feed intake = 1319 kJ day^{-1} / 10 kJ g^{-1} = 132 g day^{-1}

6.3.5 Energy requirements for reproduction and lactation

Investigations on the energy metabolism of rabbit does were started over 20 years ago by Partridge and co-workers in a well-known series of experiments (Partridge, 1986). Information on the changes of body composition in reproducing does and on the partition and utilization of dietary energy for maternal and fetal tissues synthesis and for milk production was obtained in a successive series of experiments carried out by Parigi Bini, Xiccato and co-workers using the comparative slaughter technique (Parigi Bini and Xiccato, 1993, 1998; Xiccato *et al.*, 1995, 1999, 2004, 2005b; Xiccato, 1996). The research groups of Toulouse, France (Lebas and Fortun-Lamothe), Perugia, Italy (Castellini and Dal Bosco), Valencia, Spain (Pascual and Cervera) and Kaposvár, Hungary (Szendrő, Milisits) further contributed to a more precise definition of the energy requirements and the utilization and partition of dietary energy in rabbit does, also utilizing other techniques to estimate energy requirements and body composition (ultrasound scanning, total body electrical conductivity, computerized tomography).

6.3.6 Pregnancy

Nulliparous does experience considerable variations in body composition, tissue deposition and energy retention during their first pregnancy (Parigi Bini *et al.*, 1990a, 1991a; Xiccato *et al.*, 1995; Fortun-Lamothe and Lebas, 1996; Milisits *et al.*, 1996, 1999). During early and mid-gestation (0–21 days), the LW increases similarly to that of non-pregnant does (Table 6.6).

During late pregnancy (21–30 days), the EB weight decreases as a result of protein and fat losses and a transfer of energy to the rapidly growing fetuses. At the same time, non-pregnant does continue to gain weight and retain body energy, primarily in the form of fat.

Changes in various blood plasma metabolites confirm the intense modification of energy metabolism in late pregnancy (Parigi Bini *et al.*, 1990a; Fortun *et al.*, 1994; Xiccato *et al.*, 2005b; Pascual *et al.*, 2006). In the last period of pregnancy, the glucagon level increases, while glucose, triglyceride, cholesterol and leptin levels decrease. Glucagon is involved in the control of catabolic utilization of body reserves and a similar increase in level was reported by Jean-Blain and Durix (1985). The transfer of energy from the body of doe to the fetuses leads to an energy deficit that is especially concentrated in the last 10 days of pregnancy, as reported for sows (Noblet and Close, 1980) and ewes (Rattray *et al.*, 1980). The low circulating levels of leptin and glucose immediately after kindling suggest either that body energy stores are depleted by pregnancy or that no energy is available for body tissue deposition due to the very low feed intake at kindling (Parigi Bini *et al.*, 1990a; Houseknecht and Spurlock, 2003; Xiccato *et al.*, 2005b).

The body composition of newborn kits (born dead included) can vary according to litter size, average weight of kits, parity order, physiological state and the feeding

Table 6.6. Variations in empty body (EB) composition in non-pregnant and pregnant nulliparous does (Parigi Bini *et al.*, 1990a).

	Days on trial		
Non-pregnant does	0	21	30
EB weight (kg)	2.97	3.16	3.27
Water (g kg^{-1})	611	597	576
Protein (g kg^{-1})	215	208	205
Fat (g kg^{-1})	146	162	187
EB energy (MJ kg^{-1})	10.2	10.6	11.4
	Days of pregnancy		
Pregnant does[a]	0	21	30
EB weight (kg)	2.97	3.15	2.98
Water (g kg^{-1})	611	592	596
Protein (g kg^{-1})	215	217	218
Fat (g kg^{-1})	146	160	156
EB energy (MJ kg^{-1})	10.2	10.7	10.6

[a]Excluding pregnant uterus.

regime of the mother. The body composition of newborn kits from does fed *ad libitum* is 800 (790–818) g water kg^{-1}, 120 (113–130) g protein kg^{-1}, 55 (48–61) g fat kg^{-1}, 22 (19–26) g ash kg^{-1} and 5.0 (4.3–5.6) kJ energy kg^{-1} (Parigi Bini *et al.*, 1992; Parigi Bini and Xiccato, 1993; Xiccato *et al.*, 1995; Fortun-Lamothe *et al.*, 1999). Feeding restriction of the doe reduces body fat and energy concentration and increases water of newborn kits (Xiccato *et al.*, 1992b; Fortun-Lamothe and Lebas, 1996; Fortun-Lamothe *et al.*, 1999).

The efficiency of DE utilization for maternal tissue accretion in pregnant or non-pregnant does (RE_g/DEI) has been estimated at 0.49 (Parigi Bini *et al.*, 1991a) to 0.55 (Toschi *et al.*, 2004), similarly to efficiency in growing rabbits. Lower efficiencies of dietary DE utilization for fetal growth (E_{fetus}/DEI) have been found, namely 0.31 in pregnant nulliparous does and 0.27 in lactating and pregnant does (Parigi Bini *et al.*, 1991a, 1992; Xiccato *et al.*, 1992b) (Table 6.7). Similar or lower efficiencies (0.20–0.30) for fetal growth have been observed in pigs (Walach-Janiak *et al.*, 1986). An explanation for the high energy cost of fetal growth may come from the very high protein content of the fetal tissues and the extremely rapid turnover of fetal protein (Young, 1979).

6.3.7 Lactation and concurrent pregnancy

The energy output in milk (E_{milk}) during lactation is exceptionally high in rabbits, compared to other species, due to the considerable milk production (200–300 g day^{-1}) and the high concentration in DM (300–350 g kg^{-1}), protein (100–150 g kg^{-1}) and fat (120–150 g kg^{-1}; Lebas, 1971; Partridge *et al.*, 1983, 1986b; Fraga *et al.*, 1989; Parigi Bini *et al.*, 1992; Pascual *et al.*, 1999a, 2002b; Maertens *et al.*, 2006). The chemical composition of rabbit milk changes substantially during lactation (Lebas, 1971; Pascual *et al.*, 1999a). In particular, the DM content decreases in the first 1–3 days, when colostrum becomes milk, then remains constant for about 3 weeks and finally increases as milk yield decreases. On the other hand, the composition of milk DM tends to remain unchanged, except for a constant reduction in lactose, and therefore the caloric value of milk is strictly dependent on the variation of DM content (Parigi Bini *et al.*, 1991b; Xiccato *et al.*, 1995).

Different measurements of milk energy concentration and variation during lactation are listed in Table 6.8. The average caloric value of 8.5 MJ kg^{-1} is close to the value of 8.53 MJ kg^{-1} reported by Blaxter (1989) and is about 2.9 times higher than that of cow milk (2.97 MJ kg^{-1}).

If the daily excretion of energy as milk is expressed in terms of metabolic weight, however, the average milk energy output is higher in rabbits than in cows. For example, a 4 kg doe producing 250 g milk day^{-1} excretes 751 kJ E_{milk} day^{-1} kg^{-1} $LW^{0.75}$, while a 600 kg cow producing 25 kg

Table 6.7. Efficiency of utilization of digestible energy and the doe's body energy reserves (RE_r) for fetal growth (E_{fetus}) and milk production (E_{milk}).

Authors	E_{fetus}/DEI	E_{milk}/DEI	E_{milk}/RE_r
Partridge *et al.* (1983, 1986b)	–	0.68–0.84[a]	0.94
Partridge *et al.* (1986a)	–	0.62	–
Fraga *et al.* (1989)	–	0.71	–
Parigi Bini *et al.* (1991a, b, 1992)	0.27–0.31	0.63	0.76–0.81
Xiccato *et al.* (1992b, 1995)	0.30	0.63	0.76
Pascual *et al.* (2000b)	–	0.71–0.79[a]	–

DEI, digestible energy intake.
[a]The highest values were found in does fed with high-fat-added diets.

Table 6.8. Energy concentration (MJ kg^{-1}) and variation of rabbit milk during lactation.

Authors	Week 1	Week ≥2	Final week	Average
Lebas (1971)[a]	8.10	7.11	10.22	8.02
Partridge *et al.* (1983)	8.42	9.01	10.25	9.17
Fraga *et al.* (1989)	–	7.98	–	7.98
Parigi Bini *et al.* (1991b)	–	7.75	9.84	8.27
Maertens (1992)	–	8.0	9.0–12.0	–
Xiccato *et al.* (1995)	–	8.06–8.76	–	8.42
Pascual *et al.* (1999a)[b]	9.28	9.18	11.59	–
Pascual *et al.* (2002b)[c]	–	8.85	–	–
Maertens *et al.* (2006)	8.4	8.7	10.5	–
Average	8.5	8.3	10.5	8.5

[a]Estimated energy values from chemical composition.
[b]Average of does fed diets with ether extract: 26, 99 and 117 g kg^{-1} dry matter.
[c]Average of does fed diets with ether extract: 28, 71 and 91 g kg^{-1} dry matter.

milk day^{-1} excretes only 612 kJ E_{milk} day^{-1} kg^{-1} LW$^{0.75}$.

The dietary DE is utilized very efficiently by lactating does. Based on literature (see Table 6.7), the coefficient of utilization of DE for milk production in lactating non-pregnant and lactating and concurrent pregnant does ranges from 0.60 to 0.70. The highest values (>0.75) found by Partridge *et al.* (1983, 1986b) and Pascual *et al.* (2000b) may be explained by the particular diets (high fat, high protein) used in those experiments, which could have permitted the direct passage of long-chain fatty acids from feed to milk.

The efficiency of utilization of energy retained in the doe's body reserves (RE_r) for milk production is 0.81 in lactating does (Parigi Bini *et al.*, 1991a,b) and 0.76 in lactating and pregnant does (Parigi Bini *et al.*, 1992; Xiccato *et al.*, 1992b).

The average values proposed for the utilization of dietary DE (0.65) and maternal body energy for milk production (0.78) agree with the results from other species (cattle and pigs) and the theoretical calculations by Blaxter (1989).

A calculation of the energy requirements and body balance for multiparous, lactating and non-pregnant does is provided in Box 6.2.

6.3.8 Energy and material balance during reproduction

The significant energy excretion through milk in lactating does, which is even more pronounced in selected 'hybrid' does, is not completely compensated by voluntary DEI, especially in primiparous does. This causes a consistent deficit in both body tissues and energy. During the first pregnancy, DEI decreases from 600 to 650 kJ day^{-1} kg^{-1} LW$^{0.75}$ in the first 25 days until 400–450 kJ day^{-1} kg^{-1} LW$^{0.75}$ in the last 5 days, due to the increasing volume of fetuses in the abdomen. On the day of kindling, the doe ingests only a small amount of feed or even nothing (Fig. 6.4).

Voluntary DE consumption is much higher in lactating females, which can ingest 1500–1800 kJ day^{-1} kg^{-1} LW$^{0.75}$ at the lactation peak and 1100–1300 kJ day^{-1} kg^{-1} LW$^{0.75}$ on average. The highest values are recorded by multiparous does.

After litter weaning, does quickly decrease their energy intake in a week to about 0.35–0.45 of the lactation level; that is, 500–600 kJ DE day^{-1} kg^{-1} LW$^{0.75}$ (Xiccato *et al.*, 2004, 2005b).

During lactation, therefore, the doe's body is subjected to a marked reduction in energy reserves following the mobilization of fat deposits (Fig. 6.5) (Parigi Bini *et al.*,

Box 6.2. Estimate of digestible energy (DE) requirements and body energy and fat balance in multiparous, non-pregnant and lactating does (assuming protein balance is in equilibrium).

Reference data
- Average live weight (LW) = 4.25 kg
- Empty body (EB) weight at kindling = 0.92 LW = 3.91 kg
- Days of lactation = 30 days
- Milk production = 220 g day^{-1}
- Caloric value of milk = 8.5 MJ kg^{-1} = 8.5 kJ g^{-1}
- Energy concentration of EB at kindling = 10.5 MJ kg^{-1}
- Fat concentration of EB at kindling = 160 g kg^{-1}
- Caloric value of EB fat = 36.5 kJ g^{-1}
- DE required for maintenance (DE_m) = 430 kJ day^{-1} kg^{-1} $LW^{0.75}$
- Efficiency of utilization of DE for milk energy (E_{milk}) = 0.65
- Efficiency of utilization of body energy reserves (RE_r) for E_{milk} = 0.80
- Dietary DE concentration = 11.0 MJ kg^{-1} = 11.0 kJ g^{-1}
- Maximum DE intake (DEI) = 1250 kJ day^{-1} kg^{-1} $LW^{0.75}$
- Protein balance = 0

Calculated data
- Average $LW^{0.75}$ = $4.25^{0.75}$ kg = 2.96 kg $LW^{0.75}$
- E_{milk} = 220 g day^{-1} × 8.5 kJ g^{-1} = 1870 kJ day^{-1}
- Total EB energy at kindling = 3.91 kg × 10.5 MJ kg^{-1} = 41.05 MJ
- Total EB fat at kindling = 3.91 kg × 160 g kg^{-1} = 626 g

DE requirements and intake
- DE requirement for maintenance = 430 kJ day^{-1} kg^{-1} $LW^{0.75}$ × 2.96 kg $LW^{0.75}$ = 1273 kJ day^{-1}
- DE requirement for milk production (DE_{milk}) = 1870 kJ day^{-1} / 0.65 = 2877 kJ day^{-1}
- Total DE requirement = 1273 + 2877 = 4150 kJ day^{-1} (1402 kJ day^{-1} kg^{-1} $LW^{0.75}$)
- Maximum DEI = 1250 kJ day^{-1} kg^{-1} $LW^{0.75}$ × 2.96 kg $LW^{0.75}$ = 3700 kJ day^{-1} 11.0 kJg^{-1}
- Maximum feed intake = 336 g day^{-1}
- DEI deficit = 4150 – 3700 = –450 kJ day^{-1}

Body energy and tissue balance
- DEI for milk production = DEI – DE_m = 3700 – 1273 = 2427 kJ day^{-1}
- E_{milk} from dietary DE = 2427 kJ day^{-1} × 0.65 = 1578 kJ day^{-1}
- E_{milk} from body energy reserves = 1870 – 1578 = 292 kJ day^{-1}
- Daily EB energy loss (RE_r) = –292 kJ day^{-1} / 0.80 = –365 kJ day^{-1}
- Total EB energy loss during lactation = –365 kJ day^{-1} × 30 days = –10.95 MJ
- EB energy balance = –10.95 MJ / 41.05 MJ × 100 = –26.7%
- Daily EB fat loss = –365 kJ day^{-1} / 36.5 kJ g^{-1} = –10.0 g day^{-1}
- Total EB fat loss during lactation = –10.0 g day^{-1} × 30 days = –300 g
- EB fat balance = –300 g / 626 g × 100 = –47.9%

1990b, 1991b, 1992; Xiccato *et al.*, 1992b, 1995, 1999, 2004, 2005b; Pascual *et al.*, 2000b, 2003). Unlike in other species, this energy loss remains constant throughout lactation (Parigi Bini *et al.*, 1990b) and no recovery is observed during the final phase due to the milk energy output, which remains high even after 25–30 days of lactation.

The simultaneous condition of pregnancy is responsible for a further reduction in fat content and body energy levels. It prevents the return to normal body conditions (Fortun *et al.*, 1993; Parigi Bini and Xiccato, 1993; Fortun-Lamothe and Lebas, 1996; Xiccato *et al.*, 2005b) and increases protein requirements in response to the elevated demand for protein by the fetuses and the rapid turnover of fetal protein (Parigi Bini *et al.*, 1992; Xiccato *et al.*, 1992b, 1995).

The emergence of high-performance hybrid lines with higher nutritional needs, but that are unable to ingest sufficient dietary energy, has increased rabbit doe susceptibility to the energy deficit. The

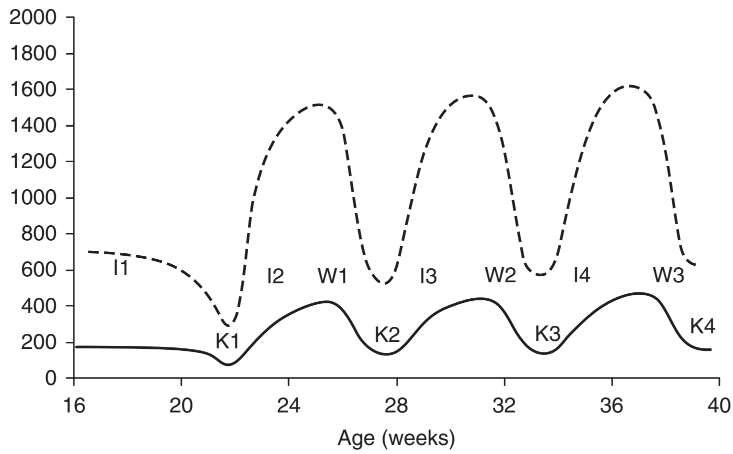

Fig. 6.4. Evolution of voluntary feed intake (g day^{-1}) (solid line) and digestible energy intake (kJ day^{-1} kg^{-1} LW$^{0.75}$) (dotted line) from the first insemination to the fourth kindling in does submitted to a 12-day post-partum remating programme (from Xiccato and Trocino, 2008). I1, I2, I3, I4: 1st, 2nd, 3rd, 4th insemination; K1, K2, K3, K4: 1st, 2nd, 3rd, 4th kindling; W1, W2, W3: 1st, 2nd, 3rd weaning.

genetic selection and crossbreeding programmes of the most common European hybrid lines aim to increase litter size at birth and daily milk production (De Rochambeau, 1990; Maertens, 1992; Khalil and Al-Saef, 2008), but until now less importance has been given to the goal of increasing voluntary feed intake and maintaining the body condition of the females (Quevedo *et al.*, 2005; Theilgaard *et al.*, 2007).

While waiting for new hybrid lines selected for higher voluntary feed intake, (i) nutritional strategies to increase feed and energy intake and (ii) management strategies to reduce body energy output and/or increase body energy recovery have been tested to keep the doe energy deficit under control.

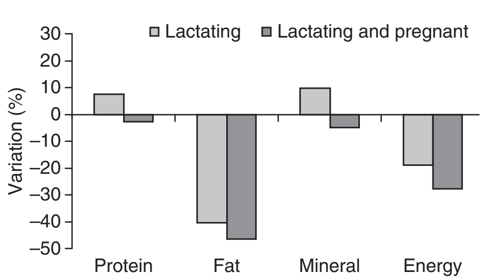

Fig. 6.5. Material and energy balance (percentage variation on the content at initial kindling) of primiparous does in different physiological states (Parigi Bini and Xiccato, 1993; Xiccato, 1996).

6.3.9 Nutritional strategies to reduce energy deficit

Feeding young does

Young does should face their first mating, pregnancy and lactation with an adequate body energy condition to support the high nutritional requirements of reproduction. From weaning (30–35 days) to puberty (10–12 weeks) and LW from 0.8 to 2.4 kg, feeding programmes and growth performance are similar to those of rabbits kept for meat production. Later, from puberty to first mating (16–18 weeks of age) and LW until 3.2–3.5 kg, the feeding programme should aim to permit correct morphologic and reproductive development and avoid overfattening (Pascual *et al.*, 2006; Rommers *et al.*, 2006; Xiccato and Trocino, 2008). In this period, voluntary feed intake decreases slightly from 800 to 700 kJ DE day^{-1} kg^{-1} LW$^{0.75}$ and daily weight gain decreases from 35 to 20 g day^{-1} (Xiccato *et al.*, 1999).

At 17 weeks of age, breeding rabbits given *ad libitum* access to a diet containing 10 MJ DE kg^{-1} may reach about 3.4 kg LW and 180 g body fat kg^{-1} LW. This condition may be excessive if the further fattening during pregnancy or the rapid overfattening (>200 g fat kg^{-1}) in 2–3 weeks in case of failure of pregnancy are considered. Overfattening can provoke subsequent dystocia and impaired reproductive performance (Partridge *et al.*, 1986a; Parigi Bini *et al.*, 1989; Viudes-de-Castro *et al.*, 1991). On the other hand, an earlier insemination (e.g. 15 weeks) is not advisable due to the still incomplete development of the endocrine and reproductive systems (Matics *et al.*, 2008).

For these reasons, feeding restriction (0.8–0.9 of *ad libitum* intake) may be applied to young does for different periods before mating to obtain a target weight at insemination. In restricted does, flushing with a lactation diet given *ad libitum* is usually performed 4–7 days before the first insemination to avoid a reduction in sexual receptivity at this time (Rommers *et al.*, 2001, 2002, 2004a,b, 2006; Bonanno *et al.*, 2004).

Feed restriction can continue also in the first part of pregnancy, especially when LW exceeds target weight, while *ad libitum* feeding with a lactation diet is recommended during the last 2 weeks of pregnancy to take into account increasing pregnancy requirements and decreasing feed intake around kindling (Rommers *et al.*, 2004b). However, restricting feed during the entire pregnancy to maintenance needs or even less (0.75) reduces body fat and energy reserves (Table 6.9) to a level that may negatively influence reproductive performance (Fortun *et al.*, 1994).

In young does, feeding restriction may also reduce voluntary feed intake in the following pregnancy and lactation and accentuate the risk of a negative energy balance between reproductive cycles (Parigi Bini *et al.*, 1991a; Maertens, 1992; Fortun-Lamothe, 1998). On the other hand, the administration of high-fibre, low-energy diets to young females before the first mating increases voluntary feed intake during growth and pregnancy, and partially decreases the body fat and energy deficit at the end of first lactation (Table 6.10) (Xiccato *et al.*,

Table 6.9. Body composition and energy concentration of primiparous does slaughtered at 28 days' of gestation. The does had been fed *ad libitum* (C), at a maintenance level (M) or at 0.75 of the maintenance level (R) during pregnancy (Fortun *et al.*, 1994).

	C does	M does	R does
Water (g kg^{-1})	590^{c}	620^{b}	640^{a}
Protein (g kg^{-1})	189	192	189
Fat (g kg^{-1})	193^{a}	156^{b}	134^{b}
Energy (MJ kg^{-1})	11.8^{a}	10.5^{b}	9.6^{c}

^{a,b,c}Means on the same row with different subscript differ ($P < 0.05$).

Table 6.10. Influence of a high-fibre, low-energy diet given from weaning until first insemination of young does on the ensuing lactation and empty body (EB) balance (Xiccato *et al.*, 1999).

	Diet fed before first insemination		
	Control diet	Low-energy diet	*P*
LW at kindling (g)	3846	3833	NS
LW at end of lactation (g)	3939	3848	NS
Milk production (g day^{-1})	206	204	NS
Feed intake (g day^{-1})	331	340	NS
DE intake (kJ day^{-1} kg^{-1} LW$^{0.75}$)	1203	1245	NS
EB gain (g)	–233	–221	NS
EB balance (variation on the composition at kindling):			
Water (%)	11	7	0.09
Protein (%)	7	6	NS
Fat (%)	–68	–59	<0.01
Energy (%)	–41	–36	<0.01

DE, digestible energy; LW, live weight; NS, not significant.

1999). A similar feeding regime does not affect reproductive performance at birth, but stimulates feed intake during lactation with consequent higher litter sizes and weights at weaning (Nizza *et al.*, 1997; Pascual *et al.*, 2002a).

Feeding reproducing does

High-energy diets have been widely tested in reproducing does to meet their high energy requirements. The effects of increasing dietary energy concentration on reproductive performance and body chemical and energy balance have been reviewed by Pascual *et al.* (2003).

During early pregnancy, increasing dietary DE concentration usually reduces DMI and does not change DEI significantly (Pascual *et al.*, 1998, 1999a, b, 2002b). During the last week of pregnancy, voluntary feed intake is limited by physical intake capacity (Pascual *et al.*, 2003).

During lactation, feeding highly digestible diets increases DEI (Partridge, 1986; Maertens and De Groote, 1988; Fraga *et al.*, 1989; Castellini and Battaglini, 1991; Barreto and de Blas, 1993; Cervera *et al.*, 1993), especially when added-fat diets are used in comparison with high-starch diets (Xiccato *et al.*, 1995; Fortun-Lamothe and Lebas, 1996; Parigi Bini *et al.*, 1996; Pascual *et al.*, 1998, 2002b). The body energy balance of does, however, is always negative and is not statistically affected by dietary treatments (Fig. 6.6). In fact, a higher dietary energy supply determines an increase of milk production, impairing its potential beneficial effect on body condition both in primiparous (Table 6.11) (Xiccato *et al.*, 1995; Fortun-Lamothe and Lebas, 1996) and multiparous does (Table 6.12) (Pascual *et al.*, 2000b).

Therefore, the limiting factor on doe productivity is not milk production, but voluntary feed intake: as DEI increases, milk production also tends to increase, thereby impairing – at least partially – the effect of increased DEI on body energy balance. An increase of 1 kJ in the DEI leads to a proportional increase in milk energy output (0.434 kJ) and a more limited reduction in the energy deficit (–0.203 kJ) (Fig. 6.7) (Xiccato, 1996).

This trend, linear throughout the interval tested, shows a DEI capable of obtaining the doe's body energy equilibrium ($RE_r = 0$) at 1585 kJ day^{-1} kg^{-1} LW$^{0.75}$. At this DEI level, the energy milk output is 711 kJ day^{-1} kg^{-1} LW$^{0.75}$, which corresponds to about 250 g day^{-1} of milk in a 4.25 kg rabbit (assuming a milk energy density of 8.5 MJ kg^{-1}). Using a

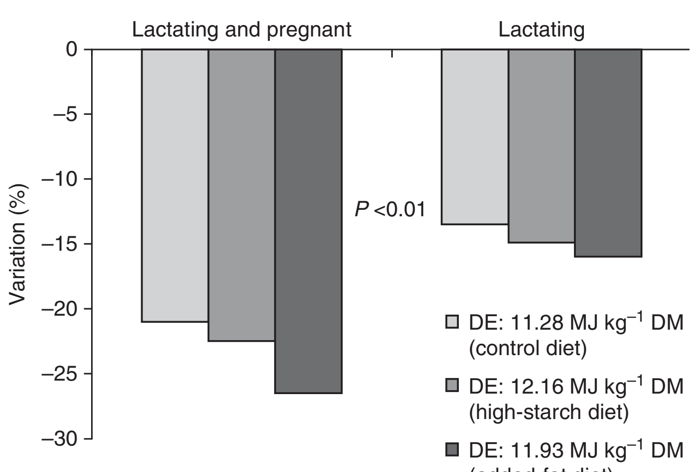

Fig. 6.6. Empty body energy balance (percentage variation on energy content at initial kindling) of does at different physiological states, given diets with increasing digestible energy (DE) concentrations (Xiccato *et al.*, 1995). DM, dry matter.

Table 6.11. Effect of dietary energy level on reproductive performance and empty body (EB) composition at day 28 of the first lactation in concurrently lactating and pregnant does (Fortun-Lamothe and Lebas, 1996).

	Dietary DE (MJ kg^{-1} DM)			
	9.9 (control diet)	12.1 (added-fat diet)	12.2 (starch diet)	Probability
Total milk production (0–21 days) (kg)	3.8^{a,b}	4.2^{a}	3.6^{b}	<0.05
Litter weight at day 28 (kg)	3.9^{b}	4.5^{a}	3.8^{b}	<0.05
EB energy (MJ kg^{-1})	7.85^{b}	8.76^{a}	9.22^{a}	<0.04

DE, digestible energy; EB, empty body.
^{a,b}Means on the same row with different subscript differ (P <0.05).

Table 6.12. Effect of dietary energy level on reproductive performance and energy balance of rabbit does at day 28 of the second lactation (Pascual *et al.*, 2000b).

	Dietary DE (MJ kg^{-1} DM)		
	11.0 (control diet)	12.4 (added-fat diet)	Probability
DE intake (kJ day^{-1} kg^{-1} LW$^{0.75}$)	1296	1445	<0.01
Milk production (g day^{-1})	191	237	<0.01
Litter weight at weaning (kg)	3.93	4.69	<0.01
EB energy gain (MJ)	−3.33	−3.42	NS

DE, digestible energy; EB, empty body; LW, live weight; NS, not significant.

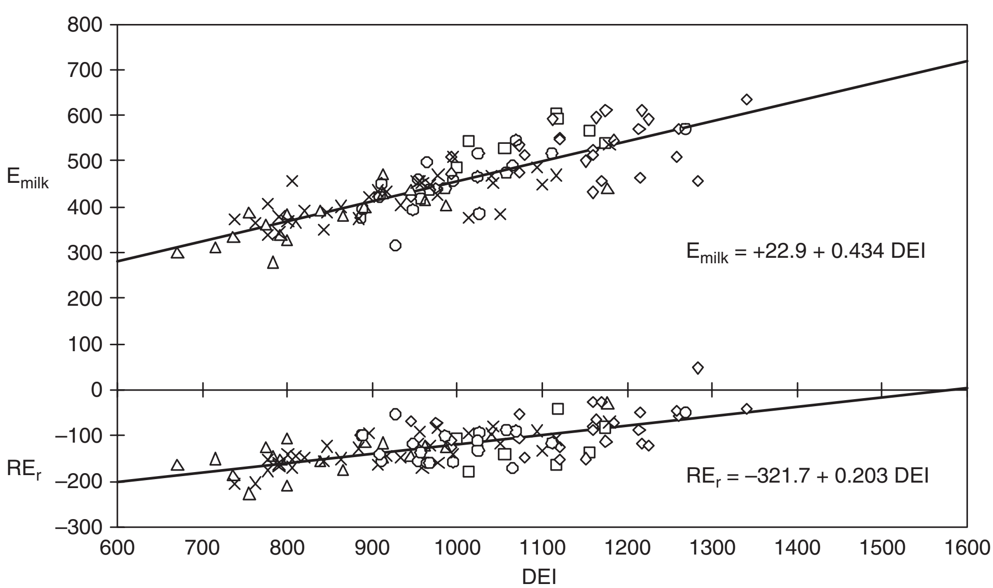

Fig. 6.7. Effect of digestible energy intake (DEI) on the energy excreted during milk production (E_{milk}) and the energy retained in the doe body (RE_r) (data expressed in kJ day^{-1} kg^{-1} LW$^{0.75}$) (Xiccato, 1996).

diet with 10.5 MJ DE kg^{-1}, this female must be able to ingest at least 150 g day^{-1} kg^{-1} LW$^{0.75}$ (i.e. about 450 g day^{-1}). Such an average voluntary intake during the entire period of lactation is unusual in primiparous and secundiparous does. Therefore, any intervention performed to stimulate energy intake will rarely provide a substantial reduction in the body energy deficit. In some cases, a contemporary increase in daily energy intake and milk production does not modify the rabbit's nutritional state.

In addition to stimulating milk production, any increase in DEI is generally associated with an increased feed intake. This leads to a faster digestive transit and a consequent reduction in the digestive utilization of the dietary energy, which makes the objective of solving the energy deficit even more difficult to achieve (Xiccato *et al.*, 1992a; de Blas *et al.*, 1995; Xiccato, 1996).

The nutritional deficit provoked by lactation also seems to be responsible for the decreased reproductive efficiency of concurrently lactating and pregnant does, and consequently for the reduction in fetal development and viability (Viudes-de-Castro *et al.*, 1991; Parigi Bini *et al.*, 1992; Fortun *et al.*, 1993; Fortun-Lamothe and Bolet, 1995; Fortun-Lamothe and Lebas, 1996). Similar negative effects have been described when feed restriction is used in reproducing rabbit does before mating and during pregnancy to avoid overfattening (Fortun *et al.*, 1994; Fortun-Lamothe, 1998).

6.3.10 Management strategies

Parity order

The occurrence of doe body energy deficit has been largely proven during the first lactation (Simplicio *et al.*, 1988; Parigi Bini *et al.*, 1989; Battaglini and Grandi, 1991; Castellini and Battaglini, 1991). Multiparous does are usually considered capable of ingesting higher amounts of feed therefore of achieving body energy and protein equilibrium. Substantial body fat and energy mobilization has, however, been observed in multiparous lactating does (Table 6.13) (Partridge *et al.*, 1983, 1986a; Pascual *et al.*, 2000b; Castellini *et al.*, 2003, 2006; Xiccato *et al.*, 2004, 2005b). Several authors have described significant increases (5–15%) in feed intake from the first to the second and from the second to the third kindling, followed by lower but not significant increases for successive parities (Parigi Bini *et al.*, 1989; Battaglini and Grandi, 1991; Castellini and Battaglini, 1991). DEI rises by 9% between the first and the second lactation, but only by 3% from

Table 6.13. Performance of lactating and concurrently pregnant does between initial and final kindling and energy balance (Xiccato *et al.*, 2004).

	Parity order			Probability	
	1st	2nd	3rd	L[a]	Q[b]
No. of does	22	20	27		
Total milk production (g)	4548	5023	5410	<0.001	NS
Total feed intake (g)					
During lactation	7276	7993	8313	<0.001	NS
From weaning to kindling	2721	2945	2888	NS	NS
DE intake (kJ day^{-1} kg^{-1} LW$^{0.75}$)					
During lactation	1099	1203	1237	<0.001	NS
From weaning to kindling	685	757	726	NS	NS
Chemical and energy balance[c]					
Water (%)	10.5	2.6	3.8	<0.001	<0.01
Protein (%)	–0.2	1.8	–1.1	NS	NS
Fat (%)	–33.0	–23.3	–20.2	<0.05	NS
Energy (%)	–20.5	–11.2	–9.2	<0.001	NS

DE, digestible energy; LW, live weight; NS, not significant.
[a]Linear component of variance.
[b]Quadratic component of variance.
[c]Change in body composition at initial kindling.

the second to the third lactation, while milk production increases by 10% and 8%, respectively (Xiccato *et al.*, 2004).

The unchanged substantial gap between dietary energy intake and milk energy output accounts for the body deficit that is also maintained at higher parities (Pascual *et al.*, 2000b; Castellini *et al.*, 2003, 2006; Xiccato *et al.*, 2004, 2005b). The total body energy of rabbit does lowers during the first lactation and remains constant until the third kindling in pregnant and lactating does, while it increases in non-pregnant does (Fig. 6.8) (Bolet and Fortun-Lamothe, 2002). In does submitted to a semi-intensive rhythm and traditional weaning, however, the body energy deficit not longer appears in females after their third kindling (Quevedo *et al.*, 2004). Different results may be ascribed to the rabbit strain (commercial hybrids or selected pure breeds) and the body balance measurement method (comparative slaughter, ultrasound technique, total body electric conductivity).

Breeding rhythm

On commercial farms, rabbit does are usually mated on a fixed day in the first weeks post-partum (PP); that is, 3–5 days PP (intensive rhythm), 10–12 or 17–19 days PP (semi-intensive rhythms) or 24–26 days PP (extensive rhythm). This determines exact theoretical intervals between two kindlings of 5, 6, 7 or 8 weeks, respectively. New schedules are now under study (post-weaning insemination associated with early weaning, alternate PP and post-weaning insemination, rhythms based on doe body condition score) that better fit doe physiology (Castellini *et al.*, 2003, 2006; Castellini, 2007; Bonanno *et al.*, 2008; Brecchia *et al.*, 2008; Cardinali *et al.*, 2008), but the most diffuse remating programme remains the semi-intensive rhythm. This rhythm is a compromise between the doe's need to recover energy between one reproductive cycle and the next and the economic demand of increasing the number of kits weaned per year (Fig. 6.9) (Mendez *et al.*, 1986; Parigi Bini *et al.*, 1989; Cervera *et al.*, 1993; Xiccato *et al.*, 2005b; Rebollar *et al.*, 2009). In fact, intensive PP insemination implies an excessive exploitation of the doe, which finally results in a reduction in reproductive performance and career length. On the other hand, extensive rhythms allow a too-low number of kindlings per year and can

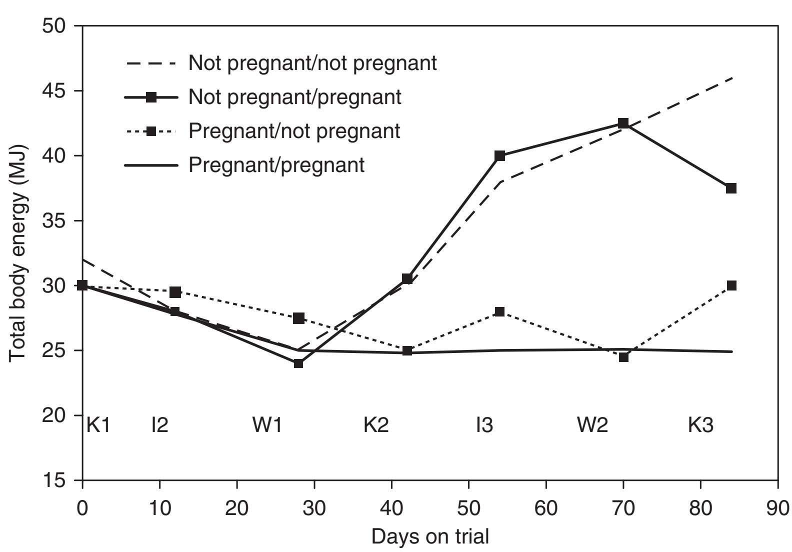

Fig. 6.8. Evolution of total empty body (EB) energy concentration (total body electrical conductivity measurements) in rabbit does from the first to third kindling, and according to their physiological state (I2, I3: 2nd, 3rd insemination; K1, K2, K3: 1st, 2nd, 3rd kindling; W1, W2: 1st, 2nd weaning) (from Bolet and Fortun-Lamothe, 2002).

cause doe overfattening, higher embryonic mortality and impairment of reproductive performance (Parigi Bini *et al.*, 1996).

The breeding system greatly affects the energy balance of lactating does, influencing both milk production and feed intake (Fig. 6.10). Does submitted to intensive reproductive rhythms begin showing decreased milk production after 15–17 days of lactation, with a sharper decrease in the last week of pregnancy (Lebas, 1972; Partridge *et al.*, 1986b; Fraga *et al.*, 1989) due to the exponential development of fetuses and hormonal changes caused by the imminent kindling that compromise milk production (Fortun-Lamothe *et al.*, 1999). The role of high prolactin and low progesterone levels in lactating does in reducing the fetal survival rate has been elucidated, and may interact with the effects of the nutritional deficit caused by

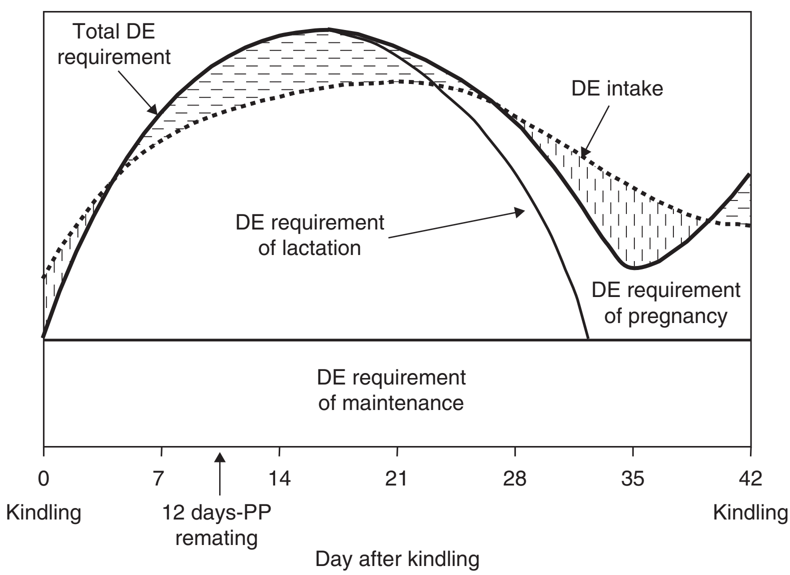

Fig. 6.9. Evolution of digestible energy (DE) requirement (full line) and of DE intake (dotted line) in does submitted to 12 days post-partum (PP) insemination. Areas with horizontal tracts indicate periods during which the female is in negative energy balance and utilizes body reserves; areas with vertical tracts indicate phases of positive balance and body energy recovery (Xiccato, 1996).

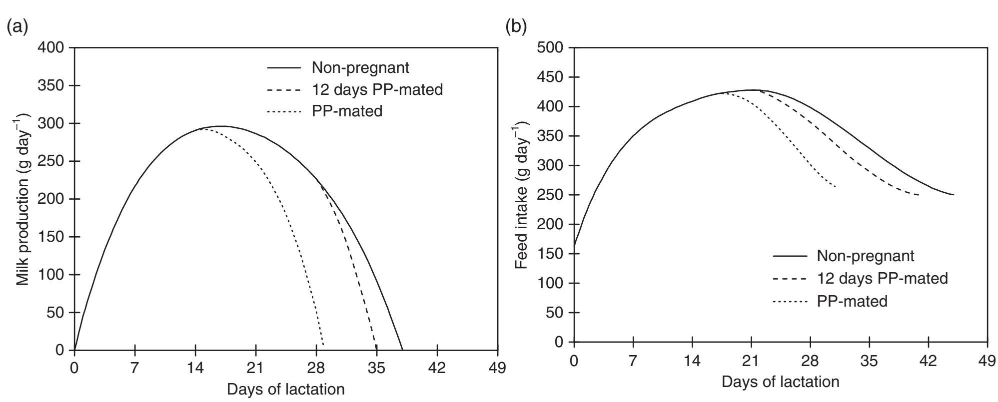

Fig. 6.10. Effect of breeding rhythm on (a) milk production and (b) feed intake (Xiccato, 1996). PP, post-partum.

lactation (Fortun-Lamothe and Prunier, 1999; Fortun-Lamothe *et al.*, 1999).

Lengthening the interval between kindlings prolongs the dry period and should permit body energy reserves to recover (Partridge *et al.*, 1984; Cervera *et al.*, 1993). In primiparous does, a severe body energy deficit has been observed within the first and second kindlings with insemination at 12 days PP (–26% of initial body energy content), but a less serious deficit (–15%) with insemination at 28 days PP (Fig. 6.11a) (Parigi Bini *et al.*, 1996). When multiparous does were submitted to early weaning (21 or 25 days), the body energy deficit disappeared in those submitted to semi-intensive (insemination at 12 days PP) and extensive (26 days PP) rhythms, but was severe in rabbits submitted to an intensive reproductive rhythm (2 days PP) (Fig. 6.11b) (Xiccato *et al.*, 2005b). The better body condition observed in this latter study, in which only the intensive rhythm provoked a substantial energy deficit, might be ascribed to the concurrent action of high parity order and early weaning age.

In primiparous and multiparous does submitted to standard (insemination at 11 days PP) or extended (insemination post-weaning at 27 days PP) reproductive rhythms, a body energy deficit was found in all does and did not change significantly with the insemination programme (Castellini *et al.*, 2006). However, the post-weaning rhythm improved reproductive performances and appeared more adapted to the doe physiology (Table 6.14).

Litter weaning age

Under field conditions, kits are usually separated from their mothers at around 32–35 days of age or even later. Previous research, in fact, reported a negative correlation between weaning weight and post-weaning mortality (Morisse, 1987; Lebas, 1993) and induced breeders to increase weight by delaying weaning age. On the other hand, more recent studies have demonstrated the possibility of successfully anticipating litter weaning age (de Blas *et al.*, 1999; Pascual, 2001; Xiccato *et al.*, 2003).

The greatest interest in early weaning lies in the possibility of reducing the doe body energy deficit by shortening the lactation length (the period of energy deficit with body energy utilized for milk synthesis) and prolonging the dry period (the period of energy surplus, with body energy restoration). In does at their first, second and third kindling, however, reducing weaning age from 32 to 21 days of age improved body energy balance (from –19% to –8% of the initial body energy content), but was unable to achieve equilibrium (Table 6.15) (Xiccato *et al.*, 2004). In multiparous does, weaning at 25 days did not prevent body energy deficit (–8% of the initial energy content), while weaning at 21 days resulted in a balance that

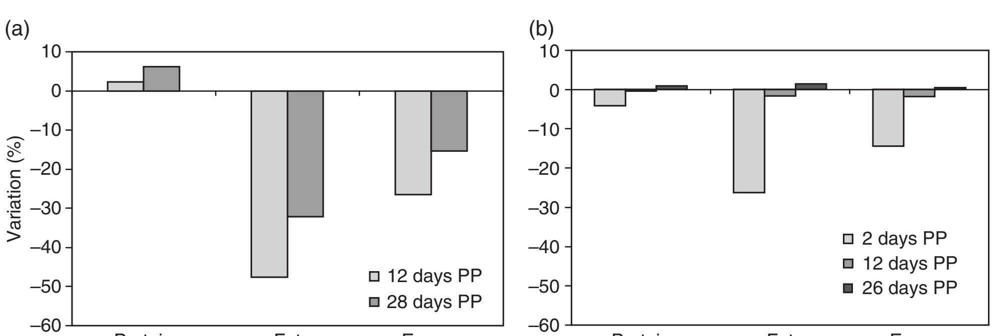

Fig. 6.11. Effect of reproductive rhythm on body protein, fat and energy balance (percentage variation between two successive kindling) at kindling in reproducing does: (a) primiparous does with litter weaning at 28 days (Parigi Bini *et al.*, 1996); (b) multiparous does, with an average of litter weaning at 21 and 25 days (Xiccato *et al.*, 2005b). PP, post-partum.

Table 6.14. Body energy balance and some productive traits in rabbit does inseminated at 11 days post-partum (control, C) or in the post-weaning (PW) period, 27 days post-partum (Castellini *et al.*, 2006).

	Primiparous does		Multiparous does		Probability	
Reproductive rhythm	C	PW	C	PW	Parity	Rhythm
DE intake during lactation (kJ day^{-1} kg^{-1} LW$^{0.75}$)	1046	1042	1220	1219	<0.01	NS
DE requirements[1] (kJ day^{-1} kg^{-1} LW$^{0.75}$)	1246	1228	1306	1313	<0.01	NS
Energy deficit (kJ day^{-1} kg^{-1} LW$^{0.75}$)[a]	162	149	86	94	<0.01	NS
Milk production (g day^{-1})	172	170	185	190	<0.01	NS
Sexual receptivity (%)	30.9	58.1	45.0	68.5	<0.01	<0.01
Fertility (%)	48.8	76.8	60.2	79.0	<0.05	<0.01
Live born (no.)	7.0	7.0	8.3	8.5	<0.01	NS
Litter size at weaning (no.)	6.2	6.0	6.9	7.1	<0.01	NS

DE, digestible energy; LW, live weight; NS, not significant.
[a]Estimated according to Parigi Bini and Xiccato (1998).

Table 6.15. Empty body balance of lactating and pregnant does between initial and final kindling (Xiccato *et al.*, 2004).

	Weaning age			
	21 days	26 days	32 days	Probability[a]
Protein (%)	1.8	−0.5	−0.8	NS
Fat (%)	−16.9	−24.5	−35.3	<0.01
Energy (%)	−8.0	−13.4	−19.4	<0.001

NS, not significant.
[a]Probability of the linear component of variance.

approached equilibrium (–3%) (Xiccato *et al.*, 2005b).

Early weaning failed definitively to avoid the energy deficit in these does because of the substantial decrease in feed intake after weaning (about 0.4–0.5 of lactation period). With standard weaning, in fact, feed intake remains at the highest levels during the last 10 days of lactation, thus partially permitting recovery of the body energy lost during the first 20 days. The sudden decrease in feed intake that occurs after early weaning reduces the daily energy surplus and delays the complete restoration of body reserves.

A negative effect on reproductive performance has also been described with a lower number of kits born and kits born alive per litter in multiparous does with weaning at 21 days (Xiccato *et al.*, 2004, 2005b). This could be ascribed to the marked effect of weaning on the metabolic and hormonal pattern at the time of fetus implantation (7–11 days of pregnancy) (Fortun-Lamothe and Bolet, 1995).

6.4 Protein Units and their Measurement

When speaking about nitrogen nutrition in rabbits, various units are available for expressing requirements (de Blas and Mateos, 1998; Fraga, 1998; Carabaño *et al.*, 2000, 2008; García *et al.*, 2005). Crude protein (CP) and apparent DP are the most commonly used units, for which both requirements and raw material composition are largely available (Villamide *et al.*, 1998; Maertens *et al.*, 2002). In reality, rabbits have specific amino acid requirements and apparent faecal and true ileal digestible amino acids would be more reliable units. However, even if increasing information is given on the amino acid concentrations of the most common raw materials, digestible amino acid requirements and concentration in feeds are barely known and even less information exists on ileal digestible amino acid (Carabaño *et al.*, 2008). In practice, due to the chemostatic regulation of appetite in rabbits, nitrogen requirements are expressed in relation to dietary energy by the DP to DE ratio, which is directly correlated to body nitrogen retention and excretion.

Besides optimizing productive performance, a correct dietary supply of protein and amino acids in growing and reproducing rabbits permits the maximization of nitrogen retention and reduces nitrogen excretion, which is of growing importance in view of controlling environmental pollution (Maertens *et al.*, 2005; Xiccato *et al.*, 2005a, 2006; Calvet *et al.*, 2008).

Table 6.16 summarizes literature data on DP requirements for maintenance (DP_m) and the efficiency of utilization of DP intake (DPI) and body protein reserves (RP_r) in the different categories of rabbits.

6.4.1 Maintenance requirements

In growing rabbits, DP_m is estimated to be 2.9 g DP day^{-1} kg^{-1} LW$^{0.75}$ (Partridge *et al.*, 1989; Fernández and Fraga, 1996; Motta Ferreira *et al.*, 1996; Fraga, 1998). Lower DP_m has been found in a new strain of laboratory rabbits (2.11–2.14 DP day^{-1} kg^{-1} LW$^{0.75}$), which was attributed to a lower basic metabolic rate (Lv *et al.*, 2009). In non-reproducing adult rabbits, since specific information is lacking, the same figures as for growing rabbits may be used for DP_m.

In lactating and concurrently lactating and pregnant does, protein requirements for maintenance have been estimated by using the comparative slaughter technique equal to 3.73 and 3.76–3.80 g DP kg^{-1} LW$^{0.75}$ (Parigi Bini *et al.*, 1991a, 1992; Xiccato *et al.*, 1992b; Parigi Bini and Xiccato, 1993).

6.4.2 Growth requirements

DP requirements vary according to the growth rate. The EB protein concentration changes from 120 g kg^{-1} (610 g kg^{-1} DM) at birth to 170 g kg^{-1} (680 g kg^{-1} DM) at weaning (35 days of age) and about 200 g kg^{-1} (590 g kg^{-1} DM) at 10–12 weeks of age. Afterwards, the body protein concentration is quite constant (200 g kg^{-1} of EBW, i.e. about 180 g kg^{-1} LW).

The efficiency of utilization of DPI for growth is estimated to be 0.56 (Partridge *et al.*, 1989; Fernández and Fraga, 1996; Motta Ferreira *et al.*, 1996; Fraga, 1998). Overall DP retention (RP/DPI) decreases linearly from 0.40 to 0.10 with increasing live weight, due to the increase in DP used for maintenance (Xiccato and Cinetto, 1988; Maertens *et al.*, 1997; Trocino *et al.*, 2000, 2001).

6.4.3 Pregnancy and lactation requirements

During the first pregnancy, rabbit does retain protein in their body in the early gestation (0–21 days), while they transfer some protein from their body to the rapidly growing fetuses in the late period of pregnancy (21–30 days) (Table 6.17)

Table 6.16. Digestible protein (DP) requirements for maintenance (DP_m) and the efficiency of utilization of DP intake (DPI) and body protein reserves (RP_r).

	Growing rabbits	Pregnant and/or lactating does	Non-reproducing does and bucks
DP_m (g day^{-1} kg^{-1} LW$^{0.75}$)	2.9	3.7	2.9
Efficiency of protein utilization:			
Body retained protein (RP/DPI)	0.56	–	0.56
RP as fetuses (P_{fetus}/DPI)	–	0.44	–
Milk protein from DP (P_{milk}/DPI)	–	0.78	–
Milk protein from body reserves (P_{milk}/RP_r)	–	0.60	–

LW, live weight.

Table 6.17. Partition of empty body gain and protein retention in pregnant does (Parigi Bini *et al.*, 1990a).

	0–21 days of pregnancy		21–30 days of pregnancy	
	Does	Pregnant uterus	Does	Pregnant uterus
Empty body gain (g)	180	193	–90	454
Retained protein (g)	44	18	–19	54

(Parigi Bini *et al.*, 1990a). This is due to the exponentially increasing protein requirements of the fetuses and the intense fetal protein turnover, which has been shown to be five times higher than that of maternal tissue, as observed in sheep by Young (1979). The efficiency of DP utilization for fetal protein synthesis is 0.42 and 0.46 in lactating and concurrently lactating and pregnant does, respectively (Parigi Bini *et al.*, 1992; Xiccato *et al.*, 1992b).

In lactating does, the coefficients of utilization of DP and maternal body protein for milk protein are estimated at 0.77 and 0.59, respectively. Similarly, in concurrently lactating and pregnant does the coefficients of utilization of DP and maternal body protein for milk protein are estimated at 0.76–0.80 and 0.60–0.61, respectively.

The high milk production and high milk protein concentration (110–130 g kg^{-1}) accounts for the high protein requirements for milk synthesis (Maertens *et al.*, 2006). When lactation and pregnancy overlap, as already described for energy, protein requirements also increase to a different extent depending on the reproductive rhythm. In concurrently pregnant and lactating does that are subjected to an intensive reproductive rhythm, limited body protein losses (5–10% of initial content) have been found (Table 6.18) (Parigi Bini *et al.*, 1992; Xiccato *et al.*, 1995, 2004). Sometimes, a negative protein balance has been reported in multiparous lactating does (Partridge *et al.*, 1986b; Pascual *et al.*, 2000b; Xiccato *et al.*, 2005b).

Table 6.18. Protein balance during the first lactation of does according to their physiological status (Xiccato *et al.*, 1995).

	Lactating does	Lactating and pregnant does
Empty body gain (g)	184	–131
Retained protein (g)	75	–38
Protein balance (%)	11	–6

6.4.4 DP to DE ratio

The dietary protein levels recommended for growing rabbits, young females and bucks range from 150 to 160 g CP kg^{-1} and from 105 to 110 g DP kg^{-1}. In reproducing does, CP from 175 to 190 g kg^{-1} and DP from 125 to 138 g kg^{-1} are recommended. These values correspond to a DP to DE ratio of 10.5–11.0 g MJ^{-1} for young rabbits and bucks and 11.5 to 12.5 g MJ^{-1} for reproducing does. The higher values are recommended for does under intensive breeding rhythms (Lebas, 1989; Maertens, 1992; Xiccato, 1996; de Blas and Mateos, 1998).

On the basis of more recent studies, the standard CP concentration of commercial diets seems to exceed rabbits' requirements around weaning and during growth (Maertens *et al.*, 1997; Trocino *et al.*, 2000, 2001; García-Palomares *et al.*, 2006a, b; Eiben *et al.*, 2008). In pregnant and lactating does, a reduction of the DP to DE ratio from 12.5 to 11.2 may decrease litter weight and size, while having less effect on the protein body balance (Table 6.19) (Xiccato *et al.,* 1992b). However, when milk production decreases (from 21 days of lactation to weaning) and the diet is adequately integrated for the most limiting amino acid, the DP to DE ratio may be lowered to 11.5 g MJ^{-1} (161 g CP kg^{-1}) without negative effects on the performance of rabbit does and their litters (García-Palomares *et al.*, 2006a).

Table 6.19. Effect of digestible protein (DP) to digestible energy (DE) ratio on reproductive performance and composition of empty body gain between first and second kindling (Xiccato *et al.*, 1992b).

	DP to DE ratio (g MJ^{-1})		
	12.5	11.2	Probability
Milk production (g day^{-1})	154	151	NS
Litter weight at 30 days (g)	4479	4367	NS
No. of kits born per litter	8.0	6.4	NS
Weight of kits born per litter (g)	474	351	<0.05
No. of kits born alive per litter	7.6	5.6	<0.10
Weight kits born alive per litter (g)	455	313	<0.05
Doe body protein gain (g)	+17	+4	NS

NS, not significant.

6.5 Amino Acid Requirements

The amino acid supply through caecotrophy has for a long time been considered adequate to support essential amino acid requirements in rabbits (de Blas and Mateos, 1998). In reality, in rabbits fed conventional diets, the contribution of soft faeces to total CP intake is only 0.15–0.18 (Fraga, 1998; Carabaño *et al.*, 2000), while limited information is available for the different amino acids. In lactating does, the contribution of caecotrophy has been found to make up 0.17 of the supply of sulphur amino acid, 0.18 of lysine and 0.21 of threonine (Nicodemus *et al.*, 1999). Recently, the microbial contribution through caecotrophy has been measured as equal to 0.23 for both tissue lysine in growing rabbits (Belenguer *et al.*, 2005) and milk lysine in lactating does (Abecia *et al.*, 2008).

On the whole, the literature on rabbit amino acid requirements is rather old and restricted to the most limiting amino acids in the diet (lysine, sulphur-containing amino acids, threonine and arginine). Therefore, the amino acid levels actually recommended are still those provided by Lebas (1989) and revised by de Blas and Mateos (1998).

The total amino acid requirements of rabbits have been mainly studied through dose–response trials (Fig. 6.12) or by using the amino acid pattern in body tissue and milk in relation to lysine according to the ideal protein concept (Table 6.20) (de Blas and Mateos, 1998; Fraga, 1998; Ball *et al.*, 2007). However, little attention has been paid to the amino acid partition and efficiency of utilization for maintenance and reproduction. More recently, specific needs for certain essential and non-essential amino acids (threonine, arginine, glutamate) have been hypothesized in order to optimize the defence mechanisms of the intestinal barrier against pathogens (Baylos *et al.*, 2008; Carabaño *et al.*, 2008; Chamorro *et al.*, 2010).

The most limiting essential amino acids in rabbit diets are methionine (and/or cystine) and lysine, immediately followed by threonine. A minimum level of 5.4 g total sulphur-containing amino acid kg^{-1} (4.0 g digestible amino acid kg^{-1}) is required to obtain adequate productivity in growing and non-reproducing rabbits. A higher level (6.3 g total amino acid kg^{-1} and 4.9 g digestible sulphur-containing amino acid kg^{-1}) is recommended for reproducing females to increase milk production, reduce the interval between parturitions and improve feed utilization efficiency (Taboada *et al.*, 1996). Recommended levels of lysine (for lactation diets with 10.5–11 MJ DE kg^{-1}) are 6.8 g total lysine kg^{-1} (5.2 g digestible lysine kg^{-1}) for maximum reproductive performance and 7.6–8.0 g total lysine (6.0–6.4 g kg^{-1} digestible lysine) kg^{-1} for maximum milk production and litter growth (Fig. 6.13) (Taboada *et al.*, 1994).

During the lactation peak (10–20 days), a minimum dietary concentration of 5.8 g total threonine kg^{-1} or 3.8 g digestible threonine kg^{-1} is necessary to maximize feed intake and

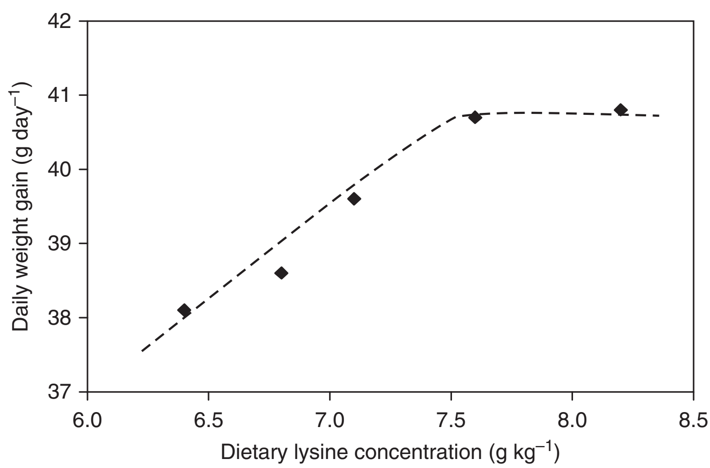

Fig. 6.12. Dose–response daily weight gain to an increasing dietary lysine concentration in growing rabbits (Taboada *et al.*, 1994).

Table 6.20. Amino acid composition of the whole body of growing rabbits and rabbit milk relative to lysine concentration (= 100) (Fraga, 1998; Carabaño *et al.*, 2000).

	Whole body	Milk
Lysine	100	100
Alanine	74	50
Arginine	108	73
Aspartic acid	121	100
Histidine	50	35
Isoleucine	51	67
Leucine	112	125
Methionine	20	33
Cystine	41	39
Glutamic acid	205	270
Glycine	121	23
Phenylalanine	65	62
Serine	74	50
Threonine	64	67
Tyrosine	50	73
Valine	62	85

milk production. The optimum supply is 6.4g total threonine kg^{-1} and 4.4g digestible threonine kg^{-1}, while higher or lower values impair both the number of weaned kits and feed efficiency (de Blas *et al.*, 1998).

6.6 Protein Retention and Nitrogen Excretion

In highly populated areas, vulnerable from a hydrogeologic point of view, animal waste can represent a potential contaminant of water and soil. The European Directive 91/676/EC aims to prevent or reduce the nitrate pollution of surface and underground water, and asks each member to state reference values for nitrogen excretion of all livestock as well as to define feeding and management strategies to control environmental pollution.

Nitrogen excretion cannot be measured directly because of the considerable loss of nitrogen (through volatile ammonia) from urine and faeces during waste stocking and treatment. According to the official methodology (ERM/AB-DLO, 1999), nitrogen excretion is quantified as the difference between nitrogen consumption and nitrogen retention in animal products; that is, for rabbits, body and fetal tissues and milk. Since nitrogen concentration in the body tissues of finishing rabbits is fairly constant (29–32g kg^{-1}) and the nitrogen of fetal tissues and milk is destined to be transferred into the body of fatteners, the farm nitrogen balance of rabbits can be calculated as the difference between the nitrogen input (dietary nitrogen) and the nitrogen output (produced rabbits) at the farm.

Various factors can affect farm nitrogen balance, both in the reproductive and in the fattening sectors (Maertens *et al.*, 2005; Xiccato *et al.*, 2005a). The role of feeding and management factors is discussed below.

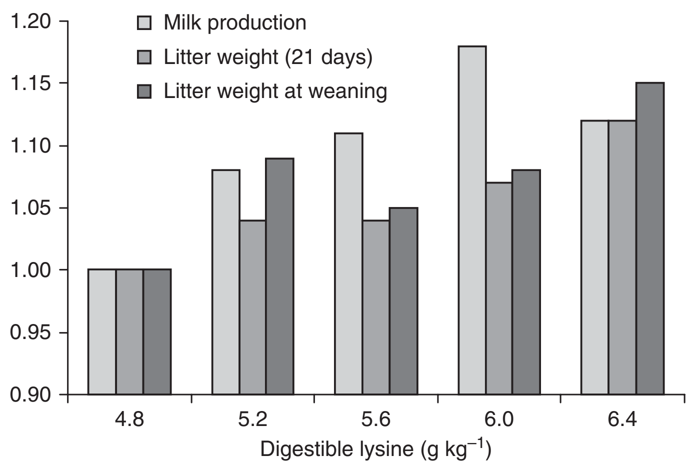

Fig. 6.13. Effect of digestible lysine concentration of the diet (g kg^{-1}) on productive traits (4.8 g kg^{-1} = 1) (from Taboada *et al.*, 1994).

6.6.1 Dietary protein level

Nitrogen excretion is strictly dependant on the dietary CP level. In fattening rabbits, once the limiting amino acid requirements are satisfied by synthetic amino acid supplementation, dietary CP may be reduced to <170 g kg^{-1}, therefore decreasing nitrogen excretion without impairing productive performance (Maertens *et al.*, 1997). Daily weight gain is impaired only at <138 g CP kg^{-1} (–0.09), but nitrogen excretion is reduced by 0.38 (Fig. 6.14).

When rabbits are fed a diet supplemented with the most limiting amino acid until slaughter at 63 days of age and 2.35 kg LW, decreasing dietary CP from 160 to 140 g kg^{-1} does not impair growth performance (García-Palomares *et al.*, 2006b). When rabbits are slaughtered later (75–90 days) at a heavier LW (2.5–3.0 kg), feeding programmes based on decreasing dietary CP would permit a better coverage of the higher protein requirements of the first growth period and reduce excretion during fattening. In fact, in this latter period feed intake is higher and dietary nitrogen concentration can be reduced without impacting performance and meat quality (Maertens *et al.*, 1997; Maertens and Luzi, 1998; Trocino *et al.*, 2000, 2001). Lowering dietary CP from 160 to 140 g kg^{-1} in the first period, from 32 to 56 days of age,

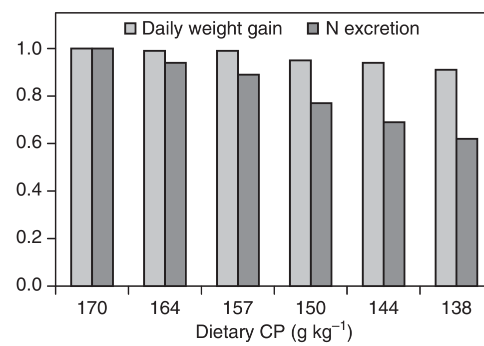

Fig. 6.14. Daily weight gain and nitrogen (N) excretion in rabbits (32–74 days of age) according to dietary crude protein (CP) concentration (170 g CP kg^{-1} = 1) (Maertens *et al.*, 1997).

reduces daily growth and body nitrogen retention (–0.06) and nitrogen excretion to a similar extent (–0.07) (Trocino *et al.*, 2000). In the second period (56–77 days), a reduction of dietary CP from 154 to 143 g kg^{-1} decreases nitrogen excretion by 0.09 without impairing daily gain and body nitrogen retention. A further decrease of dietary CP until 131 g kg^{-1} permits a lowering of nitrogen excretion by 0.15 in comparison with the control diet, while reducing growth and nitrogen retention by only 0.03.

In reproducing does, protein and amino acid requirements are largely satisfied by the current lactation diets. Therefore, as mentioned above, a reduction of dietary CP during lactation until 170 g kg^{-1} does not affect doe reproductive performance, milk yield or litter growth (Xiccato *et al.*, 1992b; García-Palomares *et al.*, 2006a). Taking into account that the lactation diet represents about a third of the total feed consumed in a closed-cycle farm (reproduction and fattening sectors), advantages in terms of reducing nitrogen excretion are of great importance.

6.6.2 Dietary energy level and DP to DE ratio

High-fibre, low-starch diets with low DE concentration have been largely used in the last decade to reduce the risk of digestive diseases such as rabbit epizootic enteropathy (Gidenne, 2003; Gidenne and García, 2006). However, when lowering DE concentration, feed intake increases and, if dietary CP concentration remains unchanged, the DP to DE ratio and nitrogen intake increase. Since growth rate is not modified and nitrogen retention remains constant, nitrogen excretion increases. As an example, when DE concentration decreases from 10.5 to 8.8 MJ kg^{-1} and dietary CP concentration is maintained at 150 g kg^{-1} with 0.70 digestibility, the DP to DE ratio increases from 10 to 12 g MJ^{-1}. As shown in Fig. 6.15, body nitrogen retention remains unchanged while daily nitrogen excretion (faecal plus urinary) increases by 0.20.

6.6.3 Numerical productivity of rabbit does and slaughter weight

Numerical productivity (i.e. the number of rabbits produced per doe per year) directly affects the amount of excreted nitrogen on a farm and is in its turn influenced by several factors. The use of more or less intensive reproductive rhythms results in great differences in reproductive efficiency (Maertens *et al.*, 2005). The number of rabbits produced per doe per year can vary from 35–40 in does submitted to extensive rhythms (post-weaning mating) to 45–50 in those undergoing intensive rhythms (mating 5–12 days PP). In a closed-cycle farm, with both reproductive and fattening sectors, nitrogen excretion can be referred to the reproducing doe, including its offspring produced during a year. In this case, excreted nitrogen per doe per year depends both on numerical productivity and on the slaughter weight of fatteners. According to the theoretical model proposed by Maertens *et al.* (2005), excreted nitrogen increases from 5.24 kg year^{-1} per doe producing 35 fatteners of 2.25 kg slaughter weight to 9.25 kg year^{-1} per doe producing 50 fatteners slaughtered at 2.75 kg (Table 6.21).

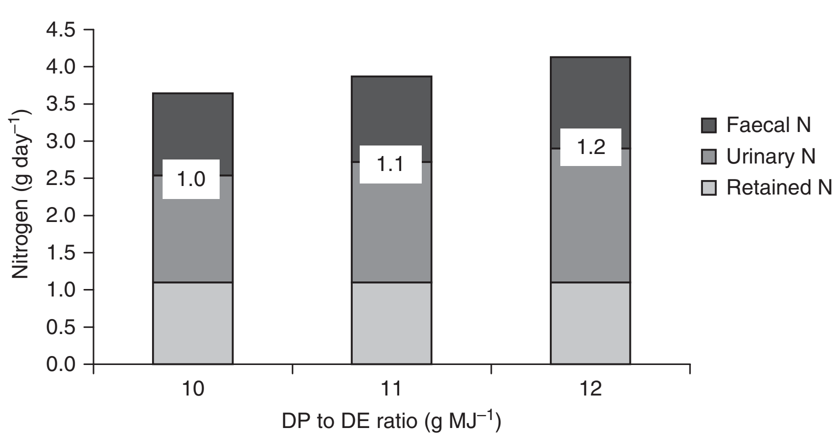

Fig. 6.15. Daily nitrogen (N) retention and excretion (faeces plus urine) according to the dietary digestible protein (DP) to digestible energy (DE) ratio (Xiccato *et al.*, 2006). The numbers on the bars represent daily nitrogen excretion (10g DP MJ^{-1} DE = 1.0).

Table 6.21. Nitrogen excretion (kg doe year^{-1}) according to doe numerical productivity (rabbits produced doe year^{-1}) and fattener slaughter weight (Maertens *et al.*, 2005).

	Rabbits produced per doe year^{-1}			
Slaughter weight (kg)	35	40	45	50
2.25	5.24	5.60	5.97	6.37
2.50	6.65	6.93	7.42	7.68
2.75	8.42	8.82	9.08	9.25

The model above is calculated on the basis of a constant dietary CP content (on average, 165 g kg^{-1}) and feed efficiency increasing with doe numerical productivity. With an average CP concentration of feeds consumed on a farm (approximately one-third lactation diet, one-third weaning diet and one-third fattening diet) of 170 g kg^{-1} and 45 rabbits produced per doe year^{-1}, nitrogen excretion of the doe and its offspring increases from 6.23 to 9.50 kg per doe year^{-1} depending on the slaughter weight (Table 6.22). A reduction in the average CP level from 170 to 160 g kg^{-1} permits a decrease of total nitrogen excretion by 0.08–0.10 (Xiccato *et al.*, 2006).

If the nitrogen excretion values listed in Table 6.21 are divided by the number of rabbits produced per year, excreted nitrogen decreases from 150 to 127 g per rabbit of 2.25 kg LW as the doe numerical productivity increases from 35 to 50 rabbits produced per doe year^{1}. With rabbits sold at 2.75 kg LW, the nitrogen excreted varies from 241 to 185 g per rabbit as the doe numerical productivity increases. If excretion is referred to by the weight (kg) of rabbits, excreted nitrogen is less variable (57–87 g N kg^{-1} rabbit produced).

The theoretical values arising from the model proposed by Maertens *et al.* (2005) have been confirmed by field data collected from 48 Italian closed-cycle farms (Xiccato *et al.*, 2005a): the does and their offspring (43 rabbits produced per doe year^{-1}, slaughtered at 2.65 kg LW) ingested on average 11.2 kg N year^{-1} and retained 3.8 kg N year^{-1}, thus excreting 7.4 kg N year^{-1}. If nitrogen excretion is expressed per rabbit or kg produced in the farm, excreted nitrogen is 173±16 g per rabbit or 65±5 g kg^{-1} (Xiccato *et al.*, 2007). The overall nitrogen or CP efficiency (retained nitrogen/ingested nitrogen) of the Italian productive system of rabbit meat is 0.34 (3.8 kg retained nitrogen per doe year^{-1}/11.2 kg ingested nitrogen per does year^{-1}). In Spain, lower values of excreted nitrogen (48 g per rabbit and 41 g kg^{-1}) and higher overall nitrogen retention (>0.40 of ingested nitrogen) have been found, because the nitrogen ingested during the reproductive and weaning phases was not included in the balance and the slaughter LW was lower (1.8 kg) (Calvet *et al.*, 2008).

Table 6.22. Nitrogen excretion (kg per doe year^{-1}) according to the average dietary crude protein (CP) level and slaughter weight of fattening rabbits (a closed-cycle farm with 45 rabbits produced per doe year^{-1}) (Xiccato *et al.*, 2006).

	Dietary CP (g kg^{-1})	
Slaughter weight (kg)	170	160
2.25	6.23	5.67
2.50	7.75	7.08
2.75	9.50	8.71

References

Abecia, L., Balcells, J., Fondevila, M., Belenguer, A., Holtrop, G. and Lobley, G.E. (2008) Contribution of gut microbial lysine to liver and milk amino acids in lactating does. *British Journal of Nutrition* 100, 977–983.

Ball, R.O., Urschel, K.L. and Pencharz, P.B. (2007) Nutritional consequences of interspecies differences in arginine and lysine metabolism. *Journal of Nutrition* 137 (Suppl.), 1626S–1641S.

Barreto, G. and de Blas, J.C. (1993) Effect of dietary fibre and fat content on the reproductive performance of rabbit does bred at two remating times during two seasons. *World Rabbit Science* 1, 77–81.

Battaglini, M. and Grandi, A. (1991) Effetto della fase fisiologica, della stagione e dell'ordine di parto sul comportamento alimentare della coniglia fattrice. In: *Proceedings IX Congresso Nazionale ASPA*, *Rome*, Vol. I, pp. 465–475.

Baylos, M., Menoyo, D., Chamorro, S., Sainz, A., Nicodemus, N., de Blas, C. and Carabaño, R. (2008) Effect of dietary level and source of glutamine on intestinal health in the postweaning period. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress*, *Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 529–534.

Belenguer, A., Balcells, J., Guada, J.A., Decoux, M. and Milne, E. (2005) Protein recycling in growing rabbits: contribution of microbial lysine to amino acid metabolism. *British Journal of Nutrition* 94, 763–770.

Blaxter, K. (1989) *Energy Metabolism in Animals and Man*. Cambridge University Press, Cambridge, UK.

Bolet, G. and Fortun-Lamothe, L. (2002) Relationship between body composition and reproductive performance in rabbit does. Cost Action 848 Joint Scientific Meeting, Ispra, 2–25 October, 2002, p. 48.

Bonanno, A., Mazza, F., Di Grigoli, A. and Alicata, M.L. (2004) Effects of restricted feeding during rearing, combined with a delayed first insemination, on reproductive activity of rabbit does. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Mexico, pp. 224–230.

Bonanno, A., Mazza, F., Di Grigoli, A. and Alicata, M.L. (2008) Body condition score and related productive responses in rabbit does. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress*, *Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 297–302.

Brecchia, G., Cardinali, R., Dal Bosco, A., Boiti, C. and Castellini, C. (2008) Effect of a reproductive rhythm based on rabbit doe body condition on fertility and hormones. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress*, *Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 309–313.

Calvet, S., Estellés, F., Hermida, B., Blumetto, O. and Torres, A.G. (2008) Experimental balance to estimate efficiency in the use of nitrogen in rabbit breeding. *World Rabbit Science* 16, 205–211.

Cambero, M.I., De La Hoz, L., Sanz, B. and Ordóñez, J.A. (1991) Lipid and fatty acid composition of rabbit meat. Part 2 – phospholipids. *Meat Science* 29, 167–176.

Carabaño, R., de Blas, J.C. and García, A.I. (2000) Recent advances in nitrogen nutrition in rabbits. *World Rabbit Science* 8 (Suppl. 1), 14–28.

Carabaño, R., Villamide, M.J., García, I., Nicodemus, N., Llorente, A., Chamorro, S., Menoyo, D., García-Rebollar, P., García-Ruiz, A.I. and de Blas, J.C. (2008) New concepts and objectives for protein-amino acid nutrition in rabbits. In: Xiccato, G., Trocino, A. and Lukefhar, S.D. (eds) *Proceedings of the 9th World Rabbit Congress*, *Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 135–155.

Cardinali, R., Dal Bosco, A., Bonanno, A., Di Grigoli, A., Rebollar, P.G., Lorenzo, P.L. and Castellini, C. (2008) Connection between body condition score, chemical characteristics of body and reproductive traits of rabbit does. *Livestock Science* 116, 209–215.

Castellini, C. (2007) Reproductive activity and welfare of rabbit does. *Italian Journal of Animal Science* 6 (Suppl.1), 743–747.

Castellini, C. and Battaglini, M. (1991) Influenza della concentrazione energetica della razione e del ritmo riproduttivo sulle performance delle coniglie. In: *Proceedings IX Congresso Nazionale ASPA*, *Rome*, Vol. I, pp. 477–488.

Castellini, C., Dal Bosco, A. and Mugnai, C. (2003) Comparison of different protocols for rabbit does: effect of litter size and mating interval. *Livestock Production Science* 83, 131–139.

Castellini, C., Dal Bosco, A. and Cardinali, R. (2006) Long term effect of post-weaning rhythm on the body fat and performance of rabbit does. *Reproduction Nutrition Development* 46, 195–204.

Cervera, C., Fernández-Carmona, J., Viudes, P. and Blas, E. (1993) Effect of remating interval and diet on the performance of female rabbits and their litters. *Animal Production* 56, 399–405.

Chamorro, S., de Blas, C., Grant, G., Badiola, I., Menoyo, D. and Carabaño, R. (2010) Effect of dietary supplementation with glutamine and a combination glutamine-arginine on intestinal health in twenty five-day-old weaned rabbits. *Journal of Animal Science* 88, 170-180.

Cheeke, P.R. (1987) *Rabbit Feeding and Nutrition*. Academic Press, Orlando, Florida, USA.

Close, W.H. (1990) The evaluation of feeds through calorimetry studies. In: Wiseman, J. and Cole, D.J.A. (eds) *Feedstuffs Evaluation*. Butterworths, London, UK, pp. 21–39.

de Blas, J.C. and Mateos, G.G. (1998) Feed formulation. In: de Blas, C. and Wiseman, J. (eds) *The Nutrition of the Rabbit*. CABI Publishing, Wallingford, UK, pp. 241–253.

de Blas, J.C., Fraga, M. and Rodriguez, J. (1985) Units for feed evaluation and requirements for commercially grown rabbits. *Journal of Animal Science* 60, 1021–1028.
de Blas, J.C., Taboada, E., Mateos, G., Nicodemus, N. and Mendez, J. (1995) Effect of substitution of starch for fibre and fat in isoenergetic diets on nutrient digestibility and reproductive performance of rabbits. *Journal of Animal Science* 73, 1131–1137.
de Blas, J.C., Taboada, E., Nicodemus, N., Campos, R., Piquer, J. and Méndez, J. (1998) Performance response of lactating and growing rabbits to dietary threonine content. *Animal Feed Science and Technology* 70, 151–160.
de Blas, J.C., Gutiérrez, I. and Carabaño, R. (1999) Destete precoz en gazapos. Situación actual y perspectivas. In: Rebollar, G., de Blas, J.C. and Mateos, G.M. (eds) *Advances en Nutrición Animal*. XV Curso de Especialización FEDNA, Ediciones Peninsular, Madrid, Spain, pp. 67–81.
De Rochambeau, H. (1990) Génétique du lapin domestique pour la production de poil et la production de viande: revue bibliographique 1984–1987. *Cuni-Science* 6, 17–48.
Eiben, Cs., Gippert, T., Gódor-Surmann, K., Podmaniczky, B. and Kustos, K. (2008) Influence of dietary protein reduction and enzyme and/or amino acid supplementation on fattening performance of rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 637–642.
ERM/AB-DLO (1999) Establishment of criteria for the assessment of the nitrogen content of animal manures. European Commission, Final Report, Luxembourg.
Fekete, S. (1992) The rabbit body composition: methods of measurement, significance of its knowledge and the obtained results. A critical review. *Journal of Applied Rabbit Research* 15, 72–85.
Fernández, C. and Fraga, M.J. (1996) Effect of fat inclusion in diets for rabbits on the efficiency of digestible energy and protein utilization. *World Rabbit Science* 4, 19–23.
Fortun, L., Prunier, A. and Lebas, F. (1993) Effect of lactation on fetal survival and development in rabbit does mated shortly after parturition. *Journal of Animal Science* 71, 1882–1886.
Fortun, L., Prunier, A., Étienne, M. and Lebas, F. (1994) Influence of the nutritional deficit of fœtal survival and growth and plasma metabolites in rabbit does. *Reproduction Nutrition Development* 34, 201–211.
Fortun-Lamothe, F. (1998) Effect of pre-mating energy intake on reproductive performance of rabbit does. *Animal Science* 66, 263–269.
Fortun-Lamothe, L. and Bolet, G. (1995) Les effets de la lactation sur les performances de reproduction chez la lapine. *INRA Production Animal* 8, 49–56.
Fortun-Lamothe, L. and Lebas, F. (1996) Effects of dietary energy level and source on foetal development and energy balance in concurrently pregnant and lactating primiparous rabbit does. *Animal Science* 62, 615–620.
Fortun-Lamothe, F. and Prunier, A. (1999) Effects of lactation, energetic deficit and remating interval on reproductive performance of primiparous rabbit does. *Animal Reproduction Science* 55, 289–298.
Fortun-Lamothe, L., Prunier, A., Bolet, G. and Lebas, F. (1999) Physiological mechanism involved in the effects of concurrent pregnancy and lactation on foetal growth and mortality in the rabbit. *Livestock Production Science* 60, 229–241.
Fraga, M.J. (1998) Protein requirements. In: de Blas, C. and Wiseman, J. (eds) *The Nutrition of the Rabbit*. CABI Publishing, Wallingford, UK, pp. 133–143.
Fraga, M.J., Lorente, M., Carabaño, R. and de Blas, J.C. (1989) Effect of diet and remating interval on milk production and milk composition of the doe rabbit. *Animal Production* 48, 459–466.
García, A.I., de Blas, J.C. and Carabaño, R. (2005) Comparison of different methods for nitrogen and amino acid evaluation in rabbit diets. *Animal Science* 80, 169–178.
García-Palomares, J., Carabaño, R., García-Rebollar, P., de Blas, J.C. and García A.I. (2006a) Effects of dietary protein reduction during weaning on the performance of does and suckling rabbits. *World Rabbit Science* 14, 23–26.
García-Palomares, J., Carabaño, R., García-Rebollar, P., de Blas, J.C., Corujo, A. and García-Ruiz, A.I. (2006b) Effects of a dietary protein reduction and enzyme supplementation on growth performance in the fattening period. *World Rabbit Science* 14, 231–236.
Gidenne, T. (2003) Fibres in rabbit feeding for digestive troubles prevention: respective role of low-digested and digestible fibre. *Livestock Production Science* 81, 105–117.
Gidenne, T. and García, J. (2006) Nutritional strategies improving the digestive health of the weaned rabbit. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Science*. ILVO, Melle, Belgium, pp. 229–238.

Houseknecht, K. L. and Spurlock, M. (2003) Leptin regulation of lipid homeostasis: dietary and metabolic implications. *Nutrition Research Reviews* 16, 83–96.
Hulot, F., Ouhayoun, J. and Dalle Zotte, A. (1992) The effects of recombinant porcine somatotropin on rabbit growth, feed efficiency and body composition. *Journal of Applied Rabbit Research* 15, 832–840.
Isar, O. (1981) Energy requirement of meat rabbits. *Lucrari stiintifice ale Institutului de Cercetai pentru Nutritia Animalelor/Ministerul Agriculturii si Industriei Alimentare, Academia de Stiinte Agricole si Silvice (Romania)* 9/10, 253–264.
Jean-Blain, C. and Durix, A. (1985) Ketone body metabolism during pregnancy in the rabbit. *Reproduction Nutrition Development* 25, 545–554.
Khalil, M.H. and Al-Saef, A.M. (2008) Methods, criteria, techniques and genetic responses for rabbit selection: a review. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress*, *Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 3–34.
Lebas, F. (1971) Composition minérale du lait de lapine. Variations en fonction du stade de lactation. *Annales des Zootechnie* 20, 487–495.
Lebas, F. (1972) Effet de la simultanéité de la lactation et de la gestation sur les performances laitières chez la lapine. *Annales des Zootechnie* 21, 129–131.
Lebas, F. (1989) Besoins nutritionnels des lapins. Revue bibliographique et perspectives. *Cuni-Sciences* 5, 1–28.
Lebas, F. (1993) Amélioration de la viabilité des lapereaux en engraissement par un sevrage tardif. *Cuniculture* 20, 73–75.
Lebas, F., Coudert, P., Rouvier, R. and De Rochambeau, H. (1984) *Le Lapin: Élevage et Pathologie*. FAO, Rome, Italy.
Lofgreen, G.P. and Garret, W.N. (1968) A system for expressing net energy requirements and feed values for growing and finishing beef cattle. *Journal of Animal Science* 27, 793–806.
Lv, J.-M., Chen, M.-LI, Qian, L.-C. and Ying, H.-Z. (2009) Requirement of crude protein for maintenance in a new strain of laboratory rabbit. *Animal Feed Science and Technology* 151, 261–267.
Maertens, L. (1992) Rabbit nutrition and feeding: a review of some recent developments. *Journal of Applied Rabbit Research* 15, 889–913.
Maertens, L. and De Groote, G. (1988) The influence of the dietary energy content on the performances of post-partum breeding does. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 3. Sandor Holdas, Hercegalom, Budapest, Hungary, pp. 42–52.
Maertens, L. and Luzi, F. (1998) Effetto della diluizione proteica nella dieta sulle performance e l'eliminazione dell'azoto dei conigli in accrescimento. *Rivista di Coniglicoltura* 35(3), 51–55.
Maertens, L., Luzi, F. and De Groote, G. (1997) Effect of dietary protein and amino acids on the performance, carcass composition and N-excretion of growing rabbits. *Annales des Zootechnie* 46, 255–268.
Maertens, L., Perez, J.M., Villamide, M., Cervera, C., Gidenne, T. and Xiccato, G. (2002) Nutritive value of raw materials for rabbits: EGRAN Tables 2002. *World Rabbit Science* 10, 157–166.
Maertens, L., Cavani, C. and Petracci, M. (2005) Nitrogen and phosphorus excretion on commercial rabbit farms: calculations based on the input-output balance. *World Rabbit Science* 13, 1–14.
Maertens, L., Lebas, F. and Szendrő, Zs. (2006) Rabbit milk: a review of quantity, quality and non-dietary affecting factors. *World Rabbit Science* 14, 205–230.
Matics, Zs., Nagy, I., Biró-Németh, E., Radnai, I., Gerencsér, Zs., Princz, Z. and Szendrő, Zs. (2008) Effect of feeding regime during rearing and age at first mating on the reproductive performance of rabbits does. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress*, *Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 399–404.
Mendez, J., de Blas, J.C. and Fraga, M. (1986) The effects of diet and remating interval after parturition on the reproductive performance of the commercial doe rabbit. *Journal of Animal Science* 62, 1624–1634.
Milisits, G., Romvári, R., Dalle Zotte, A., Xiccato, G. and Szendrő, Zs. (1996) Determination of body composition changes of pregnant does by X-ray computerised tomography. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 207–212.
Milisits, G., Romvári, R., Dalle Zotte, A. and Szendrő, Zs. (1999) Non-invasive study of changes in body composition in rabbits during pregnancy using X-ray computerized tomography. *Annales des Zootechnie* 48, 25–43.
Morisse, J.P. (1987) Factors affecting susceptibility of rabbit litters to enteritis. *Journal of Applied Rabbit Research* 10, 106–110.

Motta Ferreira, W., Fraga, M.J. and Carabaño, R. (1996) Inclusion of grape pomace, in substitution for lucerne hay, in diets for growing rabbits. *Animal Science* 63, 167–174.

Nicodemus, N., Mateos, J., de Blas, J.C., Carabaño, R. and Fraga, M. (1999) Effect of diet on amino acid composition of soft faeces and the contribution of soft faeces to total amino acid intake, through caecotrophy in lactating doe rabbits. *Animal Science* 69, 167–170.

Nizza, A., Moniello, G. and Di Lella, T. (1995) Prestazioni produttive e metabolismo energetico di conigli in accrescimento in funzione della stagione e della fonte proteica alimentare. *Zootecnica Nutrizione Animale* 21, 173–183.

Nizza, A., Di Meo, C. and Esposito, L. (1997) Influence of the diet used before and after the first mating on reproductive performance of rabbit does. *World Rabbit Science* 5, 107–110.

Noblet, J. and Close, V.H. (1980) The energy cost of pregnancy in the sow. In: Mount, L.E. (ed.) *Proceedings of the 8th Symposium on Energy Metabolism*. EEAP Publ. No. 26, Butterworth, London, pp. 335–339.

Ortiz, V., de Blas, C. and Sanz, E. (1989) Effect of dietary fibre and fat content on energy balance in fattening rabbits. *Journal of Applied Rabbit Research* 12, 159–162.

Parigi Bini, R. (1988) Recent developments and future goals in research on nutrition of intensively reared rabbits. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 3. Sandor Holdas, Hercegalom, Budapest, Hungary, pp. 1–29.

Parigi Bini, R. and Cesselli, P. (1977) Valutazione dell'escrezione urinaria di energia in conigli in accrescimento. *Rivista Zootecnica e Veterinaria* 5, 130–137.

Parigi Bini, R. and Dalle Rive, V. (1978) Utilizzazione dell'energia metabolizzabile per la sintesi di proteine e grassi nei conigli in accrescimento. *Rivista Zootecnica e Veterinaria* 6, 242–248.

Parigi Bini, R. and Xiccato, G. (1986) Utilizzazione dell'energia e della proteina digeribile nel coniglio in accrescimento. *Rivista di Coniglicoltura* 23(4), 54–56.

Parigi Bini, R. and Xiccato, G. (1993) Recherches sur l'interaction entre alimentation, reproduction er lactation chez la lapine. Une revue. *World Rabbit Science* 1, 155–161.

Parigi Bini, R. and Xiccato, G. (1998) Energy metabolism and requirements. In: de Blas, C. and Wiseman, J. (eds) *The Nutrition of the Rabbit*. CABI Publishing, Wallingford, UK, pp. 103–131.

Parigi Bini, R., Chiericato, G.M. and Lanari, D. (1974) I mangimi grassati nel coniglio in accrescimento. Digeribilità e utilizzazione energetica. *Rivista Zootecnica e Veterinaria* 2, 193–202.

Parigi Bini, R., Dalle Rive, V. and Mazzarella, M. (1978) Fabbisogni di energia netta dei conigli in accrescimento. *Rivista Zootecnica e Veterinaria* 6, 32–36.

Parigi Bini, R., Xiccato, G. and Cinetto, M. (1989) Influenza dell'intervallo parto-accoppiamento sulle prestazioni riproduttive delle coniglie fattrici. *Rivista di Coniglicoltura* 26 (7), 51–57.

Parigi Bini, R., Xiccato, G. and Cinetto, M. (1990a) Energy and protein retention and partition in pregnant and non-pregnant rabbit does during the first pregnancy. *Cuni-science* 6, 19–29.

Parigi Bini, R., Xiccato, G. and Cinetto, M. (1990b) Répartition de l'énergie alimentaire chez la lapine non gestante pendant la première lactation. In: *Proceedings 5èmes Journées de la Recherche Cunicole en France, Paris*, Vol. II. Communication 47. ITAVI, Paris, France, pp. 1–8.

Parigi Bini, R., Xiccato, G. and Cinetto, M. (1991a) Utilization and partition of digestible energy in primiparous rabbit does in different physiological states. In: *Proceedings of the 12th International Symposium on Energy Metabolism, Zurich*. Institute for Animal Sciences, Swiss Federal Institute of Technology, Zurich, Switzerland, pp. 284–287.

Parigi Bini, R., Xiccato, G. and Cinetto, M. (1991b) Utilizzazione e ripartizione dell'energia e della proteina digeribile in coniglie non gravide durante la prima lattazione. *Zootecnica Nutrizione Animale* 17, 107–120.

Parigi Bini, R., Xiccato, G., Cinetto, M. and Dalle Zotte, A. (1992) Energy and protein utilization and partition in rabbit does concurrently pregnant and lactating. *Animal Production* 55, 153–162.

Parigi Bini, R., Xiccato, G., Cinetto, M., Dalle Zotte, A., Carazzolo, A., Castellini, C. and Stradaioli, G. (1996) Effect of remating interval and diet on the performance and energy balance of rabbit does. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 253–258.

Partridge, G.G. (1986) Meeting the protein and energy requirements of the commercial rabbit for growth and reproduction. In: *Proceedings of the 4th World Congress of Animal Feeding, Madrid*, Vol. IX. Editorial Garsi, Madrid, Spain, pp. 271–277.

Partridge, G.G., Fuller, M. and Pullar, J. (1983) Energy and nitrogen metabolism of lactating rabbits. *British Journal of Nutrition* 49, 507–516.

Partridge, G.G., Allan, S.J., Findlay, M. and Corrigal, W. (1984) The effects of reducing the remating interval after kindling on the reproductive performance of the commercial doe rabbit. *Animal Production* 39, 465–472.

Partridge, G.G., Daniels, Y. and Fordyce, R.A. (1986a) The effects of energy intake during pregnancy in doe rabbits on pup birth weight, milk output and maternal body composition change in the ensuing lactation. *Journal Agricultural Science, Cambridge* 107, 697–708.

Partridge, G.G., Lobley, G.E. and Fordyce, R.A. (1986b) Energy and nitrogen metabolism of rabbits during pregnancy, lactation, and cuncurrent pregnancy and lactation. *British Journal of Nutrition* 56, 199–207.

Partridge, G.G., Garthwaite, P.H. and Findlay, M. (1989) Protein and energy retention by growing rabbits offered diets with increasing proportions of fibre. *Journal Agricultural Science, Cambridge* 112, 171–178.

Pascual, J.J. (2001) Early weaning of rabbits: a review. *World Rabbit Science* 9, 165–170.

Pascual, J.J., Cervera, C., Blas, E. and Fernández-Carmona, J. (1998) Effect of high fat diets on the performance and food intake of primiparous and multiparous rabbit does. *Animal Science* 66, 491–499.

Pascual, J.J., Cervera, C., Blas, E. and Fernández-Carmona, J. (1999a) Effect of high fat diets on the performance, milk yield and milk composition of multiparous rabbit does. *Animal Science* 68, 151–162.

Pascual, J.J., Tolosa, C., Cervera, C., Blas, E. and Fernández-Carmona, J. (1999b) Effect of diets with different digestible energy content on the performance of rabbit does. *Animal Feed Science and Technology* 81, 105–117.

Pascual J.J., Castella, F., Cervera, C., Blas, E. and Fernández-Carmona, J. (2000a) The use of ultrasound measurement of perirenal fat thickness to estimate changes in body condition of young female rabbits. *Animal Science* 70, 435–442.

Pascual, J.J., Cervera, C. and Fernández-Carmona, J. (2000b) The effect of dietary fat on the performance and body composition of rabbits in their second lactation. *Animal Feed Science and Technology* 86, 191–203.

Pascual, J.J., Cervera, C. and Fernández-Carmona, J. (2002a) A feeding programme for young rabbit does based on lucerne. *World Rabbit Science* 10, 7–14.

Pascual, J.J., Motta, W., Cervera, C., Quevedo, F., Blas, E. and Fernández-Carmona, J. (2002b) Effect of dietary energy source on performance and perirenal fat thickness evolution of primiparous rabbit does. *Animal Science* 75, 267–279.

Pascual, J.J., Cervera, C., Blas, E. and Fernández-Carmona, J. (2003) High-energy diets for reproductive rabbit does: effect of energy source. *Nutrition Abstracts and Reviews, Series B: Livestock Feeds and Feeding* 73, 27R–39R.

Pascual, J.J., Blanco, J., Piquer, O., Quevedo, F. and Cervera, C. (2004) Ultrasound measurements of perirenal fat thickness to estimate the body condition of reproducing rabbit does in different physiological status. *World Rabbit Science* 12, 7–21.

Pascual, J.J., Xiccato, G. and Fortun-Lamothe, L. (2006) Strategies for doe's corporal condition improvement – relationships with litter viability and career length. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Science*. ILVO, Melle, Belgium, pp. 247–258.

Perez, J.M., Lebas, F., Gidenne, T., Maertens, L., Xiccato, G., Parigi Bini, R., Dalle Zotte, A., Cossu, M.E., Carazzolo, A., Villamide, M.J., Carabaño, R., Fraga, M.J., Ramos, M.A., Cervera, C., Blas, E., Fernández, J., Falcão e Cunha, L. and Bengala Freire, J. (1995) European reference method for *in vivo* determination of diet digestibility in rabbits. *World Rabbit Science* 3, 41–43.

Quevedo, F., Pascual, J.J., Cervera, C. and Moya, V.J. (2004) Efecto del número de parto sobre la condición corporal y la productividad de las conejas lactantes. In: *Proceedings XXIX Symposium de Cunicultura, Lugo, Spain*, pp. 137–141.

Quevedo, F., Cervera, C., Blas, E., Baselga, M., Costa, C. and Pascual, J.J. (2005) Effect of selection for litter size and feeding programme on the development of rearing rabbits does. *Animal Science* 80, 161–168.

Rattray, P.V., Trigg, T.E. and Urlich, C.F. (1980) Energy exchanges in twin-pregnancy ewes. In: Mount, L.E. (ed.) *Proceedings of the 8th Symposium on Energy Metabolism*. EEAP Publ. No. 26, Butterworth, London, pp. 325–328.

Rebollar, P.G., Pérez-Cabal, M.A., Perada, P.L., Arias-Álvarez, M. and García-Rebollar, P. (2009) Effects of parity order and reproductive management on the efficiency of rabbit productive systems. *Livestock Science* 121, 227–233.

Rommers, M.J., Kemp, B., Meijerhof, R. and Noordhuizen, J.P.T.M. (2001) Effect of different feeding levels during rearing and age at first insemination on body development, body composition, and puberty characteristics of rabbit does. *World Rabbit Science* 9, 101–108.

Rommers, J.M., Meijerhof, R., Noordhuizen, J.P.T.M. and Kemp, P. (2002) Relationships between body weight at first mating and subsequent body development, feed intake, and reproductive performance of rabbits does. *Journal of Animal Science* 80, 2036–2042.

Rommers, M.J., Meijerhof, R., Noordhuizen, J.P.T.M. and Kemp, B. (2004a) Effect of feeding program during rearing and age at first insemination on performance during subsequent reproduction in young rabbits. *Reproduction Nutrition Development* 44, 321–332.

Rommers, M.J., Meijerhof, R., Noordhuizen, J.P.T.M. and Kemp, B. (2004b) The effect of level of feeding in early gestation on reproductive success in young rabbit does. *Animal Reproduction Science* 81, 151–158.

Rommers, J., Maertens, L. and Kemp, B. (2006) New perspectives in rearing systems for rabbit does. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Science*. ILVO, Melle, Belgium, pp. 21–51.

Santomá, G., de Blas, J.C., Carabaño, R. and Fraga, M. (1989) Nutrition of rabbits. In: Haresign, W. and Lewis, D. (eds) *Recent Advances in Animal Nutrition*. Butterworths, London, UK, pp. 97–138.

Scheele, C.W., Van Den Broek, A. and Hendricks, F.A. (1985) Maintenance requirements and energy utilisation of growing rabbits at different temperatures. In: *Proceedings of the 10th Energy Metabolism Symposium*, *Airle*. EEAP Publication no. 32, Rowman and Littlefield, Totowa, New Jersey, USA, pp. 202–205.

Simplicio, J.B., Fernández-Carmona, J. and Cervera, C. (1988) The effect of the high ambient temperature on the reproductive response of the commercial doe rabbit. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 3. Sandor Holdas, Hercegalom, Budapest, Hungary, pp. 36–41.

Taboada, E., Méndez, J., Mateos, G.G. and de Blas, J.C. (1994) The response of highly productive rabbits to dietary lysine content. *Livestock Production Science* 40, 329–337.

Taboada, E., Méndez, J. and de Blas, J.C. (1996) The response of highly productive rabbits to dietary sulphur amino acids content for reproduction and growth. *Reproduction Nutrition and Development* 36, 191–203.

Theilgaard, P., Sánchez, J.P., Pascual, J.J., Friggens, N.C. and Baselga, M. (2007) Effect of body fatness and selection for prolificacy on survival of rabbit does assessed using a cryopreserved control population. *Livestock Science* 103, 65–73.

Toschi, I., Rapetti, L., Bava, L., Crovetto, G.M. and Castrovilli, C. (2003) Energy utilization of diets with different starch content in nulliparous does fed different levels of intake. In: *Proceedings of the 16th Symposium on Energy Metabolism*. EAAP Publ. 109, pp. 441–444.

Toschi, I., Cesari, V., Rapetti, L., Bava, L., Grilli, G. and Castrovilli, C. (2004) Energy utilization and partition of nulliparous rabbit does in the last third of pregnancy. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Mexico, pp. 1002–1007.

Trocino, A., Xiccato, G., Sartori, A. and Queaque, P.I. (2000) Feeding plans at different protein levels: effects on growth performance, meat quality and nitrogen excretion in rabbits. *World Rabbit Science* 8 (Suppl. 1), 467-474.

Trocino, A., Xiccato, G., Sartori, A. and Queaque, P.I. (2001) Phase feeding plans based on low protein diets in growing rabbits. In: *Proceedings XIV Congresso Nazionale ASPA*, *Firenze*. ASPA Publishing, Firenze, Italy, pp. 430–432.

Villamide, M.J., Maertens, L., de Blas, J.C. and Perez, J.M. (1998) Feed formulation. In: de Blas, C. and Wiseman, J. (eds) *The Nutrition of the Rabbit*. CABI Publishing, Wallingford, UK, pp. 89–101.

Viudes-de-Castro, P., Santacreu, M.A. and Vicente, J.S. (1991) Effet de la concentration énergétique de l'alimentation sur les pertes embryonnaires et foetales chez la lapine. *Reproduction Nutrition Development* 31, 529–534.

Walach-Janiak, M., Raj, St. and Fandrejewski, H. (1986) The effect of pregnancy on protein, water and fat deposition in the body of gilts. *Livestock Production Science* 15, 249–260.

Webster, A.J.F. (1989) Bioenergetics, bioengineering and growth. *Animal Production* 48, 249–269.

Xiccato, G. (1996) Nutrition of lactating does. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 29–50.

Xiccato, G. and Cinetto, M. (1988) Effect of nutritive level and of age on feed digestibility and nitrogen balance in rabbit. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 3. Sandor Holdas, Hercegalom, Budapest, Hungary, pp. 96–103.

Xiccato, G. and Trocino, A. (2008) Nutrition and feeding of breeding does and bucks. In: Fekete, S. Gy. (ed.) *Veterinary Nutrition and Dietetics*. Pro Scientia Veterinaria Hungarica Foundation, Budapest, Hungary, pp. 755–779.

Xiccato, G., Cinetto, M. and Dalle Zotte, A. (1992a) Effetto del livello nutritivo e della categoria di conigli sulla digeribilità degli alimenti e sul bilancio azotato. *Zootecnica Nutrizione Animale* 18, 35–43.

Xiccato, G., Parigi Bini, R., Cinetto, M. and Dalle Zotte, A. (1992b) The influence of feeding and protein levels on energy and protein utilization by rabbit does. *Journal of Applied Rabbit Research* 15, 965–972.

Xiccato, G., Parigi Bini, R., Dalle Zotte, A., Carazzolo, A. and Cossu, M.E. (1995) Effect of dietary energy level, addition of fat and physiological state on performance and energy balance of lactating and pregnant rabbit does. *Animal Science* 61, 387–398.

Xiccato, G., Bernardini, M., Castellini, C., Dalle Zotte, A., Queaque, P.I. and Trocino, A. (1999) Effect of post-weaning feeding on performance and energy balance of female rabbit at different physiological states. *Journal of Animal Science* 77, 416–426.

Xiccato, G., Trocino, A., Sartori, A. and Queaque, P.I. (2003) Effect of weaning diet and weaning age on growth, body composition and caecal fermentation of young rabbits. *Animal Science* 77, 101–111.

Xiccato, G., Trocino, A., Sartori, A. and Queaque, P.I. (2004) Effect of doe parity order and litter weaning age on the performance and body energy deficit of rabbit does. *Livestock Production Science* 85, 239–251.

Xiccato, G., Schiavon, S., Gallo, L., Bailoni, L. and Bittante, G. (2005a) Nitrogen excretion in dairy cow, beef and veal cattle, pig, and rabbit farms in Northern Italy. *Italian Journal of Animal Science* 4 (Suppl. 3), 103–111.

Xiccato, G., Trocino, A., Boiti, C. and Brecchia, G. (2005b) Reproductive rhythm and litter weaning age as they affect rabbit doe performance and body energy balance. *Animal Science* 81, 289–296.

Xiccato, G., Trocino, A. and Nicodemus, N. (2006) Nutrition of the young and growing rabbit: a comparative approach with the doe. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Science*. ILVO, Melle, Belgium, pp. 239–246.

Xiccato, G., Trocino, A., Fragkiadakis, M. and Majolini, D. (2007) Enquête sur les élevage de lapins en Vénétie: résultats de gestion technique et estimation des rejets azotés. In: *Proceedings 12èmes Journées de la Recherche Cunicole*. Le Mans, France, pp. 167–169.

Young, M. (1979) Transfer of amino acids. In: Chamberlain, G.V.P. and Wilkinson, A.W. (eds) *Placental Transfer*. Pitman, London, UK, pp. 142–158.

7 Minerals, Vitamins and Additives

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7.1. Mineral Requirements of Rabbits

Rabbit meat is rich in protein and low in energy, with an ash content similar to or greater than that of other domestic species (Ouhayoun and Lebas, 1987). Publications have shown that rabbit meat is poor in sodium and iron and rich in potassium and phosphorus when compared to meat from other domestic species (Combes, 2004; Hermida *et al.*, 2006). Mean values (mg 100 g^{-1} fresh loin or hind leg muscles) of 55 for sodium, 400 for potassium, 9.0 for calcium, 234 for phosphorus, 28 for magnesium, 0.83 for iron, 0.89 for zinc, 0.09 for copper, 0.08 for selenium and 0.03 for manganese have been reported. For the whole carcass, Lombardi-Boccia *et al.* (2005) reported lower values for some of the trace minerals (0.38 for iron, 0.55 for zinc and 0.03 mg 100 g^{-1} for copper).

Compared with that from other mammals, rabbit milk is high in ash, and especially in calcium, phosphorus and sodium. This is not surprising since the bones of the newborn pups are immature at birth and need extensive mineralization (Widdowson, 1974). In addition, the low level of lactose in rabbit milk has to be compensated for by a high sodium concentration in order to maintain, within an adequate range, the osmotic pressure values (Coates *et al.*, 1964). The macromineral composition of rabbit milk as compared to that of other mammals is presented in Table 7.1.

7.1.1 Macrominerals

Macrominerals are defined as those elements that are required in grams per day and are expressed as g kg^{-1}. The definition includes calcium, phosphorus, magnesium, sodium, potassium, chloride and sulphur. Currently, only calcium, phosphorus and sodium are taken into account in the practical formulation of rabbit diets.

Calcium

Calcium is the main component of the skeleton. Over 0.98 of the total body calcium is present in bones and teeth. In addition, calcium plays a key role in numerous organic processes such as heart function, muscle contraction, blood coagulation and electrolyte equilibrium in serum. Furthermore, doe milk is rich in calcium. Therefore, the dietary requirements for this mineral are expected to be greater for fast-growing young animals and rabbit does in late gestation or at the peak of milk production than for later maturing rabbits or those at maintenance.

Table 7.1. Macromineral composition of rabbit milk (g kg^{-1}) compared with that of other mammals.

	Cow[a]	Sheep[a]	Sow[b]	Rabbit[c]	Rabbit[d]
Sodium	0.45	0.45	0.5	0.96	0.84
Potassium	1.50	1.25	0.84	1.86	2.01
Calcium	1.20	1.9	2.2	4.61	2.76
Magnesium	0.12	0.15	–	0.27	0.45
Phosphorus	0.90	1.5	1.6	2.78	2.44
Chloride	1.10	1.2	–	0.66	–

[a]Guegen *et al.* (1988).
[b]Partridge and Gill (1993).
[c]El-Sayiad *et al.* (1994).
[d]Maertens *et al.* (2006).

When compared to other domestic species, the metabolism of calcium in the rabbit presents important differences: (i) calcium is absorbed in direct proportion to its concentration in the diet, regardless of metabolic needs and, therefore, blood levels of calcium rise with increasing intake (Chapin and Smith, 1967); and (ii) urine is the main route used by the rabbit to eliminate any excess.

Calcium absorption is not tightly controlled in the rabbit. Intestinal absorption is very efficient (Cheeke and Amberg, 1973) and serum concentrations of ionized and total calcium (3–4 mmol l^{-1} and 13–15 mg dl^{-1}, respectively) are high compared to those of other mammals (Warren *et al.*, 1989). The blood calcium level is controlled by parathyroid hormone and 1,25-OH D_3, similarly to other mammalian species (Bas *et al.*, 2005). Diets low in calcium (Barr *et al.*, 1991) or vitamin D (Bourdeau *et al.*, 1986) reduce urinary excretion and induce a rapid renal tubular mechanism to conserve the mineral, indicating the importance of homeostatic mechanisms within the kidneys to maintain serum calcium levels (Redrobe, 2002). Excess calcium is excreted through the urine as a white, thick, creamy precipitate that is deposited beneath the cages. Swick *et al.* (1981) proposed that the filtration of calcium, as well as the crystals formed in the process of eliminating excess calcium, might damage the structure of the kidneys, occasionally producing the observed red pigmentation of urine. Prolonged feeding of excess calcium (>40 g kg^{-1}) increases the risk of urolithiasis (Kamphues, 1991) and may result in calcification of the soft tissues (Löliger and Vogt, 1980; Cheeke, 1987), particularly when vitamin D intake is high (Kamphues, 1991). Furlan *et al.* (1997b) estimated that, in most instances, 5 g calcium kg^{-1} covers the nutritional requirements of rabbits from 35 to 90 days of age.

High milk-producing does might suffer a syndrome similar to that of milk fever in dairy cows. During late gestation and early lactation, does may show a drop in calcium (from 14 to <7 mg 100 ml^{-1}) and other mineral (phosphorus and magnesium) levels in plasma that results in loss of appetite, tetany, muscle tremors, ear flapping, animals lying on their sides and eventually death (Barlet, 1980). Injection of calcium gluconate induces a rapid recovery within 2 h. It is possible that a modification of the electrolyte equilibrium of the diet towards a negative balance (acidotic diets) will benefit rabbit does under these circumstances, as it does in dairy cattle and other species.

Phosphorus

Phosphorus is a major constituent of the bones. It also plays an important role in many reactions related to energy metabolism. In most mammalian species, inorganic phosphorus is absorbed at the duodenal and jejunum level, a mechanism that is modulated by endocrine (calcitriol, triiodothyronine) and nutritional factors (Barlet *et al.*, 1995). However, the role of 1,25-OH D_3 and other vitamin D metabolites on phosphorus absorption in the rabbit is largely unknown. Borowitz and Granrud (1993) reported the existence of an active mechanism for phosphorus transport at the duodenum and proximal jejunum in 3-month-old rabbits. Because horses and rabbits have a somewhat similar digestive tract, Cheeke (1987) proposed that similar absorption values should be used for both species. It is likely that phosphorus absorption is more efficient in younger animals than in adults because of the higher requirements at younger ages (Marounek *et al.*, 2003).

A major factor influencing phosphorus availability from plant materials in non-ruminant animals is the presence of phytates and phytases. Phytates are phosphorus-rich complexes that are not degraded by endogenous enzymes. In the rabbit, phytate phosphorus is well utilized because of phytase production by the microorganisms of the caecum (Marounek *et al.*, 2009). Most of the phosphorus is recycled through soft faeces followed by coprophagy and, therefore, should result in an almost complete utilization of phytate phosphorus. In addition, natural phytases present in wheat and other ingredients, as well as in other exogenous sources, may facilitate phytate hydrolysis and absorption of phosphorus in the upper part of the digestive tract (Marounek *et al.*, 2003). Swick *et al.* (1981) found a coefficient of apparent phosphorus digestibility in rabbits fed a maize-soybean meal diet of 0.75, which indicates that the utilization of phosphorus from vegetable sources is close to that of dicalcium phosphate. The utilization of phosphorus contained in lucerne seems rather low in rabbits compared to that of pigs (Cheeke *et al.*, 1985; Cromwell, 1992). This is surprising, since most of the phosphorus in lucerne is found in the leaves rather than in the stems. However, the absorption of soluble phosphorus in the caecum and the recycling of the phosphorus of the digesta through soft faeces and caecotrophy seem to be incomplete. Marounek *et al.* (2003) found that the digestibility of phytate phosphorus in rabbits fed diets containing cereals, lucerne meal, oilseed meals and sugarbeet pulp averaged 0.82. However, the apparent digestibility of total phosphorus was 0.48 at 7 weeks and 0.35 at 10 weeks of age. Similarly, Gutierrez *et al.* (2000) found an apparent phosphorus digestibility of 0.31 in 8-week-old New Zealand rabbits. Gutierrez *et al.* (2000) showed that the inclusion of 2000 FTU kg^{-1} of an *Aspergillus niger* phytase into a diet that contained 4.2 g total phosphorus kg^{-1} improved phosphorus digestibility by 24% in growing rabbits. GuoXian *et al.* (2004) and Eiben *et al.* (2008) demonstrated that phytases supplemented at 800–1000 FTU kg^{-1} to fattening rabbit diets containing 3.5 g total phosphorus kg^{-1} resulted in similar growth and feed conversion to those obtained with conventional diets and, in fact, eliminated the need for inorganic supplements.

There is growing interest in controlling the excretion of phosphorus through feed manipulation to reduce environmental pollution. Studies conducted under commercial conditions have estimated phosphorus excretion of 4.76 kg P_2O_5 year^{-1} doe (for a 2.5 kg doe producing 45 fatteners year^{-1}) and 26 g P_2O_5 kg^{-1} per fattener rabbit (0.8–2.5 kg body weight) (Maertens *et al.*, 2005). A decrease in dietary phosphorus levels (depending on the age and physiological status of the rabbits) is a promising tool to reduce phosphorus excretion. Studies conducted with fryers (Steenland, 1991) and breeding does (Lebas and Jouglar, 1990) have demonstrated that rabbit performance is not reduced when dietary phosphorus is decreased below former practical recommendations. In fact, these authors found that 5 g phosphorus kg^{-1} was adequate for all types of production. Moreover, Ritskes-Hoitinga *et al.* (2004) found that 1 g phosphorus kg^{-1}, included as dicalcium phosphate in semi-purified diets, supported growth and bone development in rabbits. In addition, this low level of phosphorus prevented kidney calcification. Unfortunately, the available information on phosphorus requirements in rabbits fed commercial diets is scarce. Moreover, in order to achieve these low dietary phosphorus levels, the inclusion of some raw materials rich in phosphorus (i.e. grains, grain by-products) in the diet should be limited, an alternative that might not be economically feasible (Maertens, 1999).

A dietary relationship of calcium to available phosphorus of 2:1 to 1.5:1 is widely accepted in practical feeding (Vandelli, 1995). In fact, rabbit milk maintains a constant 2:1 calcium to phosphorus ratio throughout the lactation period (El-Sayiad *et al.*, 1994; Maertens *et al.*, 2006). However, because of the renal mechanism existing in the rabbit to conserve both calcium and phosphorus and maintain mineral homeostasis (Redrobe, 2002), the need to closely maintain this relationship is not evident and, at least in fatteners, does not seem to be critical. Diets for growing rabbits with a calcium to phosphorus ratio of 12:1 have not shown any detrimental effect in terms of performance (Chapin and Smith, 1967). However, calcium in excess of requirements may decrease phosphorus absorption and, therefore, create an artificial deficiency in this mineral when low dietary phosphorus levels are used. On the other hand, an increase in dietary phosphorus levels from 4 to 8 g kg^{-1}, at a constant calcium concentration (5 g kg^{-1}), changes the route of calcium excretion from urine to faeces through the formation of insoluble complexes of dicalcium phosphate. Consequently, the intestinal absorption of both minerals is reduced (Ritskes-Hoitinga *et al.*, 2004). Under practical conditions it is more common to find dietary excesses of calcium than of phosphorus. Excess calcium is more detrimental to rabbit health if marginal levels of phosphorus are used. Assane *et al.* (1993) observed an increase of phosphorus and magnesium in the blood at the end of the gestation period when the calcium to phosphorus ratio in feed was 1:1 as compared with 2:1 (Table 7.2). Assane *et al.* (1994) reported positive calcium and phosphorus balances with dietary calcium to phosphorus ratios of 2:1 and 1:1. However, calcium retention increased in both non-pregnant and pregnant does with the higher calcium level. Moreover, the best reproductive performance was obtained when the calcium to phosphorus ratio was 2:1.

Practical recommendations on dietary levels of calcium and phosphorus vary according to age, breed, productivity and diet composition. For growing-fattening rabbits, the recommendations vary from 4 to 10 g for calcium and from 2.2 to 7 g for phosphorus (Table 7.3) (NRC, 1977; AEC, 1987; Schlolaut, 1987; INRA, 1989; Mateos, 1989; Burgi, 1993; Mateos and Piquer, 1994; Vandelli, 1995; Xiccato, 1996; Mateos and de Blas, 1998; Lebas, 2004; Maertens and Luzi, 2004). Calcium and phosphorus requirements are higher for lactating does than for growing rabbits or non-lactating does, because rabbit milk is particularly rich in both minerals. Average contents of calcium and phosphorus in rabbit milk is approximately three to five times higher than those in cow milk (Burgi, 1993; El-Sayiad *et al.*, 1994). At maximal milk production the doe can excrete up to 2 g of calcium. Practical recommendations in doe feeds vary from 7.5 to 15 g for calcium and from 4.5 to 8 g for phosphorus, according to the same authors (Table 7.3).

Table 7.2. Influence of the dietary calcium to phosphorus (C:P) ratio on calcium, phosphorus and magnesium levels in the serum of gestating rabbit does (Assane *et al.*, 1993).

C:P ratio	Premating	Week 3 of gestation	Pre-partum	SEM
Calcaemia (mg l^{-1})				
1:1[a]	123*	120**	111***	3.7
2:1[b]	145*	125**	114***	6.2
Phosphataemia (mg l^{-1})				
1:1	38	39	41	6.3
2:1	43*	38*	25**	5.9
Magnesaemia (mg l^{-1})				
1:1	28	27	27	3.7
2:1	31*	23**	19**	4.7

SEM, standard error of the mean.
[a]5.2 g calcium and 5.1 g phosphorus kg^{-1} diet.
[b]8.3 g calcium and 3.9 g phosphorus kg^{-1} diet.
*Means in the same row with different superscripts differ (P< 0.05).

Table 7.3. Macromineral recommendations (g kg^{-1} as-fed) for intensively reared rabbits.

	Calcium	Phosphorus	Sodium	Chloride	Potassium
Growing-fattening rabbits					
NRC (1977)	4	2.2	2	3	6
AEC (1987)	8	5	3	–	–
Schlolaut (1987)[a]	10	5	–	–	10
INRA (1989)	4	3	3	3	6
Mateos (1989)	4.0–8.0	3.0–5.0	3	–	6.0–9.0
Burgi (1993)	5	3	–	–	–
Mateos and Piquer (1994)	5.5	3.5	3.5	–	–
Vandelli (1995)	4.0–8.0	3.0–5.0	–	–	–
Xiccato (1996)[b]	8.0–9.0	5.0–6.0	2	3	–
Mateos and de Blas (1998)	3.0–10.0	3.0–7.0	2.0–2.3	2.8–4.8	6.5–10
Lebas (2004)	7–8	4–4.5	2.2	2.8	<15
Maertens and Luzi (2004)	8	5	2.5	3	8
Lactating does					
NRC (1977)	7.5	5.0	2.0	3.0	6.0
AEC (1987)	11.0	8.0	3.0	–	–
INRA (1989)	11.0	8.0	3.0	3.0	9.0
Schlolaut (1987)[a]	10.0	5.0	–	–	10.0
Lebas (1990)	12.0	7.0	2.0	3.5	9.0
Mateos and Piquer (1994)	11.5	7.0	–	–	–
Vandelli (1995)	11.0–13.5	6.0–8.0	–	–	–
Maertens (1996)	12.0	5.5	–	3.0	–
Xiccato (1996)[b]	13.0–13.5	6.0–6.5	2.5	3.5	–
Mateos and de Blas (1998)	10.0–15.0	4.5–7.5	2.2–2.5	2.8–4.8	6.5–10
Lebas (2004)	12	6	2.5	3.5	<18
Maertens and Luzi (2004)	12	5.5	2.5	3	10

[a]Angora rabbits.
[b]Young does.

Dietary calcium and phosphorus levels below requirements will lead to rickets (young rabbits), osteomalacia (adults), lack of fertility (does) and abnormal behaviour. Adequate calcium supplementation rapidly reverses the problem caused by calcium deficiency in growing rabbits (Mehrotra *et al.*, 2006). Excess calcium (>13 g kg^{-1}) does not increase bone mass (Gilsanz *et al.*, 1991), but might result in calcification of the soft tissues (Kamphues, 1991) and reduced phosphorus absorption. Excess phosphorus (>9 g kg^{-1}) may depress feed intake and impair prolificacy in does (Chapin and Smith, 1967; Lebas and Jouglar, 1984, 1990). In all cases, excess phosphorus has detrimental effects on the environment.

The recommended concentrations of calcium and phosphorus in complete diets for rabbits are presented in Table 7.4. These values are based on a literature review and practical experience.

Table 7.4. Requirements of calcium and phosphorus for rabbits (g kg^{-1} as-fed basis).

	Calcium	Phosphorus
Breeding does		
Recommendation	10.5	6.0
Acceptable commercial range	10.0–12.5	5.5–7.0
Growing rabbits (1–2 months of age)		
Recommendation	6.0	4.0
Acceptable commercial range	4.5–7.6	3.3–4.6
Finishing rabbits (>2 months of age)		
Recommendation	4.0	3.0
Acceptable commercial range	3.0–6.0	3.0–4.5

Other macrominerals

Magnesium is a major component of the bones (0.7 of total body magnesium is in the skeleton) and also acts as a cofactor in many energy metabolism reactions. Deficiency produces poor growth, alopecia, hyperexcitability, convulsions, poor fur texture and fur chewing. The magnesium requirements for growing rabbits vary from 0.3 (NRC, 1977; INRA, 1989) to 3 g kg^{-1} (Lebas, 2004; Maertens and Luzi, 2004). Evans *et al.* (1983a,b) found that 3.4 g kg^{-1} fulfilled requirements, but that 1.7 g kg^{-1} was insufficient. Excess dietary magnesium is eliminated through the urine. Therefore, extra supplementation with magnesium rarely induces severe side effects (Plamenac *et al.*, 2008). The content and apparent digestibility of magnesium in most raw materials is high and the need to add extra magnesium to commercial rabbit diets has not been established.

Potassium plays a key role in the regulation of the acid–base balance in organisms and is a cofactor of numerous enzymes. Symptoms of deficiency include muscle weakness, paralysis and respiratory distress. Potassium ion (K^+) deficiency in rabbits might appear when diarrhoea is present (Licois *et al.*, 1978). Current estimates indicate that 6 g potassium kg^{-1} avoids symptoms of deficiency. Because most of the ingredients used in rabbit diets are rich in K^+ (i.e. soybean meal, lucerne, molasses), deficiency is difficult to envisage. Excess K^+ (>10 g kg^{-1}) may occur when a high proportion of heavily fertilized, early mature lucerne is used. Lebas (2004) recommended dietary K^+ levels for growing rabbits and lactating does of <15–20 and 18 g kg^{-1}, respectively. Surdeau *et al.* (1976) observed a higher incidence of nephritis when the K^+ level of the diet was >8 g kg^{-1}. Furthermore, Evans *et al.* (1983a) reported reduced feed intake with K^+ levels >10 g kg^{-1}. In addition, excess K^+ antagonizes magnesium absorption, although the importance of this problem has not been ascertained in the rabbit. Practical recommendations range between 6.0 and 10 g kg^{-1}.

Sodium is involved in the regulation of pH and osmotic pressure. In contrast to K^+, sodium ions (Na^+) concentrate in the plasma, outside the cells. Sodium is essential for the absorption of luminal nutrients such as glucose and amino acids (Schultz and Zalusky, 1964, 1965). Intestinal brush-border membranes contain a Na^+-phosphate co-transport system, which catalyses the entry of phosphate and Na^+ into the intestinal epithelial cell. The requirements for Na^+ have not been studied in breeding does. In fattening rabbits, however, Harris *et al.* (1984b) and Furlan *et al.* (1997a) reported that 1.0 g sodium kg^{-1} met the requirements for growth from 35 to 90 days. A deficit in Na^+ may impair the efficiency of digestive processes and/or the absorption of amino acids, as has been demonstrated in pigs (Patience *et al.*, 1985). Chamorro *et al.* (2007) observed that a reduction in Na^+ from 2.6 to 1.6 g kg^{-1} impaired ileal digestibility of methionine and cystine, although dry matter and protein digestibility were not affected. Under practical conditions, 2.0–2.3 and 2.2–2.5 g sodium kg^{-1} are used for fryers and does, respectively. Excess Na^+ in the feed (>8–10 kg sodium chloride (NaCl) kg^{-1} diet) or the presence of salt in the drinking water (3000 mg kg^{-1}) is detrimental to growth (Harris *et al.*, 1984b; Marai *et al.*, 2005).

Chloride is also involved in acid–base regulation. In addition, this ion concentrates in the gastric cells. It is secreted as hydrogen chloride and is involved in the solubility of mineral salts and protein digestion. Practical diets for high-producing rabbits are unlikely to be deficient in chloride ions (Cl^-), because NaCl and lysine hydrochloride are routinely used in feed formulations as a source of Na^+ and lysine, respectively, and indirectly serve as a supplement for Cl^-. The Cl^- requirements have been estimated as within the 1.7–3.2 g kg^{-1} range, but excess (4.7 g kg^{-1}) does not impair performance (Colin, 1977). Practical levels vary between 2.8 and 4.8 g kg^{-1}.

It is well known that the relationship between Na^+, K^+ and Cl^- (the electrolyte balance) affects animal performance. In addition to influencing resistance to thermal stress, leg score, kidney function and incidence of milk fever, a large negative value may decrease feed intake, whereas a positive value may increase problems around farrowing. Chiericato and Rizzi (2004, 2005) found that increasing the electrolyte balance ($Na^+ + K^+ - Cl^-$) of breeding rabbit diets from 270 to 350 mEq kg^{-1} tended to increase the mortality rate of does at farrowing, but did not affect milk production or feed intake up to 21 days of lactation. Similarly, Rizzi *et al.* (2005) did not observe any effect on the performance of bucks during three reproductive cycles with electrolyte balances of 270 or 350 mEq kg^{-1} diet. No information on variation of this balance on the productivity of growing rabbits is available. However, this species is particularly vulnerable to acid loads. In fact, rabbit urine is more alkaline than that of rats or other mammals fed diets with similar electrolyte balances. Therefore, care should be taken to avoid electrolyte imbalances that might result in nephritis and decrease feed intake.

Sulphur is one of the more abundant elements in nature. Sulphur is a component of chondroitin sulphate, a major component of cartilage, tendons, blood vessel walls and bones. In addition, sulphur is a constituent of numerous organic substances such as haemoglobin, glutathione, coenzyme A and the amino acids methionine and cystine. Practical diets include over 2.0 g sulphur kg^{-1} but, in general, no supplemental sources are used under practical conditions. There are no reports in the literature indicating any benefit of sulphur supplementation on rabbit performance, although inorganic S^{2-} can be incorporated into microbial protein in the hindgut and used for protein accretion. Furthermore, there are no reports on the effects of excess sulphur on rabbit performance. In laying hens and dairy cows, however, excess sulphur reduces performance. Consequently, care should be taken when including high levels of rapeseed meal, sunflower meal or distillers' dried grains with solubles in rabbit diets, especially in does.

7.1.2 Trace minerals

Trace minerals are defined as those elements required in mg per day and needs are expressed as mg kg^{-1} or ppm of the diet. The definition includes iron, copper, manganese, zinc, selenium, iodine and cobalt. Other trace elements that are required by the rabbit but are not supplemented under practical conditions are molybdenum, fluorine and chromium. The trace minerals mentioned in the first group are routinely added to rabbit diet as salts through a premix.

Iron is a major constituent of enzymes involved in oxygen transport and metabolism. Therefore, deficiency may result in impaired haemoglobin formation and anaemia. The mechanisms for transporting iron to milk or to the fetus are poor, especially in the pig, but rabbits are capable of absorbing reasonable amounts of iron through the placenta. Rabbits have sufficient iron reserves at birth, provided the doe has received a properly supplemented diet. Therefore, rabbits are not as dependent as piglets on an exogenous supply of iron for survival. Even though milk is poor in iron, no deficiency is expected in young rabbits because they have free access to doe feed during the milking period. Since most ingredients used in feeds are rich in iron (i.e. soil-contaminated lucerne, macromineral sources and trace mineral premix) an iron deficiency is not expected to develop early in life. El-Masry and Nasr (1996) reported beneficial effects in does when 80 mg iron kg^{-1} was added to diets that already contained 129 mg kg^{-1} diet. Does fed the iron-supplemented diet produced more milk and had greater litter sizes and litter weights than controls. However, the low productivity and high mortality of the animals on trial, as well as the small number of replicates and the composition of the premix used, do not allow the results of this trial to be adopted with confidence.

Recommendations for iron reported in the literature vary from 30 to 100 mg kg^{-1} (Table 7.5), with greater levels for does and fur-producing animals (Schlolaut, 1987). Under commercial conditions, all of the commercial premixes supply extra iron (15–105 mg kg^{-1}) (Table 7.6) and most are within the 30–50 mg kg^{-1} diet range. At these levels, iron requirements for all types of production are easily met, especially when calcium carbonate and dicalcium phosphate are used as sources of calcium and

Table 7.5. Micromineral requirements of rabbits (mg kg^{-1} diet).

	NRC (1977)	INRA (1989)	Mateos and Piquer (1994)[a]	Xiccato (1996)[b]	Lebas (2004)	Maertens and Luzi (2004)
Growing-fattening						
Copper	3	5	5	10	6	10
Iodine	0.2	0.2	1.1	0.2	–	0.2
Iron	–	50	35	50	50	–
Manganese	8.5	8.5	5	5	8	8.5
Zinc	–	50	60	25	25	25
Cobalt	0	0.1	0.25	0.1	–	0.1
Selenium	0	–	0.01	0.15	–	–
Lactating does						
Copper	5	5	5	10	10	10
Iodine	1	0.2	1.1	0.2	–	0.2
Iron	30	100	35	100	100	–
Manganese	15	2.5	2.5	5	12	8.5
Zinc	30	70	60	50	50	50
Cobalt	1	0.1	0.25	0.1	–	0.1
Selenium	0.08	–	0.01	0.15	–	–

–, not determined.
[a]Same feed for does and fryers.
[b]Young does.

Table 7.6. Trace mineral recommendations for commercial rabbit diets (mg kg^{-1} diet).

	Commercial premixes[a]		Recommended levels[b]	Maximum level[c]
	Mean ± SD	Range		
Copper	9.9 ± 4.8	4–25	5–10	25
Iodine	1.0 ± 0.4	0.25–2	0.2–0.5	10
Iron	46.6 ± 20.2	15–105	35–50	750
Manganese	28.2 ± 18.4	5–75	8–15	150
Zinc	65.5 ± 22.7	40–140	50–60	150
Cobalt	0.38 ± 0.16	0.10–0.70	0.25	2
Selenium	0.09 ± 0.09	0–0.35	0.05	–

SD, standard deviation.
[a]n = 29 (>0.9 of total feed production for rabbits). The range indicates the maximum and minimum levels used commercially.
[b]Author recommendations under practical conditions. The higher values of the range are more appropriate for lactating rabbits.
[c]According to European Commission regulation (EC) 1134/2003.

phosphorus, respectively. In fact, the need for extra iron supplementation in commercial diets for fatteners is questionable.

Copper is a major component of metalloenzymes involved in energy and iron metabolism and in collagen and hair formation. Faecal excretion is the main route of copper output in rabbits (Skrivanova *et al.*, 2002). Deficiency will manifest as retarded growth, grey hair, bone abnormalities and anaemia, among other symptoms. When fed in excess, copper is accumulated in the liver (Cavalcante *et al.*, 2002) but not in muscle tissues. Therefore, copper has little effect on the oxidative stability status of rabbit meat (Skrivanova *et al.*, 2001). Published recommendations vary between 3 and 10 mg copper kg^{-1} (Table 7.5), with higher levels recommended for fur production (25 mg kg^{-1} diet; Schlolaut, 1987) and breeding does. Practical levels used in Iberian rabbit commercial premixes vary from 4 to 25 mg kg^{-1} (Table 7.6). Even at the lower levels of inclusion, no deficiency symptoms are expected because of the high copper content of most raw materials used in rabbit feeds and the potential for accumulation in the liver. As a precaution, the use of forages high in sulphur and molybdenum should be avoided. The interaction between copper and molybdenum for absorption is well known in sheep, and the presence of sulphur exacerbates this antagonism.

In addition to its role as an essential nutrient, copper is used worldwide as a growth promoter in poultry and pigs. Indeed, several reports (Bassuny, 1991; Ayyat *et al.*, 1995; Abbo el-Ezz *et al.*, 1996; Onifade and Abu, 1998; Aboul-Ela *et al.*, 2000; Skrivanova *et al.*, 2001) have reported that supplementation with 100–400 mg $CuSO_4$ kg^{-1} improves growth performance in rabbits. The beneficial effects are more noticeable in young animals under poor sanitation status and in the presence of digestive diseases such as enteritis and enterotoxaemia (Patton *et al.*, 1982). There are, however, conflicting reports. For example, King (1975) and Harris *et al.* (1984a) did not find any benefit for the rabbit with supplementation of $CuSO_4$. Fekete *et al.* (1988) observed an improvement in the growth of rabbits fed a high-protein diet (180 g crude protein kg^{-1}) when 100 mg of extra copper kg^{-1} was added, but no effects were observed when 140 g crude protein kg^{-1} was used. Aboul-Ela *et al.* (2000) reported a reduction in mortality in response to copper supplementation in rabbits fed a high-fibre, low-energy diet, but not in those fed a high-energy, low-fibre diet. In any event, the use of $CuSO_4$ as a growth promoter is not permitted in rabbit feeds in the European Union (EU), which precludes its utilization at high levels in commercial feeds. In fact the level of dietary copper is limited to 25 mg kg^{-1} (Table 7.6). Cavalcante *et al.* (2002) did not find any difference in copper bioavailability between organic and inorganic sources of copper (oxide, sulfate, carbonate and a chelate) in rabbits.

Manganese acts as a coenzyme in amino acid metabolism and in the formation of the cartilage matrix. Deficiency results in poor consistency of the bones, which may result in brittle bones and leg problems. Manganese deficiency also results in reproductive failure in most domestic species, but information on rabbit does is not available. In fact, even in the absence of supplementary manganese, its concentration in uterine tissues remains stable. Hidiroglou *et al.* (1978) observed that, when fed in excess, manganese concentrates in the kidney, liver and spleen, but not in the reproductive tract of rabbits. The response of rabbits to manganese is probably more similar to that of pigs than poultry. Published values on manganese requirements for rabbits vary between 2.5 and 15 mg kg^{-1} (Table 7.5), similar to those of pigs. Commercial mineral premixes from the Iberian Peninsula include levels between 5 and 75 mg kg^{-1} (Table 7.6). Based on field information and cost, the advisable manganese levels is around 8–15 mg kg^{-1}.

Zinc is a component of numerous enzymes and is involved in the biosynthesis of nucleic acids and in cell division processes. Higher levels of zinc are recommended for reproduction and fur and hair production than for maintenance or meat production. Because of the relatively high phytase production by hindgut microorganisms, dietary phytates are less detrimental for zinc absorption in rabbits than in other non-ruminant species. Therefore, a lower requirement must be expected for rabbits than for pigs or poultry (Swick *et al.*, 1981). No recent trials on the zinc requirements of rabbits have been published in the literature, but levels of use vary between 25 and 60 mg kg^{-1}, with the higher values proposed for does and bucks. Practical commercial diets contain a wider range of zinc (40–140 mg kg^{-1}). Zinc oxide is the most commonly used source because it is less reactive and has a higher zinc concentration than sulfate and carbonate salts. No differences in zinc bioavailability between inorganic and organic sources have been reported in rabbits (Guimaraes and Motta, 2000). Because of zinc's environmental impact, the maximum level allowed in the EU for rabbit feeds is 150 mg kg^{-1}. In addition, the adverse effect of high zinc intake on copper availability has to be considered (Maret and Sandstead, 2006).

Selenium was considered a toxic element but, in 1957, the essentiality of this nutrient was demonstrated. Diseases such as white muscle, liver degeneration and exudative diathesis and impaired reproduction and poor immunity have been associated with selenium deficiency. In most species the role of selenium is closely linked to vitamin E. Selenium is a constituent of the enzyme glutathione peroxidase (GSH), which plays a role in the detoxification of peroxides formed during metabolic processes. However, rabbit tissues are less dependent on selenium for the disposal of peroxides than tissues from other mammals. Lee *et al.* (1979) observed that, in the rabbit, most of the existing GSH does not have selenium as a cofactor. Moreover, Erdélyi *et al.* (2000) did not observe any relationship between selenium status and GSH activity in the liver, kidney, pancreas, genital organs and femoral muscle of rabbits. Similarly, supplementation with 0.5 mg selenium kg^{-1} does not improve plasma tocopherol or the oxidative stability of spermatozoa (Castellini *et al.*, 2002). As observed in pig and poultry meats and in eggs, feeding supplemental organic selenium (0.12–0.50 mg kg^{-1}) to fattening rabbits increases the selenium content of meat (95.5–395 μg kg^{-1}). However, extra selenium supplementation has limited potential to improve the oxidative stability status of rabbit meat (Dokoupilová *et al.*, 2007). Therefore, the rabbit is more dependent on vitamin E and less on selenium than other mammals in reducing the oxidation load on tissues (Jenkins *et al.*, 1970). On the other hand, Struklec *et al.* (1994) observed improved fetal and birth weight when does received 0.1 mg supplemental selenium kg^{-1}, but no further improvement was observed with 0.3 mg selenium kg^{-1}. Commercial premixes without supplemental selenium have been marketed for years in Europe without any evidence of impaired productivity in does or growing-fattening rabbits (Mateos, 1989; Mateos and Piquer, 1994; Robledo *et al.*, 1999; Lebas, 2004). Since no detailed experiments have recently been conducted on this subject, and taking into account other potential effects of selenium as a constituent of various enzymes complexes, it is advisable to include a small amount of supplemental selenium (0.05 mg kg^{-1}) in the feeds of rabbits.

Iodine is a component of the thyroid hormones that regulates energy metabolism. No requirements for iodine have been established for any type of production in rabbits. Iodine deficiency results in goitre. The incidence of this disease increases when goitrogens are present in the diet. *Brassica* species, such as cabbage, turnips and rape seeds, are rich in goitrogens and therefore their use will increase iodine requirements. Does are probably more sensitive to iodine deficiency than growing-fattening rabbits. Literature requirements vary from 0.2 to 1.1 mg kg^{-1} (Table 7.5). Practical premixes in Spain and Portugal include between 0.25 and 2 mg kg^{-1}. At these levels of inclusion, no goitre or other classic symptoms of deficiency have ever been detected. If marine salt is used as a source of iodine, the requirements are fully satisfied and a supplemental source of iodine is no longer required.

Cobalt requirements are often overestimated for non-ruminants. The only metabolic role currently accepted for cobalt is as a component of vitamin B_{12}. Therefore, similar symptoms of deficiency are observed in cases of cobalt or vitamin B_{12} deficiency. Since animals do not have the enzymes required to attach cobalt to the molecule to form vitamin B_{12}, the supply of this mineral is ineffective in non-ruminants without access to faeces. Rabbits, however, depend on cobalt to produce vitamin B_{12}. In fact, the existing bacteria in the rabbit hindgut are more efficient than the bacteria of other non-ruminant and ruminant species in the production of vitamin B_{12} (Simnett and Spray, 1965a, b). Furthermore, the requirements for cobalt are relatively greater for ruminants than for rabbits (Underwood, 1977), because rumen microorganisms use large amounts of cobalt to synthesize non-active compounds. In addition, ruminants require extra amounts of vitamin B_{12} (and consequently of cobalt) for propionic acid metabolism, a volatile fatty acid that is a major energy-yielding source for these species. In the case of cobalt deficiency, the rate of propionate clearance from the blood is depressed and, as a consequence, feed intake and productivity are decreased (McDowell, 2003).

Literature requirements for cobalt in rabbits generally vary between 0 and 0.25 mg kg^{-1}, although the National Research Council (NRC, 1977) recommended 1.0 mg kg^{-1}. Practical premixes for rabbits in Spain and Portugal contain between 0.1 and 0.7 mg cobalt kg^{-1}, and one-third of them include >0.4 mg. Cobalt deficiency, even in unsupplemented vitamin B_{12} diets, is unlikely to occur, especially under non-intensive production systems. Based on the above data, supplementation of rabbit diets with 0.25 mg cobalt kg^{-1} is recommended.

The current trace mineral composition (average and range) of commercial premixes used in Spain and Portugal for rabbits is depicted in Table 7.6. This table also presents recommended values from the authors and the current maximum levels allowed in the EU.

7.2 Vitamin Requirements of Rabbits

Vitamins are defined as a group of complex organic compounds that are present in minute amounts in natural feeds and are essential for nutrient metabolism and life. Deficiency causes a decrease in performance and often pathological symptoms of disease. Vitamins and trace minerals differ in their nature: vitamins are organic and trace minerals are inorganic.

In a review of published data, Combes (2004) showed that rabbit meat contains on average the following amounts of vitamins 100 g^{-1} of product: 0.186 mg vitamin E, 0.082 mg thiamine, 0.125 mg riboflavin, 9.6 mg niacin, 0.34 mg pyridoxine, 0.60 mg pantothenic acid, 6.85 μg cobalamin, 5 μg folic acid and 0.7 μg biotin. Vitamin A was only found in trace amounts, whereas vitamin C was essentially absent. These values are similar to those found for other meats, except for thiamine and folic acid, which are higher in pig and beef meat, respectively (Dalle Zotte, 2004).

Except for choline, vitamins are required in minute amounts and requirements are expressed as IU, mg kg^{-1} or ppm. All vitamins have essential functions in the organism: most act as metabolic catalysts of organic processes. Not all vitamins are essential in a strict sense. Some can be derived from other substances obtained through metabolic changes. For example: vitamin C can be synthesized in several species including the rabbit; choline is synthesized by mammals and avian species, but often in smaller amounts than required for optimal growth; niacin can be obtained from tryptophan, although the process is quite inefficient; most B vitamins are synthesized by microorganisms of the gut and recycled into the body; and vitamin D can be obtained from precursors by the action of ultraviolet light on the skin. Therefore, many vitamins do not fit the classic definition of vitamins.

Vitamins are classified on the basis of their solubility. Vitamins A, D, E and K are soluble in fat, whereas all the others (B complex, vitamin C) are soluble in water. Fat-soluble vitamins are absorbed with dietary lipids, probably by similar mechanisms. In general, they are stored in the body (predominantly in the liver and fat tissues) in appreciable amounts. Water-soluble vitamins are not stored but rapidly excreted, the exception being vitamin B_{12}. In addition, both groups differ in their excretion patterns: fat-soluble vitamins are excreted primarily in faeces via the bile, whereas water-soluble vitamins are excreted mainly through the urine. A continuous supply is therefore more important for water- than for fat-soluble vitamins. Because rabbits have a functional hindgut, the need for supplementation is much higher for fat- than for water-soluble vitamins. In fact, the benefits of adding B-complex vitamins to commercial rabbit feeds have not been experimentally demonstrated. However, the production of B vitamins may not meet requirements in highly producing rabbits and, thus, extra supplementation is often convenient and sometimes required.

7.2.1 Fat-soluble vitamins

Vitamin A

Vitamin A, as such, is only found in ingredients of animal origin or synthetic supplements. Plants contain a series of precursors, the carotenoids, with variable vitamin A activity. In the rabbit, β-carotene, the most important precursor of vitamin A found in vegetables, is converted into vitamin A in the intestinal mucosa. In most domestic species, the process is not very efficient. In the rabbit, Bondi and Sklan (1984) estimated a conversion efficiency of around 1700 IU vitamin A mg^{-1} β-carotene, similar to that of chickens. The process, which requires the presence of a copper-dependent enzyme, is more efficient at low β-carotene intakes. For this reason, vitamin A toxicity in rabbits is more likely to occur when vitamin A, rather than the precursor, is supplemented in the diet (Deeb *et al.*, 1992). Vitamin A participates in numerous metabolic reactions and is involved in vision, bone development, maintenance of epithelial integrity, reproduction and the immunological response. A particular problem in young rabbits that responds to vitamin A supplementation is hydrocephaly. It results from defective bone growth with stenosis of the cerebral aqueduct and elevated cerebrospinal fluid pressure, which may affect nerve function. In addition, vitamin A deficiency reduces fertility and milk production in does and increases abortion rates and resorption of fetuses (Cheeke *et al.*, 1984).

Vitamin A plasma levels in the rabbit are around 150 μg 100^{-1} ml, a value somewhat higher than that for most domestic species (Cheeke, 1987). This value is very variable, because vitamin A is stored in the liver and released as needed. In addition, Kerti *et al.* (2005) showed that caecotrophy increases the retinoid blood levels in rabbits fed diets supplemented with 10,000 IU vitamin A, but that the retinoid content of the liver and kidney was not affected.

Vitamin A requirements for growth and reproduction have not been experimentally determined. Values in the literature vary from 6000 to 10,000 IU (Table 7.7). In practice, feeding levels of 6000 IU for growing-fattening rabbits and 10,000 IU for breeders appear to be sufficient under commercial conditions. The liver can store large quantities of vitamin A. If the amounts supplied are in excess of requirements, the organ becomes overloaded and toxicity symptoms may appear. The adverse effects of a high supplementation of vitamin A (50,000 IU) in growing rabbits include reductions in plasma calcium, bone weight, bone ash and body weight gain (Albar, 1998). Rabbit does are particularly sensitive to vitamin A excess, with symptoms of toxicity similar to those observed for deficiency (Cheeke *et al.*, 1984; Grobner *et al.*, 1985; Moghaddam *et al.*, 1987; Deeb *et al.*, 1992). Therefore, high doses of vitamin A supplied continuously through feed or water to increase immune status and combat stress or other field problems should be avoided. The NRC (1987) recommends a maximum of 16,000 IU to be added to rabbit diets as an upper safe level.

Several authors have observed some benefits when the diets of sows and dairy cows are supplemented with β-carotene, irrespective of vitamin A status (Byers *et al.*, 1956; Czarnecki *et al.*, 1992; Chew, 1994a,b). Cows receiving extra β-carotene show more intense oestrus, increased conception rates and a reduced incidence of follicular cysts. The suggestion is that β-carotene has a specific function in reproduction, independent of its role as a precursor of vitamin A. The mechanism is not known. In the cow, β-carotene is absorbed intact through the intestinal wall and concentrates in the ovarian follicles, where it may exert a beneficial action on reproduction. However, other authors have not found any benefit of β-carotene supplementation other than as a source of vitamin A (Wang *et al.*, 1982, 1988). In the rabbit, this theory has been tested by several authors (Parigi Bini *et al.*, 1983; Elmarimi *et al.*, 1989; Kormann *et al.*, 1989; Besenfelder *et al.*, 1996) with conflicting results. In some cases, the injection or addition through feed of 30–40 mg β-carotene kg^{-1} improved doe conception and the survival rate of lactating kids. In others, no benefits were noted. The DSM

Table 7.7. Vitamin requirements of rabbits (mg kg^{-1}, unless otherwise indicated).

	NRC (1977)	INRA (1989)	Mateos and Piquer (1994)[a]	Xiccato (1996)[b]	Lebas (2004)	Maertens and Luzi (2004)[a]
Growing-fattening						
Vitamin A (mIU)	0.58	6	10	6	6	10
Vitamin D (mIU)	–	0.9	1	0.8	1	1
Vitamin E	40	50	20	30	30	50
Vitamin K_3	–	0	1	2	1	2
Niacin	180	50	31	50	50	50
Pyridoxine	39	2	0.5	2	2	2
Thiamine	–	2	0.8	2	2	2
Riboflavin	–	6	3	6	6	2
Folic acid	–	5	0.1	5	5	5
Pantothenic acid	–	20	10	20	20	20
Cyanocobalamin	–	0.01	0.01	0.01	0.01	0.01
Choline	1200	–	300	50[c]	200	100
Biotin	–	0.2	10	0.2	–	–
Lactating does						
Vitamin A (mIU)	–	12	0.01	10	10	10
Vitamin D (mIU)	–	0.9	1	1	1	1
Vitamin E	30	50	20[d]	50	50	50
Vitamin K_3	–	2	1	2	2	2
Niacin	–	–	31	–	40	50
Pyridoxine	–	–	0.5	–	2	2
Thiamine	–	–	0.8	–	2	2
Riboflavin	–	–	3	–	6	2
Folic acid	–	–	0.1	–	5	5
Pantothenic acid	–	–	10	–	20	20
Cyanocobalamin	–	0	0.01	–	0.01	0.01
Choline	–	–	300	100[d]	100	100
Biotin	–	–	0.01	–	–	–

[a]Common feed for does and fryers.
[b]Young does.
[c]As choline chloride.
[d]Increase to 50 mg kg^{-1} for high-producing does.

(2006) recommends supplementing doe feeds with 10–20 mg β-carotene kg^{-1} in order to improve prolificacy. In contrast to cattle, horses and poultry, rabbits are 'white fat' animals and they are not capable of storing carotenoids. Therefore, it is unlikely that supplementation of β-carotene to diets rich in vitamin A through the feed can improve fertility. The β-carotene molecule will be split at the intestinal mucosa by a 15,15′-dioxygenase and converted into a single molecule of vitamin A. Kerti *et al.* (2005) showed that carotenoids are found in considerable amounts in the hard and soft faeces of adult female rabbits fed standard diets containing 10,000 IU vitamin A and 13.1 mg total carotenoids kg^{-1}. However, no significant amounts were detected in tissues such as blood, liver and kidneys. Moreover, the ovarian follicles of rabbits fed β-carotene showed no detectable levels of β-carotene or other carotenoids (Kormann *et al.*, 1989). Therefore, the only explanation could be that the mechanism by which β-carotene exerts its benefits in rabbits is different from that observed in the cow. Kormann *et al.* (1989) speculated that cleavage of β-carotene may yield a biologically active metabolite of an 'as yet' unknown nature. Based on the lack of agreement among authors on the influence of β-carotene on reproduction and the cost of supplementation, caution is needed. Therefore, it is advisable not to make any recommendation until additional research confirm the benefits of β-carotene supplementation of rabbit diets.

Vitamin D

Vitamin D is synthesized by the animal when exposed to sunlight. The two major natural sources are cholecalciferol (vitamin D_3 of animal origin) and ergocalciferol (vitamin D_2 of plant origin). Vitamin D_3 is preferred to vitamin D_2 by rabbit tissues.

Vitamin D, after dihydroxylation in the liver and kidney, acts as a hormone and plays a central role in the metabolism of calcium and phosphorus, as in other mammalian species, influencing bone mineralization and mobilization. The classic symptoms of deficiency are rickets in growing animals and osteomalacia in adults. Under normal circumstances (calcium levels in excess of requirements) rabbits are very efficient in absorbing calcium, a process that seems to be quite independent of vitamin D status. Bourdeau *et al.* (1986) showed that the net intestinal absorption of calcium and phosphorus in adult rabbits is similar for rabbits deficient in vitamin D and those with vitamin D-supplemented diets. Fébel and Huszar (2000) found that the injection of a large dose of 100,000 IU cholecalciferol did not affect calcium and phosphorus excretion via the faeces. In addition, excess vitamin D_3 increased renal tubular reabsorption of calcium, but did not affect that of inorganic phosphorus. Levels of vitamin D_3 as low as 2300 IU kg^{-1} are detrimental to rabbit productivity, with increased fetal mortality, depressed appetite, diarrhoea, ataxia, paralysis and death (Ringler and Abrams, 1970; Kubota *et al.*, 1982; Lebas, 1987; Zimmerman *et al.*, 1990).

Excess vitamin D, rather than deficiency, is more likely to be a problem under practical conditions. The excess causes resorption of bones and calcification of soft tissues such as the arteries, liver and kidneys (Löliger and Vogt, 1980; Kamphues, 1991). The incidence of problems because of excess dietary vitamin D_3 is more acute when calcium is fed in excess of requirements. Consequently, the recommended level of vitamin D_3 for rabbits is low and should not, under practical conditions, exceed 1000–1300 IU (Table 7.7). Most of the commercial premixes surveyed (Table 7.8) were within this range, but >0.3 (ten out of 29) appeared to have an excess of this vitamin.

Vitamin E

Vitamin E activity is found in a series of eight compounds of plant origin: four tocopherols and four tocotrienols that occur as α, β, γ and Δ forms. The various forms of vitamin E existing in nature have different biological activity, with the natural source isomer, called *RRR*-α-tocopherol or *d*-α toco-

Table 7.8. Premix composition and practical vitamin recommendations for commercial rabbit feeds (mg kg^{-1} diet as-fed basis, unless otherwise indicated).

	Commercial premixes[a]		Recommendation	
	Mean ± SD	Range	Does	Fatteners
Vitamin A (mIU)	9.2 ± 1.3	6–12.5	10	6
Vitamin D (mIU)	1.2 ± 0.4	0.6–2	0.9	0.9
Vitamin E	24.9 ± 10.3	10–50	50	15
Vitamin K_3	1.16 ± 0.6	0–3.3	2	1
Niacin	28.1 ± 11.1	11–50	35	15
Pyridoxine	1.32 ± 0.9	0–4	1.5	0.5
Thiamine	1.11 ± 0.6	0–2	1	0.8
Riboflavin	3.65 ± 1.6	0.5–8	5	3
Folic acid	0.23 ± 0.3	0–1	1.5	0.1
Pantothenic acid	10.1 ± 5.8	0–22	13	10
Cyanocobalamin	0.013 ± 0.01	0–0.1	0.012	0.01
Choline	251 ± 92	0–450	200	100
Biotin	0.014 ± 0.03	0–0.1	0.08	0.01

[a]Vitamin premix composition (*n* = 29).

pherol, the most active. Synthetic forms of α-tocopherol are known as *all-rac*-α-tocopherol or *dl*-α-tocopherol, and include an eight-isomer equimolar mix of which only one isomer is identical to the *d*-α tocopherol found in nature. Current evidence in pigs and poultry indicates that natural sources have approximately twice as much bioactivity than synthetic sources of vitamin E (Lauridsen *et al*., 2002; Wilburn *et al*., 2008; Boler *et al*., 2009). Major functions of vitamin E are the synthesis of prostaglandins, blood clotting, stability of membrane structure and modulation of the immune response. As discussed previously, the functions of vitamin E are closely related to those of selenium for most species, but the role of selenium in rabbit tissues is less important than that of vitamin E. In fact, selenium does not seem to have a sparing effect on vitamin E requirements in the rabbit.

The main signs of vitamin E deficiency are muscular dystrophy in the growing rabbit and poor reproductive performance, with increased abortion rates and stillbirths in the pregnant doe (Yamini and Stein, 1989). Furthermore, problems related to myocardial damage, exudative diathesis, hepatosis, oedema, ulcerations and an increased incidence of mastitis, mammitis and agalaxia have been reported in rabbits fed vitamin E-deficient diets. No recent experiments are available on the vitamin E requirements of rabbits under intensive production systems. Recommended levels in the literature and practical supplementation values are based either on old data (Ringler and Abrams, 1971) or on extrapolation from other species. Until more information is available, it seems advisable to recommend 15 and 50 mg vitamin E kg^{-1} for fatteners and does, respectively. However, vitamin E recommendations might depend on the amount and fatty acid profile of the fat source used, because the fatty acid composition of cell membranes is modified as well as their susceptibility to oxidation. In cases of impaired immunity (Fortun-Lamothe and Drouet-Viard, 2002), high incidence of infections and inflammation of the reproductive organs (Castellini *et al*., 2007) and in the presence of coccidiosis (Diehl and Dristler, 1961) it might be advisable to increase these levels. In addition, Castellini *et al*. (2007) reported than vitamin E-enriched diets (200 mg vitamin E kg^{-1} diet) reduce the production of free radicals and improve semen quality (viability, membrane integrity and motility of spermatozoa), particularly after storage. The inclusion of 200 mg vitamin E kg^{-1} in diets supplemented with unsaturated fat sources (30 g kg^{-1}) has been found to reduce oxidative damage of the jejunal mucosa and lipid oxidation of the liver (Rey *et al*., 1997) and muscle tissues in rabbits (López-Bote *et al*., 1997).

Unlike other fat-soluble vitamins, vitamin E does not accumulate to toxic levels in the liver and the excess is excreted via bile and urine. The absorption of dietary vitamin E varies with rabbit age and the duration of the supplementation period. In adult rabbits the absorption seems to be a saturable process (Castellini *et al*., 2000), whereas in young rabbits the continuous administration of a dose seven to ten times higher than required increases the concentration of vitamin E in plasma and muscle tissue (Oriani *et al*., 2001). The concentration of the degradation product of α-tocopherol (α-CEHC) in urine may serve as indicator of adequate α-tocopherol supply in rabbit bucks. On the other hand, meat cuts from rabbits fed diets supplemented with high levels of vitamin E (>200 mg kg^{-1} diet) have been found to have greater stability, better colour, lower dripping losses and longer shelf-life than cuts from control animals (Bernardini *et al*., 1996; Castellini *et al*., 1998, 2001; Corino *et al*., 1999; Dal Bosco *et al*., 2004; Lo Fiego *et al*., 2004).

Vitamin K

As indicated for other fat-soluble vitamins, the term ‘vitamin K’ is used to describe a group of compounds that share the common characteristic of antihaemorrhagic effects. These compounds can be of vegetable (phylloquinone or K_1), microbial or animal (menaquinones or K_2) origin. Vitamin K is involved in the mechanism of blood coagulation. It is required for the synthesis of prothrombin and other plasma-clotting factors.

Deficiency may result in haemorrhagic conditions and lameness in growing rabbits and in placental haemorrhage and abortion of kits in pregnant does (NRC, 1977). Other vitamin K-dependent enzymes have now been discovered, indicating that this vitamin has more roles than previously thought. For example, osteocalcin, a metabolite involved in the mineralization and formation of bone, has a vitamin K-dependent calcium-binding amino acid that facilitates the binding of osteocalcin to hydroxyapatite in bone (McDowell, 2000).

Most ingredients used in feeds are poor sources of vitamin K; the exception is lucerne meal, which may contain up to 20–25 mg vitamin K kg^{-1}. However, a considerable number of microorganisms present in the rumen and hindgut synthesize large amounts of vitamin K and, consequently, the faeces contain substantial amounts of the vitamin even if none is present in the feed. Therefore, the requirement of rabbits for this vitamin is partly satisfied through coprophagy.

Rabbit requirements for vitamin K are difficult to evaluate. In fact, no studies have been conducted in this respect. Most commercial feeds include levels of vitamin K close to 1 mg kg^{-1} (Table 7.8), an amount that should suffice in most situations. However, in cases of subclinical coccidiosis, the use of sulpha or other drugs, or the inclusion of antimetabolites in the feed (i.e. mouldy ingredients, amprolium), an increase in vitamin K supplementation is advisable, especially in diets for pregnant does.

7.2.2 Water-soluble vitamins

Vitamin C

Vitamin C (ascorbic acid) plays an important role in many biochemical reactions in which oxygen is incorporated into the substrate. It is involved in the biosynthesis of collagen (hydroxylation of lysine and proline) and carnitine, and stimulates phagocytic activity of leukocytes. Vitamin C is synthesized from D-glucose in the liver by most mammals, including the rabbit. Therefore, it is not strictly considered a vitamin for these species (Jennes *et al.*, 1978). In the body, ascorbic acid is found mainly in aqueous compartments such as plasma and seminal fluid. However, extra supplementation with vitamin C does not reduce the number of tocopherol radicals within the membranes of spermatozoa (Castellini *et al.*, 2007). Vitamin C has been shown to act as a pro-oxidant or as an antioxidant depending on the vitamin E status of the tissue (Chen, 1989). More recent studies (Castellini *et al.*, 2003; Lo Fiego *et al.*, 2004) have shown that when vitamins C and E are supplemented simultaneously, the deposition of vitamin E in the muscles and organs of the rabbit increases, indicating that vitamin E is protected from oxidation by the presence of vitamin C.

It has been reported that vitamin C reduces the effects of stress in many species. Under adverse conditions, such as hot weather, intensive production, high stocking density, poor transport and weaning and in the presence of subclinical diseases, the synthesis of ascorbic acid from glucose might be inadequate; consequently, the concentration of vitamin C in the plasma is reduced. Under these circumstances exogenous supplementation with ascorbic acid may be useful (Mahan *et al.*, 1994; Zakaria and Al-Anezi, 1996). No direct confirmation of these effects has been reported in the rabbit, although rabbits under heat stress show a reduced vitamin C concentration in plasma (Verde and Piquer, 1986). In fact, Ismail *et al.* (1992a,b) found a reproductive response in rabbits fed vitamins C and E when subjected to high ambient temperature, but, because of the experimental design used, the effects of vitamin E and C were confounded. In stressful situations, Xiccato (1996) recommended supplementing the diet with 50–100 mg vitamin C kg^{-1}. In all cases, any supplement of this vitamin must be added to the premix in a protected form, because ascorbic acid is easily oxidized, especially under moist conditions and when exposed to contact with copper, iron and other oligoelements.

B vitamins

Appreciable amounts of water-soluble vitamins are supplied to the rabbit through caecotrophy. In fact, caecotrophy meets rabbit requirements for maintenance and average levels of production (NRC, 1977; Harris *et al.*, 1983). However, fast-growing fryers and high-producing does may respond to additional supplementation of B vitamins, namely thiamine (B_1), riboflavin (B_2), pyridoxine (B_6) and niacin (Maertens, 1996; Xiccato, 1996; Lebas, 2004).

Few recent detailed studies have been conducted on the requirements of rabbits for B vitamins. Dietary ingredients used in rabbit diets, such as lucerne meal, wheat middlings and soybean meal, are excellent sources of most B vitamins (Cheeke, 1987). Even when semi-purified diets are used, the classic symptoms of deficiency are seldom observed; no reports are available using high-producing rabbits as experimental animals. Therefore, most of the recommendations for intensive production have been extrapolated from other species or are based on field observations.

Choline is utilized by the organism as a building unit and as an essential component of other molecules involved in the regulation of many metabolic processes. Choline is essential for: (i) building and maintenance of cell structure as a component of phospholipids; (ii) fat metabolism in the liver, preventing abnormal lipid accumulation; (iii) formation of acetylcholine, which allows the transmission of nerve impulses; and (iv) donation of labile methyl groups for the formation of methionine, betaine and other metabolites. Unlike all other vitamins of the B group, choline is synthesized in the liver and acts more as a structural constituent than as a coenzyme. As in other species, betaine is used in practice to replace part of the choline requirements (methyl donor).

In the rabbit, choline deficiency results in retarded growth, fatty liver and necrosis of the kidney tubules (McDowell, 2000). In addition, progressive muscular dystrophy has been reported in fryers fed diets deficient in choline (NRC, 1977). Recommendations for supplementation vary widely (Table 7.7), but unfortunately no recent reports have been published on the requirements of rabbits for this vitamin. Under practical conditions, choline is supplemented through the premix at levels between 0 and 450 mg kg^{-1} (Table 7.8). Based on this information, and taking into account data from other species, a choline supplement of 200 mg kg^{-1} diet should suffice for most situations.

Folic acid is necessary for the transfer of single-carbon units, a role analogous to that of pantothenic acid in the transfer of two-carbon units. Therefore, folic acid is important for the biosynthesis of nucleic acids and for cell division. Folic acid has attracted the attention of scientists because of studies showing an improvement in number of piglets born alive when gestating sows are fed a diet supplemented with choline (Lindemann, 1993; Matte and Giard, 1996). In pigs, the response in litter size with folic acid supplementation seems to be a result of improved embryo and fetal survival. However, no information is available on the folic acid requirements for reproduction in does. In fact, the NRC (1977) does not add any information on the requirements for this vitamin in rabbits. The study of El-Masry and Nasr (1996) indicated that additional supplementation of doe diets with 5 mg of folic acid may improve performance and prolificacy. However, as mentioned before, the experimental conditions used in this research were not representative of modern rabbit production (poor productivity, high mortality at weaning, small number of replicates, high variability and inappropriate composition of the premix used for the control diet). Consequently, this information has to be viewed cautiously.

Values recommended in the literature vary from 0.1 to 5 mg kg^{-1} (Table 7.7). This wide variability indicates the lack of information on the vitamin requirement for rabbits. Commercial premixes used in Spain have a folic acid content varying from 0 to 1 mg kg^{-1}, without any evidence of deficiency even at the lowest level (Table 7.8). Based on the available information, and until further information becomes available, 0.1 and 1.5 mg kg^{-1} are recommended for growing-fattening and does, respectively.

Biotin (vitamin H) is involved in many metabolic reactions, including the interconversions of protein to carbohydrate and carbohydrate to fat. It plays a role in maintaining normal blood glucose when carbohydrate intake is low. Deficiency is detected by abnormal function of the thyroid and adrenal glands, reproductive tract and nervous system (McDowell, 2000). The more obvious clinical signs are dermatitis and secondary lameness. In the rabbit, no deficiency signs have been reported even in the absence of exogenous supplementary biotin. Only when raw egg white, which contains avidin (an antivitamin H), has been fed have a loss of hair and dermatitis been noted (NRC, 1977). In any case, there is no information on the requirements for reproduction and growth under intensive rearing conditions. Recommended values in the literature vary from 0 to 0.2 mg kg^{-1} diet, with greater values for young rabbits. Commercial premixes used in Spain vary in biotin content from 0 to 0.1 mg kg^{-1}, although most do not include any supplement at all (Table 7.8). Data available for pigs indicate a benefit from biotin supplementation on hoof cracks, growth and reproduction (Kornegay, 1986; Lewis *et al.*, 1991). Therefore, until more information is available, 0.01 and 0.08 mg kg^{-1} are recommended for fatteners and does and rearing kits, respectively.

Thiamine is a coenzyme of certain reactions of the citric acid cycle. The classic symptoms of deficiency are neurological disorders, cardiovascular damage and lack of appetite. In the rabbit, a mild ataxia and flaccid paralysis have been reported when extremely low thiamine diets are used (NRC, 1977). Recommended values in the literature are presented in Table 7.7. Commercial premixes include between 0 and 2 mg kg^{-1} diet. Until more information is available, it is recommended to supplement the diets of does and fatteners with 1.0 and 0.8 mg thiamine kg^{-1}, respectively (Table 7.8).

Riboflavin is required as a coenzyme in many metabolic processes. Most flavoproteins (flavin adenine dinucleotide, flavin mononucleotide) contain vitamin B_2 and, therefore, this vitamin is involved in the release of food energy and the assimilation of nutrients. Typical symptoms of deficiency involve the eyes, skin and nervous system. Milk is rich in riboflavin and deficiency should not therefore be expected in suckling rabbits. Diets deficient in riboflavin have more negative effects on early embryonic mortality than on fertility. Riboflavin concentrates in the uterus in the early pregnancy of sows, and a massive increase in dietary riboflavin may improve embryo survival and litter size (Bazer and Zavy, 1988). Moreover, Pettigrew *et al.* (1996) observed an increase in farrowing in early pregnant sows that were extra-supplemented with riboflavin, but no increases in litter size were detected. No reports are available on the requirements of highly productive rabbits for this vitamin. Values recommended in the literature vary between 2 and 6 mg kg^{-1} (Table 7.7). Spanish and Portuguese premixes provide between 0.5 and 8 mg kg^{-1} (Table 7.8). Based on the few available data, 3 and 5 mg kg^{-1} is recommended for fryers and does, respectively.

Niacin is involved in many metabolic reactions such as electron transport, which yields energy to the animal. It plays a role in tissue integrity, especially of the skin, gastrointestinal tract and nervous systems. Deficiency is characterized by hair loss, dermatitis, diarrhoea, lack of appetite and ulcerative lesions. Therefore, in cases of deficiency, bacterial infection and enteric conditions are likely to develop. In the rabbit, substantial amounts of niacin are synthesized by the hindgut microorganisms, which add to satisfy requirements. In addition, a small amount of this vitamin can be derived from dietary tryptophan, although the process is very inefficient. In all cases, rabbits seem to respond to extra niacin supplementation (NRC, 1977). Recommended values in the literature vary between 31 (Mateos and Piquer, 1994) and 180 mg kg^{-1} (NRC, 1977). These large discrepancies are inexplicable, even in the absence of experimental information. Practical observations indicate that the NRC (1977) recommendations grossly overestimate rabbit requirements. In fact, no symptoms of deficiency have been reported when rabbit does are supplemented with as low as 10–15 mg niacin kg^{-1} diet.

Pyridoxine refers to a group of three related compounds: pyridoxine, pyridoxal and pyridoxamine, with equivalent activity in mammals. This vitamin plays a role in the Krebs cycle and in amino acid, carbohydrate and fatty acid metabolism. Synthesis of niacin from tryptophan, conversion of linoleic to arachidonic acid, formation of adrenalin from phenylalanine and tyrosine, incorporation of iron into haemoglobin and antibody formation are some of the reactions in which pyridoxine is involved. Pyridoxine deficiency produces retarded growth, dermatitis, convulsions, anaemia, scaly skin, alopecia, diarrhoea and a fatty liver, among other symptoms.

In the rabbit, pyridoxine deficiency causes inflammation around the eyes and nose, scaly thickening of the skin around the ears, alopecia in the forelegs and skin desquamation (Bräunlich, 1974). Supplemental values recommended in the literature vary from 0.5 mg kg^{-1} for lactating does and growing rabbits (Mateos and Piquer, 1994), 39 mg kg^{-1} for growing-fattening (NRC, 1977) and up to 400 mg kg^{-1} for growing angora rabbits (Schlolaut, 1987). Again, this extreme range of recommendations is equivocal and is partly due to a lack of experimental data. Spanish and Portuguese premixes included in the survey contained between 0 and 4 mg pyridoxine kg^{-1}. Most of the commercial premixes (25 out of 29) contained between 1 and 2 mg pyridoxine kg^{-1} and no clinical symptoms have ever been detected in the field using these low levels (Mateos and Piquer, 1994). Based on current field observations, 0.5 and 1.5 mg kg^{-1} are recommended for fatteners and does, respectively.

Pantothenic acid is a constituent of coenzyme A and acyl carrier proteins, key metabolites in tissue metabolism. Pantothenic acid deficiency reduces growth and produces symptoms such as skin lesions, nervous disorders, gastrointestinal disturbances, impairment of adrenal function and decreased resistance to infection. No deficiency symptoms have ever been described in the rabbit (McDowell, 2000) because of deficiency in this vitamin. Kulwich *et al.* (1953) observed that caecotrophs have up to six times more pantothenic acid than hard faeces. Literature requirements vary from 10 to 20 mg kg−1 (Table 7.7). Contents of Spanish and Portuguese premixes vary from 0 to 22 mg kg^{-1} diet. Until new data are available, 10 and 13 mg kg^{-1} are recommended for growers and does, respectively (Table 7.8).

Vitamin B_{12} (cyanocobalamin) was the last but the most potent vitamin to be discovered. It is synthesized in nature only by microorganisms and is not found in feeds of plant origin. As mentioned before, cobalt is the prosthetic group of this molecule (being the only role known for this micromineral). Vitamin B_{12} is involved as a coenzyme in reactions such as the formation of one-carbon units (methyl group synthesis). Therefore, vitamin B_{12} is metabolically related to choline, methionine and folacin, among other essential nutrients. Symptoms of deficiency include anaemia, loss of appetite, rough skin, diarrhoea and reduced litter size. Rabbits are capable of producing substantial amounts of vitamin B_{12} through coprophagy, provided that cobalt is available, and no deficiency symptoms have ever been described when commercial diets are used. Values recommended in the literature vary from 0 to 0.01 mg kg^{-1}. Commercial premixes contain between 0 and 0.1 mg kg^{-1} (Table 7.8). On the basis of current knowledge, 0.01 to 0.012 mg kg^{-1} is recommended for growers and does, respectively.

The vitamin composition of 29 premixes marketed in Spain and Portugal that correspond to more than 0.90 of the total feed compound produced in these two countries is presented in Table 7.8. Furthermore, the recommended levels of vitamins of premixes intended for commercial rabbit diets are included in this table.

7.3 Additives

A large number of feed additives are used in rabbit feeding worldwide to improve certain characteristics of the feed or to enhance animal performance. The list is very broad and includes anticoccidial drugs, growth promoters, preservative agents, enzymes, flavours, prebiotics, probiotics, acidifiers and pellet binders. Some (i.e. antibiotics) are not legally permitted in many countries, including the EU-27, and some (i.e. enzymes, probiotics) may need to pass through a registration process prior to commercialization. A selection of additives authorized by EU legislation and more likely to be used in conventional rabbit feeds is now presented.

7.3.1 Anticoccidial drugs

Coccidiosis is one of the most important diseases affecting rabbitries. Intestinal and liver coccidiosis may cause diarrhoea and death. In most commercial situations the disease occurs subclinically, with growth retardation and impairment of feed conversion. Consequently, anticoccidial drugs are frequently used in intensive production systems as a prophylactic therapy to reduce losses caused by coccidiosis. The production of rabbits in wire cages together with the use of preventive medication reduces the incidence of the disease to manageable levels in modern rabbitries.

Several products are available to control coccidiosis. Those used in the EU include robenidine (Licois and Coudert, 1980), salinomycin (Gaca-Lagodzinska *et al.*, 1994; Paefgen *et al.*, 1996) and diclazuril (Table 7.9) (Vanparis *et al.*, 1989; Van Meirhaeghe *et al.*, 1996). Other products that have been shown to be effective but that are not registered in the EU are metichlorpindol and the combination of metichlorpindol and methylbenzoquate. Some ionophores, successfully used in poultry, are toxic for rabbits (e.g. narasin, monensin) and cross-contamination with feeds from other species may cause problems (Salles *et al.*, 1994).

Most of the available coccidiostats to control the disease have some side effects, especially when the recommended doses are not followed. For example, robenidine can taint the muscles and, more specifically, the liver of the rabbits, and might not be as effective as other drugs in controlling hepatic coccidiosis. Furthermore, if cross-contamination occurs with layer feeds, a taint may appear in egg yolks. Salinomycin, in excess of recommended levels, decreases feed intake, which is a quite common side effect for most ionophore drugs and also for other therapeutic drugs (Okerman and Moermans, 1980; Peeters *et al.*, 1980; Morisse *et al.*, 1989). In addition, ionophore cross-contamination may be responsible for toxicity in animals other than the target species. Horses, turkeys, guinea fowls and broiler breeders are the species more greatly affected by toxicity of ionophores when cross-contamination occurs.

7.3.2 Antibiotics and growth promoters

Currently, bacitracin, colistin, apramycin and tiamulin are registered as therapeutic drugs in most European countries. All of them require veterinary prescription for use in rabbit feeds. Several antibiotics, most of them effective against Gram-positive microorganisms, have been shown to improve rabbit growth and feed efficiency at low levels of inclusion. Flavophospholipol (2–4 mg kg^{-1}), avoparcin (10–20 mg kg^{-1}), bacitracin (50–100 mg kg^{-1}) and virginiamycin (30 mg kg^{-1}) are some of the antibiotics effective in improving rabbit

Table 7.9. Anticoccidial drugs permitted for rabbits in the European Union.

Name	Amount (mg kg^{-1} diet)	EU registration status (Annex 1)	Withdrawal period (days)
Robenidine	50–66	All types	5
Salinomycin	20–25	Growing-fattening	5
Diclazuril	1	All types	1

performance (Escribano *et al.*, 1982; Mateos, 1989; Maertens *et al.*, 1992; Abecia *et al.*, 2005; Ayed and Saïd, 2008). However, the use of any antibiotic as a growth promoter is forbidden in the EU.

Many antibiotics used as therapeutic drugs in other species have side effects on rabbit performance (Licois, 1980; Thilsted *et al.*, 1981). Ampicillin, lincomycin and other drugs disturb the normal microflora of the intestines, increasing mortality and depressing growth (Mateos, 1989; Morisse *et al.*, 1989; Lafargue-Hauret *et al.*, 1994; Licois, 1996).

7.3.3 Probiotics and prebiotics

Because of uncertainty regarding the use of antibiotics and growth promoters in animal feeds, new lines of products, more acceptable to the consumer, are appearing in the market. These are used to reduce the incidence of enteric diseases and to improve feed intake and digestibility. Probiotics, such as yeasts (Maertens and De Groote, 1992) and *Bacillus* (Maertens *et al.*, 1994), and prebiotics, such as oligosaccharides (Morisse *et al.*, 1992; Lebas, 1993) and yeast cell walls, are finding a place in rabbit feeding. A review by Falçao-e-Cunha *et al.* (2007) reported the results published in the literature on the use of these additives in rabbits.

Probiotics are supplements that contain beneficial live or reviable microorganisms. It is thought that these supplements colonize the gut, contributing to the maintenance of the flora equilibrium (Maertens and De Groote, 1992). The objective is to create a gut barrier against pathogens. The mechanism of action of probiotics has not been elucidated, but might include: (i) reduction of toxin production; (ii) stimulation of enzyme production by the host; (iii) production of some vitamins or antimicrobial substances; (iv) competition for adhesion to epithelial cells and increased resistance to colonization; and (v) stimulation of the immune system of the host (Simon *et al.*, 2003; Falçao-e-Cunha *et al.*, 2007). A summary of 13 trials conducted with probiotics in fattening rabbits and four trials in does from 1991 to 2006 has been published (Falçao-e-Cunha *et al.*, 2007). At this moment, *Bacillus cereus* var. *toyoi* and *Saccharomyces cerevisiae* NCYC Sc 47 are registered for rabbits in the EU (Falçao-e-Cunha *et al.*, 2007). Some probiotics have been shown to benefit rabbit performance (de Blas *et al.*, 1991; Onifade *et al.*, 1999; Trocino *et al.*, 2005; Pinheiro *et al.*, 2007), but none works under all systems of production (Aoun *et al.*, 1994; Maertens *et al.*, 1994; Michelan *et al.*, 2002).

Prebiotics are non-digestible food ingredients that can selectively stimulate certain intestinal bacteria with potential benefits for the health of the rabbit. The main commercial oligosaccharides available on the market are fructo-, α-galacto-, transgalacto-, mannan- and xylo-oligosaccharides (Falçao-e-Cunha *et al.*, 2007). The main advantages of prebiotics over probiotics are the lack of problems when heat is applied during feed processing or with the acidity of the stomach. In addition, prebiotics are not live organisms, which facilitates the legal registration process. Prebiotics selectively stimulate the beneficial bacteria of the caecum microflora. Supplementation of rabbit feeds with certain oligosaccharides increases volatile fatty acids in the caeca of weanling rabbits, decreasing the caecal ammonia concentration. In addition, to stimulate the beneficial microflora of the gut, prebiotics may prevent the adhesion of pathogens to the mucosa and stimulate the immune response (Forchielli and Walker, 2005; Falçao-e-Cunha *et al.*, 2007). Several authors (Aguilar *et al.*, 1996; Lebas, 1996) have observed a decrease in mortality and an improvement in performance when oligosaccharides are added to rabbit feeds. The beneficial effects of these additives are more evident when rabbits are reared under poor commercial conditions than under clean experimental conditions (Mourao *et al.*, 2006). The changes in the intestinal environment produced by the inclusion of prebiotics in the diet may prevent or reduce the incidence of colibacillosis (Peeters *et al.*, 1992). However, beneficial effects are not always observed with the use of these additives (Guidenne, 1995; Pinheiro *et al.*, 2009). Thus, more research and information are needed prior to recommending their inclusion in rabbit diets.

7.3.4 Enzymes

Extensive research conducted in poultry throughout the world has clearly demonstrated that adding exogenous enzymes to diets rich in cereals such as wheat and barley improves bird performance (Bedford and Morgan, 1996; Gracia *et al.*, 2003; García *et al.*, 2008). The mode of action of enzymes has not been fully elucidated, but might be related to modifications of the intestinal environment, including changes in the viscosity of the digesta (Bedford, 1995; Lázaro *et al.*, 2003). This may allow a better contact between nutrients, endogenous enzymes and the absorptive mucosa, and therefore a better use of the diet. In addition, non-starch polysaccharides may coat the nutrients contained in the grain and the addition of cell-wall-degrading enzymes (e.g. xylanases, β-glucanases) may release nutrients facilitating their digestion (Classen, 1996; Cowan *et al.*, 1996). Furthermore, enzyme supplementation increases the rate of passage in poultry, which may improve feed intake (Lázaro *et al.*, 2003) and reduce the growth of *Clostridium* species and other anaerobes in the gut.

The use of enzymes as feed additives has not been extensively studied in the rabbit (Falçao-e-Cunha *et al.*, 2007). Rabbits are very efficient in the utilization of nutrients (except for fibre), probably because of coprophagy (Marounek *et al.*, 1995; Guidenne and Lacois, 2005). Makkar and Singh (1987) found that the activity of proteases and amylases was higher in the caecum of rabbits than in the rumen. Marounek *et al.* (1995) reported that the caecal contents of 4-week old rabbits contained most of the total activity of pectinase (0.43), amylase (0.45), lactase (0.57), xylanase (0.65), cellulase (0.69), β-glucosidase (0.70) and urease (0.80) present in the rabbit digestive tract, and that these values increased with age. Consequently, the beneficial effects of carbohydrase supplementation are expected to be very limited. In fact, most published results with exogenous enzymes in rabbit diets have not found any improvement in performance (Tor-Aghydye *et al.*, 1992; Fernández *et al.*, 1996; Remois *et al.*, 1996; Pinheiro and Almeida, 2000; Falçao-e-Cunha *et al.*, 2004, 2007). Moreover, Bolis *et al.* (1996) found a negative effect on nutrient digestibility when a commercial protease was added to the diet. Others, however, have found benefits when an enzyme cocktail is added to diets for rabbits under extensive (Bhatt *et al.*, 1996) and intensive production (García-Ruíz *et al.*, 2006) systems, especially in young animals. The inclusion of proteases in the diet reduced rabbit mortality during the first 14 days of the fattening period (García-Palomares *et al.*, 2006a, b). García-Palomares *et al.* (2006a) and Chamorro *et al.* (2005) found that nitrogen flow reaching the terminal ileum decreased with enzyme inclusion, reducing the colony counts of highly pathogenic *Clostridium perfringens* in the caecum. García-Palomares *et al.* (2006b) supplemented a starter diet for early weaned rabbits (25 days old) with a carbohydrase cocktail (β-glucanase, β-xylanase, α-amylase and pectinase) and found a significant improvement in digestibility, growth rate and feed efficiency from 25 to 39 days of age and decreased mortality for the entire fattening period.

Therefore, at present, the addition of exogenous carbohydrase and protease enzymes to rabbit feeds should be limited to diets for the first 14 days after weaning. Similarly, the use of phytases may have some merits in rabbit feeds (Gutierrez *et al.*, 2000), although the benefits are probably less than those observed for other non-ruminant species.

References

Abbo el-Ezz, Z.R., Salem, M.H., Sassan, G.A., El-Komy, A.G. and Abd El Moula, E. (1996) Effect of different levels of copper sulphate supplementation on some physical traits of rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 59–64.

Abecia, L., Balcells, J., Fondevila, M., Belenguer, A. and Calleja, L. (2005) Effect of therapeutic doses of antibiotics in the diet on the digestibility and caecal fermentation in growing rabbits. *Animal Research* 54, 307–314.

Aboul-Ela, S., Abd El-Galil, K. and Ali, F.A. (2000) Effect of dietary fiber and energy levels on performance of post-weaning rabbits. *World Rabbit Science* 8 (Suppl. 1), 61–75.

AEC (1987) Tables AEC. *Recommendations for Animal Nutrition*, 5th edn. Rhône-Poulenc, Commentry, France.

Aguilar, J.C., Roca, T. and Sanz, E. (1996) Fructo-oligosaccharides in rabbit diets. Study of efficiency in suckling and fattening periods. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 73–77.

Albar, A. (1998) Effects of ascorbic acid on hypervitaminosis in rabbits. *University of Aden Journal of Natural and Applied Sciences* 2, 13–19.

Aoun, M., Grenet, L., Mousset, J.L. and Robart, P. (1994) Effet d'une supplementation avec de l'oxytetracycline et des levures vivantes sur les performance d'engraissement. In: *6èmes Journées de la Recherche Cunicole*. INRA-ITAVI, La Rochelle, France, pp. 277–284.

Assane, M., Gongnet, G.P., Coulibaly, A. and Sere, A. (1993) Influence du rapport calcium/phosphore de la ration sur la calcemie, la phosphatemie et la magnesiemie de la lapine en gestation. *Reproduction Nutrition Development* 33, 223–228.

Assane, M., Gongnet, G.P., Coulibaly, A., Sere, A. and Gaye, O. (1994) Effect of calcium phosphorus ratio on phosphocalcic balance and reproduction performance in rabbit in Sahelian environment. *Revue de Medicine Veterinaire* 145, 651–657.

Ayed, M.H. and Saïd, B.B. (2008) Effect of tiamulin or rescue-kit (R) on diet utilisation, growth and carcass yield of growing rabbits. *World Rabbit Science* 16, 183–188.

Ayyat, M.S., Marai, I.F.M. and Alazab, A.M. (1995) Copper protein nutrition of New Zealand White rabbits under Egyptian conditions. *World Rabbit Science* 3, 113–118.

Barlet, J.P. (1980) Plasma calcium, inorganic phosphorus and magnesium levels in pregnant and lactating rabbits. *Reproduction Nutrition Development* 20, 647–651.

Barlet, J.P., Davicco, M.J. and Coxam, V. (1995) Physiologie de l'absorption intestinale du phosphore chez l'animal. *Reproduction Nutrition Development* 35, 475–489.

Barr, D.R., Sadowski, D.L., Jie, H. and Bourdeau, J.E. (1991) Characterizing renal and intestinal adaptations to dietary calcium deprivation in growing female rabbit. *Mineral and Electrolyte Balance* 17, 32–40.

Bas, S., Bas, A., López, I., Estepa, J.C., Rodríguez, M. and Aguilera-Tejero, E. (2005) Nutritional secondary hyperparathyroidism in rabbits. *Domestic Animal Endocrinology* 28, 380–390.

Bassuny, S.M. (1991) The effect of copper sulfate supplement on rabbit performance under Egyptian conditions. *Journal of Applied Rabbit Research* 14, 93–97.

Bazer, F.W. and Zavy, M.T. (1988) Supplemental riboflavin and reproductive performance of gilts. *Journal of Animal Science* 66 (Suppl.), 324 (Abstr.).

Bedford, M.R. (1995) Mechanism of action and potential environmental benefits from the use of feed enzymes. *Animal Feed Science and Technology* 53, 145–155.

Bedford, M.R. and Morgan, A.J. (1996) The use of enzymes in poultry diets. *World's Poultry Science Journal* 52, 61–68.

Bernardini, M., Dal Bosco, A., Castellini, C. and Miggiano, G. (1996) Dietary vitamin E supplementation in rabbit: antioxidant capacity and meat quality. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 137–140.

Besenfelder, U., Solti, L., Seregi, J., Muller, M. and Brem, G. (1996) Different role of β-carotene and vitamin A in the reproduction of rabbits. *Theriogenology* 45, 1583–1591.

Bhatt, R.S., Bhasin, V. and Bhatia, D.R. (1996) Effect of Kemzyme on the performance of German angora weaners. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 97–99.

Boler, D.D., Gabriel, S.R., Yang, H., Balsbaugh, R., Mahan, D.C., Brewer, M.S., McKeith, F.K. and Killefer, J. (2009) Effect of different dietary levels of natural source vitamin E in grow-finishing pigs on pork quality and shelf-life. *Meat Science* 83, 723–730.

Bolis, S., Castrovilli, C., Rigoni, M., Tedesco, D. and Luzi, F. (1996) Effect of enzyme addition in diet on protein and energy utilization in rabbit. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 111–115.

Bondi, A. and Sklan, D. (1984) Vitamin A and carotene in animal nutrition. *Progress in Food Nutrition Science* 8, 165–191.

Borowitz, S.M. and Granrud, G.S. (1993) Ontogeny of intestinal phosphate absorption in rabbits. *American Journal of Physiology* 262, 847–853.

Bourdeau, J.E., Schwer-Dymerski, D.A., Stern, P.H. and Langman, C.B. (1986) Calcium and phosphorus metabolism in chronically vitamin D deficient laboratory rabbits. *Mineral and Electrolyte Metabolism* 12, 176–185.

Bräunlich, K. (1974) *Vitamin B6*. Report No. 1451, Hoffmann-La Roche, Basel, Switzerland.

Burgi, A.R. (1993) Nutriçao mineral de coelhos. *Zootecnia* 31, 89–95.

Byers, J.H., Jones, I.R. and Bone, J.F. (1956) Carotene in the ration of dairy cattle. II. The influence of suboptimal levels of carotene intake upon the microscopic aspect of selected organs. *Journal of Dairy Science* 39, 1556–1564.

Castellini, C., Dal Bosco, A., Bernardini, M. and Cyril, H.W. (1998) Effect of dietary vitamin E on the oxidative stability of raw and cooked rabbit meat. *Meat Science* 50, 153–161.

Castellini, C., Dal Bosco, A. and Bernardini, M. (2000) Effect of α-tocopheryl acetate and ascorbic acid on rabbit semen: vitamin content and oxidation status of rabbit semen. In: Blasco, A. (ed.) *Proceedings of the 7th World Rabbit Congress, Valencia*. Valencia University Publications, Valencia, Spain, pp. 105–110.

Castellini, C., Dal Bosco, A. and Bernardini, M. (2001) Improvement of lipid stability of rabbit meat by vitamin E and C administration. *Journal of the Science of Food and Agriculture* 81, 46–53.

Castellini, C., Lattaioli, P., Dal Bosco, A. and Beghelli, D. (2002) Effect of supranutritional level of α-tocopheryl acetate and selenium on rabbit semen. *Theriogenology* 58, 1723–1732.

Castellini, C., Lattaioli, P. Dal Bosco, A., Minelli, A. and Mugnai, C. (2003) Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids. *Reproduction Nutrition Development* 43, 91–103.

Castellini, C., Mourvaki, E., Dal Bosco, A. and Galli, F. (2007) Vitamin E biochemistry and function: a case study in male rabbit. *Reproduction in Domestic Animals* 42, 248–256.

Cavalcante, S.G., Ferreira, W.M., Valente, S.S., Santiago, G.S., Dias, J.C.C.A and Naranjo, A.P. (2002) Bioavailability of copper from different sources in rabbits. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* 54, 290–294.

Chamorro, S., Gómez-Conde, M.S., Pérez de Rozas, A.M., Badiola I., Carabaño R. and de Blas C. (2005) Efecto del nivel y tipo de proteína en piensos de gazapos sobre parámetros productivos y salud intestinal. *Proceedings XXX Symposium de Cunicultura de ASESCU*. Valladolid, Spain, pp. 135–142.

Chamorro, S., Gómez-Conde, M.S., Centeno, C., Carabaño, R. and de Blas, J.C. (2007) Effect of dietary sodium on digestibility of nutrients and performance in growing rabbits. *World Rabbit Science* 15, 141–146.

Chapin, R.E. and Smith, S.E. (1967) Calcium requirement of growing rabbits. *Journal of Animal Science* 26, 67–71.

Cheeke, P.R. (1987) *Rabbit Feeding and Nutrition*, 1st edn. Academic Press, London, UK.

Cheeke, P.R. and Amberg, J.W. (1973) Comparative calcium excretion by rats and rabbits. *Journal of Animal Science* 37, 450–454.

Cheeke, P.R., Patton, N.M., Diwyanto, K., Lasmini, A., Nurhadi, A., Prawirodigdo, S. and Sudaryanto, B. (1984) The effect of high dietary vitamin A levels on reproductive performance of female rabbits. *Journal of Applied Rabbit Research* 7, 135–137.

Cheeke, P.R., Bronson, J., Robinson, K.L. and Patton, N.M. (1985) Availability of calcium, phosphorus and magnesium in rabbit feeds and mineral supplements. *Journal of Applied Rabbit Research* 8, 72–74.

Chen, L.H. (1989) Interaction of vitamin E and ascorbic acid (Review). *In Vivo* 3, 199–209.

Chew, B.P. (1994a) Beta-carotene appears to improve reproductive performance. *Feedstuffs* 66, 14–15.

Chew, B.P. (1994b) Beta-carotene, other carotenoids push immunity defense. *Feedstuffs* 66, 17–51.

Chiericato, G.M. and Rizzi, C. (2004) Study of the effect of the dietary mineral content on the reproductive performance of rabbits of both sexes and on the zootechnical performance of their litters. *Rivista di Coniglicoltura* 41, 44–48.

Chiericato, G.M., Rizzi, C. and Verdiglione, R. (2005) Effeto di diete con different equilibrio electrolitio gulle prestazioni produttive e sul biochimismo ematico di coniglie fattrici. *Rivista di Coniglicottua* 42, 45–48.

Classen, H.L. (1996) Cereal grain starch and exogenous enzymes in poultry diets. *Animal Feed Science and Technology* 62, 21–27.

Coates, M.E., Gregory, M.E. and Thompson, S.Y. (1964). The composition of rabbit's milk. *British Journal of Nutrition* 18, 583–586.

Colin, M. (1977) Effet d'une variation du taux de chlore dans l'alimentation du lapin en croissance. *Annales de Zootechnie* 26, 99–103.

Combes, S. (2004) Valeur nutritionalle de la viande de lapin. *INRA Production Animales* 17, 373–383.

Corino, C., Pastorelli, G., Pantaleo, L., Oriani, G. and Salvatori, G. (1999) Improvement of color and lipid stability of rabbit meat by dietary supplementation with vitamin E. *Meat Science* 52, 285–289.

Cowan, W.D., Jorsbak, A., Hastrup, T. and Rasmussen, P.B. (1996) Influence of added microbial enzymes on energy and protein availability of selected feed ingredients. *Animal Feed Science and Technology* 60, 311–319.

Cromwell, G.L. (1992) The biological availability of phosphorus in feedstuffs for pigs. *Pig News and Information* 13, 75N–78N.

Czarnecki, R., Iwanska, S., Falkowska, A., Delikator, B., Karmelita, M. and Pycio, Z. (1992) Effects of β-carotene containing caromix on reproductive performance of primiparous sows. *World Review of Animal Production* 27, 3–30.

Dal Bosco, A., Castellini, C., Bianchi, L. and Mugnai, C. (2004) Effect of dietary alpha-linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. *Meat Science* 66, 407–443.

Dalle Zotte, A. (2004) Le lapin doit apprivoiser le consommateur: avantages dietétiques. *Viandes Production Carnés* 23, 161–167.

de Blas, C., García, J. and Alday, S. (1991) Effects of dietary inclusion of a probiotic (Paciflor) on performance of growing rabbits. *Journal of Applied Rabbit Research* 14, 148–150.

Deeb, B.J., Digiacomo, R. and Anderson, R.J. (1992) Reproductive abnormalities in rabbits due to vitamin A toxicity. *Journal of Applied Rabbit Research* 15, 973–984.

Diehl, J.F. and Dristler, B.G. (1961) Vitamin E saturation test in coccidiosis infected rabbits. *Journal of Nutrition* 74, 495–499.

Dokoupilova, A., Marounek, M., Skrivanova, V. and Brezina, P. (2007) Selenium content in tissues and meat quality in rabbits fed selenium yeast. *Czech Journal of Animal Nutrition* 52, 165–169.

DSM (2006) Recommended vitamin supplementation levels for domestic animals. In: *Feed Milling Yearbook*, pp. 124–126.

Eiben, C.S., Gippert, T., Gódor-Surmann, K. Podmaniczky, B. and Kustos, K. (2008) Effect of dietary phosphorus reduction and phytase supplementation on growth of rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 201.

Elmarimi, A.A., Ven, E. and Bardos, L. (1989) Preliminary study o the effects of vitamin A and β-carotene on growth and reproduction of female rabbits. *Journal of Applied Rabbit Research* 12, 163–168.

El-Masry, K.A. and Nasr, A.S. (1996) The role of folic acid and iron in reproductive performance of New Zealand White does and their kits. *World Rabbit Science* 4, 127–131.

El-Sayiad, G.A., Habbeb, A.A. and Maghawry, A.M. (1994) A note on the effects of breed, stage of lactation and pregnancy status on milk composition of rabbits. *Animal Production* 58, 153–157.

Erdélyi, M., Virag, G.Y. and Mézes, M. (2000) Effect of supranutritional additive selenium supply on the tissue concentration and the activity of gluthatione peroxidase enzyme in rabbit. In: Blasco, A. (ed.) *Proceedings of 7th World Rabbit Congress*, *Valencia*, Vol. C. Valencia University Publications, Valencia, Spain, pp. 183–189.

Escribano, F., García Alfonso, J., Lozon, C. and Mateos, G.G. (1982) Avotan avoparcina, un nuevo estimulante de crecimiento en conejos en crecimiento-cebo. In: *VII Symposium de Cunicultura, Santiago de Compostela*. ASESCU, 7, 241–249.

Evans, E., Jebelian, V. and Rycquart, W.C. (1983a) Effects of potassium and magnesium levels upon performance of fryer rabbits. *Journal of Applied Rabbit Research* 6, 49–50.

Evans, E., Jabelian, V. and Rycquart, W.C. (1983b) Further evaluation of the magnesium requirements of fryer rabbits. *Journal of Applied Rabbit Research* 6, 130–131.

Falçao-e-Cunha, L., Reis, J., Freire, J.B. and Castro-Solla, L. (2004) Effects of enzyme addition and source of fiber on growth and fibrolytic activities of growing-finishing rabbits. In: Becerril, C.M. and Pro, A. (eds)

Proceedings of the 8th World Rabbit Congress, Puebla. Colegio de Postgraduados, Montecillo, Spain, pp. 1532–1537.

Falçao-e-Cunha, L., Castro-Solla, L., Maertens, L., Marounek, M., Pinheiro, V., Freire, J. and Mourao, J.L. (2007) Alternatives to antibiotic growth promoters in rabbit feeding: a review. *World Rabbit Science* 15, 127–140.

Fébel, H. and Huszar, S. (2000) Examination of the effect of vitamin D_3 on Ca and P metabolism in the rabbit with isotope method. *Magyar Állatorvosok Lapja* 122, 209–213.

Fekete, S. Gippert, R., Hillár, I. and Szilagyi, M. (1988) Effect of dietary copper suphate concentration on digestion, growth rate and some blood parameters of broiler rabbits. In: *Proceedings of the 4th World Rabbit Congress, Budapest*. Sandor Holdas, Hercegalom, Budapest, Hungary, pp. 198–205.

Fernández, C., Merino, M.J. and Carabaño, R. (1996) Effect of enzyme complex supplementation on diet digestibility and growth performance in growing rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 163–166.

Forchielli, M.L. and Walker, W.A. (2005) The role of gut-associated lymphoid tissues and mucosal defense. *British Journal of Nutrition* 93, s41–s48.

Fortun-Lamothe, L. and Drouet-Viard, F. (2002) Review: II – diet and immunity: current state of knowledge and research prospects for the rabbit. *World Rabbit Science* 10, 25–39.

Furlan, A.C., Scapinello, C., Murakami, A.E., Moreira, I., Martins, E. and Cavalieri, F.L.B. (1997a) Exigencia nutricional de sodio de coelhos en crescimento. *Revista da Sociedade Brasileira de Zootecnia* 26, 299–303.

Furlan, A.C., Scapinello, C., Murakami, A.E., Moreira, I., Martins, E. and Cavalieri, F.L.B. (1997b) Exigencia nutricional de calcio de coelhos en crescimento. *Revista da Sociedade Brasileira de Zootecnia* 26, 294–298.

Gaca-Lagodzinska, K., Provot, F. and Coudert, P. (1994) Tolerance de la salinomycine et efficacite contre trois coccidioses du lapin. In: *Proceedings 6èmes Journées de la Recherche Cunicole*. INRA-ITAVI, La Rochelle, France, pp. 67–72.

García, M., Lázaro, R., Latorre, M.A., Gracia, M.I. and Mateos, G.G. (2008) Influence of enzyme supplementation and heat processing of barley on digestive traits and productive performance of broilers. *Poultry Science* 87, 940–948.

García-Palomares, J., Carabaño, R., García-Rebollar, P., de Blas, C., Corujo, A. and García Ruíz, A.I., (2006a) Effects of a dietary protein reduction and enzyme supplementation on growth performance in the fattening period. *World Rabbit Science* 14, 231–236.

García-Palomares, J., Carabaño, R., García-Rebollar, P., de Blas, J.C. and García, A.I. (2006b). Effects of dietary protein reduction during weaning on the performance of does and suckling rabbits. *World Rabbit Science* 14, 23–26.

García-Ruiz, A.I., García Palomares, J., García-Rebollar, P., Chamorro, S., Carabaño, R. and de Blas, C. (2006) Effect of protein source and enzyme supplementation on ileal protein digestibility and fattening performance in rabbits. *Spanish Journal of Agricultural Research* 4, 297–303.

Gilsanz, V., Roe, T.F., Antunes, J., Carlson, M. Duarte, M.L. and Goodman, W.G. (1991) Effect of dietary calcium on bone-density in growing rabbits. *American Journal of Physiology* 260, E471–E476.

Gracia, M.I., Aranibar, M.J., Lazaro, R., Medel, P. and Mateos, G.G. (2003) Alpha-amylase supplementation of broiler diets based on corn. *Poultry Science* 82, 436–442.

Grobner, M.A., Cheeke, P.R. and Patton, N.M. (1985) A note on the effect of a high dietary vitamin A level on the growth of fryer rabbits. *Journal of Applied Rabbit Research* 8, 6.

Gueguen, L., Lamandd, M. and Mewchy, F. (1988) Nutrition mineral. In: Jarrige, R. (ed.) *Alimenttion des Bovines, Ovins and Caprines*. Institut National de la Recherche Agronomique, Paris, France, pp. 97–111.

Guidenne, T. (1995) Effect of fibre level reduction and glucooligosaccharide addition on the growth performance and caecal fermentation in the growing rabbit. *Animal Feed Science and Technology* 56, 253–263.

Guidenne, T. and Licois, D. (2005) Effect of a high fibre intake on the resistance of the growing rabbit to an experimental inoculation with an enteropathogenic strain of *Escherichia coli*. *Animal Science* 80, 281–288.

Guimaraes, C.S. and Motta, F.W. (2000) Bioavailability of dietary zinc sources for fattening rabbits. In: Blasco, A. (ed.) *Proceedings of 7th World Rabbit Congress, Valencia*. Valencia University Publications, Valencia, Spain, pp. 255–261.

GuoXian, Z., ZhiHua, F., YuDing, W., YunQi, L. and GuanZhong, L. (2004) The effects of supplemental microbial phytase in diets on the growth performance and mineral excretion of rex-rabbits. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 1114–1120.

Gutierrez, I., García, J., Carabaño, R., Mateos, G.G. and de Blas, J.C. (2000) Effect of exogenous phytase on phosphorus and nitrogen digestibility in growing-finishing rabbits. In: Blasco, A. (ed.) *Proceedings of 7th World Rabbit Congress*, *Valencia*. Valencia University Publications, Valencia, Spain, pp. 277–281.

Harris, D.J., Cheeke, P.R. and Patton, N.M. (1983) Effect of supplemental vitamins on fryer rabbit performance. *Journal of Applied Rabbit Research* 6, 29–131.

Harris, D.J., Cheeke, P.R. and Patton, N.M. (1984a) Effect of supplemental copper on postweaning performance of rabbits. *Journal of Applied Rabbit Research* 7, 10–12.

Harris, D.J., Cheeke, P.R. and Patton, N.M. (1984b) Effect of feeding various levels of salt on growth performance, mortality and feed preferences of fryer rabbits. *Journal of Applied Rabbit Research* 7, 117–119.

Hermida, M., González, M., Miranda, M. and Rodríguez-Otero, J.L. (2006) Mineral analysis in rabbit meat from Galicia (NW Spain). *Meat Science* 73, 635–639.

Hidiroglou, M., Ho, S.K., Ivan, M. and Shearer, D.A. (1978) Manganese status of pasturing ewes, of pregnant ewes and doe rabbits on low manganese diets and of dairy cows with cystic ovaries. *Canadian Journal of Comparative Medicine* 42, 100–107.

INRA (1989) *L'Alimentation des Animaux Monogastriques: Porc, Lapin, Volailles*, 2nd edn. INRA, París, Cedex, France, pp. 281.

Ismail, A., Shalash, S., Kotby, E., Cheeke, P.R. and Patton, N.M. (1992a) Hypervitaminosis A in rabbits. 3. Reproductive effects and interactions with vitamins E and C and ethoxyquin. *Journal of Applied Rabbit Research* 15, 1206–1218.

Ismail, A., Shalash, S., Kotby, E. and Cheeke, P.R. (1992b) Effects of vitamin A, C and E on the reproductive performance of heat stressed female rabbits in Egypt. *Journal of Applied Rabbit Research* 15, 1291–1300.

Jenkins, K.J., Hidiroglow, M., Mackay, R.R. and Proulx, J.G. (1970) Influence of selenium and linoleic acid on the development of nutritional muscular dystrophy in beef calves, lambs and rabbits. *Canadian Journal of Comparative Medicine* 50, 137–146.

Jennes, R., Birney, E.C. and Ayaz, K.L. (1978) Ascorbic acid and L-gulonolactone oxidase in lagomorphs. *Comparative Biochemistry and Physiology* 61, 395–399.

Kamphues, J. (1991) Calcium metabolism if rabbits as an etiological factor for urolithiasis. *Journal of Nutrition* 121, 595–596.

Kerti, A., Bardos, L., Deli, J. and Olah, P. (2005) Relationship of retinoid and carotenoid metabolism with caecotrophy in rabbits. *Acta Veterinaria Hungarica* 53, 309–318.

King, J.O.L. (1975) The feeding of copper sulphate to growing rabbits. *British Veterinary Journal* 131, 70–75.

Kormann, A.W., Riss, G. and Weiser, H. (1989) Improved reproductive performance of rabbit does supplemented with dietary β-carotene. *Journal of Applied Rabbit Research* 12, 15–21.

Kornegay, E.T. (1986) Biotin in swine production: a review. *Livestock Production Science* 14, 65–89.

Kubota, M., Ohno, K., Shiina, Y. and Suda, T. (1982) Vitamin D metabolism in pregnant rabbits: differences between the maternal and fetal response to administration of large amounts of vitamin D_3. *Endocrinology* 110, 1950–1956.

Kulwich, R., Struglia, L. and Pearson, P.B. (1953) The effect of coprophagy in the excretion of B vitamins by the rabbit. *Journal of Nutrition* 49, 639–645.

Lafargue-Hauret, P., Javrin, D., Ricca, V. and Rouillere, H. (1994) Toxicite de l'amoxicilline chez le lapin. In: *Proceedings 6èmes Journées de la Recherche Cunicole*. INRA-ITAVI, La Rochelle, France, pp. 81–84.

Lauridsen, C., Engel, H., Craig, A.M. and Traber, M.G. (2002) Relative bioactivity of dietary RRR- and all-rac-alpha-tocopherol acetates in swine assessed with deuterium-labeled vitamin E. *Journal of Animal Science* 76, 1216–1231.

Lázaro, R., García, M., Araníbar, M.J. and Mateos, G.G. (2003) Effect of enzyme addition to wheat-, barley- and rye-based diets on nutrient digestibility and performance of laying hens. *British Poultry Science* 44, 256–265.

Lebas, F. (1987) Feeding conditions for top performance in the rabbit. In: Auxilia, T. (ed.) *Report EUR 10983 EN*. Commission of the European Community, Brussels, Belgium, pp. 27–39.

Lebas, F. (1990) Recherche et alimentation des lapines. *Cuniculture* 17, 12–15.

Lebas, F. (1993) Incidence du Profeed sur l'efficacité alimentaire chez le lapin en croissance. *Cuniculture* 20, 169–172.
Lebas, F. (1996) Effects of fructo-oligosaccharides origin on rabbit's growth performance in 2 seasons. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 211–215.
Lebas, F. (2004) Reflections on rabbit nutrition with special emphasis on feed ingredients utilization. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 686–736.
Lebas, F. and Jouglar, J. (1984) Apports alimentaires de calcium et de phosphore chez la lapine reproductrice. In: Finzi, A. (ed.) *Proceedings of the 3rd World Rabbit Congress, Rome*. WRSA, Rome, Italy, pp. 461–466.
Lebas, F. and Jouglar, J.Y. (1990) Influence du taux de phsphore alimentaire sur les performances de lapines reproductrices. In: *5èmes Journées Recherche Cunicole*. Communication 48, INRA, ITAVI, Paris, France.
Lee, Y.H., Layman, D.K. and Bell, R.R. (1979) Selenium dependent and non selenium dependent glutathione peroxidase activity in rabbit tissue. *Nutrition Reports International* 20, 573–578.
Lewis, A.J., Cromwell, G.L. and Pettigrew, J.F. (1991) Effects of supplemental biotin during gestation and lactation on reproductive performance of sows: a cooperative study. *Journal of Animal Science* 69, 207–214.
Licois, D. (1980) Action toxique de certains antibiotiques chez le lapin. *Recueil Médecine Vétérinaire* 156, 915–919.
Licois, D. (1996) Resques associes a l'utilization des antibiotiques chez le lapin: une mini revue. *World Rabbit Science* 4, 63–68.
Licois, D. and Coudert, P. (1980) Action de la robenidine sur l'excretion des oocystes de differentes especes de coccidies du lapin. In: Camps, J. (ed.) *Proceedings of the 2nd World Rabbit Congress*. Asociación Española de Cunicultura, Barcelona, Spain, pp. 285–289.
Licois, D., Coudert, P. and Mongin, P. (1978) Changes in hydromineral metabolism in diarrhoeic rabbits. 2. Study of the modifications of electrolyte metabolism. *Annales Recherches Vétérinaires* 9, 1–10.
Lindemann, M.D. (1993) Supplemental folic acid: a requirement for optimizing swine reproduction. *Journal of Animal Science* 71, 239–246.
Lo Fiego, D.P., Santoro, P., Machioni, P., Mazzoni, D., Piattoni, F., Tassone, F. and De Leonibus, E. (2004) The effect of dietary supplementation of vitamin C and E on the α-tocopherol content of muscles, liver and kidney, on the stability of lipids, and on certain meat quality parameters of the longissimus dorsi of rabbits. *Meat Science* 67, 319–327.
Löliger, H.C. and Vogt, H. (1980) Calcinosis of kidneys and vessels in rabbits. In: Camps, J. (ed.) *Proceedings of the 2nd World Rabbit Congress*. Asociación Española de Cunicultura, Barcelona, Spain, pp. 284 (Abstr.).
Lombardi-Boccia, G., Lanzi, S. and Aguzzi, A. (2005) Aspects of meat quality: trace elements and B vitamins in raw and cooked meats. *Journal of Food Composition and Analysis* 18, 39–46.
López-Bote, C.J., Rey, A.I., Sanz, M., Gray, J.I. and Buckley, D.J. (1997) Dietary vegetable oils and α-tocopherol reduce lipid oxidation in rabbit muscle. *Journal of Nutrition* 127, 1176–1182.
Maertens, L. (1996) Nutrition du lapin. Connaissances actuelles et acquisitions recentes. *Cunicultura* 23, 33–35.
Maertens, L. (1999) Towards reduced feeding costs, dietary safety and minimal mineral excretion in rabbits: a review. *World Rabbit Science* 7, 65–74.
Maertens, L. and De Groote, G. (1992) Effect of a dietary supplementation of live yeast on the zootechnical performance of does and weanling rabbits. *Journal of Applied Rabbit Research* 15, 1079–1086.
Maertens, L. and Luzi, F. (2004) I fabbisogni alimentary del coniglio da carne. *Coniglicoltura* 5, 20–25.
Maertens, L., Moermans, R. and De Groote, G. (1992) Flavomycin for early weaned rabbits: response of dose on zootechnical performance. Effect on nutrient digestibility. *Journal of Applied Rabbit Research* 15, 1270–1277.
Maertens, L., Van Renterghem, R. and De Groote, G. (1994) Effects of dietary inclusion of Paciflor (Bacillus CIP 5832) on the milk composition and performance of does on caecal and growth parameters of their weanlings. *World Rabbit Science* 2, 67–73.
Maertens, L., Cavani, C. and Petracci, M. (2005) Nitrogen and phosphorus excretion on commercial rabbit farms: calculations based on the input-output balance. *World Rabbit Science* 13, 3–16.
Maertens, L., Lebas, F. and Szendrö, Zs. (2006) Rabbit milk: a review of quantity and non-dietary affecting factors. *World Rabbit Science*, 14, 205–230.

Mahan, D.C., Lepine, A.J. and Dabrowski, K. (1994) Efficacy of magnesium-L-ascorbyl 2-phosphate as a vitamin C source for weanling and growing-finishing swine. *Journal of Animal Science* 72, 2354–2361.
Makkar, H.P.S. and Singh, B. (1987) Comparative enzymatic profiles of rabbit caecum and bovine rumen content. *Journal of Applied Rabbit Research* 10, 172–174.
Marai, I.F.M., Habeeb, A.A.M. and Gad, A.E. (2005) Tolerance of imported rabbits grown as meat animals to hot climate and saline drinking water in the subtropical environment of Egypt. *Animal Science* 81, 115–123.
Maret, W. and Sandstead, H.H. (2006) Zinc requirements and the risks and benefits of zinc supplementation. *Journal of Trace Elements in Medicine and Biology* 20, 3–18.
Marounek, M., Vovk, S.J. and Skrivanova, V. (1995) Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *British Journal of Nutrition* 73, 463–469.
Marounek, M., Duskova, D. and Skrivanova, V. (2003) Hydrolysis of phytic acid and its availability in rabbits. *British Journal of Nutrition* 89, 287–294.
Marounek, M., Brenova, N., Suchorska, O. and Mrazek, J. (2009) Phytase activity in rabbit caecal bacteria. *Folia Microbiologica* 54, 111–114.
Mateos, G.G. (1989) Minerales, vitaminas, antibióticos, anticcodiósicos y otros aditivos en la alimentación del conejo. In: de Blas, C. (ed.) *Alimentación del Conejo*, 2nd edn. Mundi Prensa, Madrid, Spain, pp. 177.
Mateos, G. and de Blas, C. (1998) Feed formulation. In: de Blas, C. and Wiseman, J. (eds) *The Nutrition of the Rabbit*. Nottingham University Press, Nottingham, UK.
Mateos, G. and Piquer, J. (1994) Diseño de programas alimenticios para conejos: aspectos teóricos y formulación práctica. *Boletín de Cunicultura* 17, 16–31.
Matte, J.J. and Giard, C.L. (1996) Le besoin en acide folique chez la truie gravid. *Journées Recherche Porcine en France* 28, 365–370.
McDowell, L.R. (2000) *Vitamins in Animal Nutrition*, 2nd edn. Iowa State University Press, Ames, Iowa, USA, pp. 793.
McDowell, L.R. (2003) *Minerals in Animal and Human Nutrition*, 2nd edn. Elsevier Science, Amsterdam, Países Bajos, pp. 644.
Mehrotra, M., Gupta, S.K., Kumar, K., Awasthi, P.K., Pandey, C.M. and Godbole, M.M. (2006) Calcium deficiency-induced secondary hyperparathyroidism and osteopenia are rapidly reversible with calcium supplementation in growing rabbit pups. *British Journal of Nutrition* 95, 582–590.
Michelan, A.C., Scapinello, C., Natali, M.R.M., Furlan, A.C., Sakaguti, E.S., Faria, H.G., Santolin, M.L.R. and Hernandes, A.B. (2002) Utilizaçao de probiotico, ácido organic e antibiotic em dietas para coelhos em crescimento: ensaio de digestiblidade, avaliaçao da morfometria intestinal e desempenho. *Revista Brasileira de Zootecnia* 31, 2227–2237.
Moghaddam, M.F., Cheeke, P.R. and Patton, N.M. (1987) Toxic effects of vitamin A on reproduction in female rabbits. *Journal of Applied Rabbit Research* 10, 65–67.
Morisse, J.P., Le Gall, G., Boilletot, E. and Maurice, R. (1989) Intoxication alimentaire chez le lapin par des residus d'antibiotiques. *Cuniculture* 16, 288–290.
Morisse, J.P., Maurice, R., Boiletot, E. and Cotte, J.P. (1992) Effect of a fructo-oligo-saccharides compound in rabbit experimentally infected with *E. coli* 0103. *Journal of Applied Rabbit Research* 15, 1137–1143.
Mourao, J.L., Pinheiro, V., Alves, A., Guedes, C.M., Pinto, L., Saavedra, M.J., Spring, and Kocher, A. (2006) Effect of mannan oligosaccharides on the performance, intestinal morophology and caecal fermentation of fattening rabbits. *Animal Feed Science and Technology* 126, 107–120.
NRC (1977) *Nutrient Requirements of Rabbits*. National Academy of Science, National Research Council, Washington, DC, USA.
NRC (1987) *Vitamin Tolerance of Animals*. National Academy of Science, National Research Council, Washington, DC, USA.
Okerman, F. and Moermans, R.J. (1980) L'influence du coccidiostatique salinomycin en tant qu'additif des aliment composes sur les resultants de production des lapins de chair. *Revue de l'Agriculture* 33, 1311–1322.
Onifade, A.A. and Abu, O.A. (1998) Productive response of rabbits to supplemental copper in a diet based on tropical feedstuffs. *Journal of Applied Animal Research* 13, 129–135.
Onifade, A.A., Obiyan, R.I., Onipede, E., Adejunmo, D.O., Abu, O.A. and Babatunde, G.M. (1999) Assessment of the effects of supplementing rabbit diets with a culture of *Saccharomyces cerevisiae* using growth performance, blood composition and clinical activities. *Animal Feed Science and Technology* 77, 25–32.

Oriani, G., Corino, C., Pastorelli, G., Pantaleo, L., Ritieni, A. and Salvatori, G. (2001) Oxidative status of plasma and muscle in rabbits supplemented with dietary vitamin E. *Journal of Nutritional Biochemistry* 12, 138–143.

Ouhayoun, J. and Lebas, F. (1987) Composition chimique de la viande de lapin. *Cuniculture* 14, 33–45.

Paefgen, D., Scheuermann, S.E. and Raether, W. (1996) The anticoccidial activity of saco in fattening rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 103–106.

Parigi Bini, R., Cinetto, M. and Carotta, N. (1983) The effect of β-carotene on the reproductive performance of female rabbits. In: *Proceedings of the 5th World Conference on Animal Production*, Vol. 2, pp. 231–232.

Partridge, G.G. and Gill, B.P. (1993) New approaches with weaner diets. In: Garnsworthy, P.C. and Cole, D.J.A. (eds) *Recent Advances in Animal Nutrition*. Nottingham University Press, Nottingham, UK, pp. 221–248.

Patience, J.F., Austic, R.E. and Boyd, R.D. (1985) The effect of sodium bicarbonate or potassium bicarbonate on acid-base status and protein and digestibility in swine. *Nutrition Research* 6, 263–273.

Patton, N.M., Harris, D.J., Grobner, M.A., Swick, R.A. and Cheeke, P.R. (1982) The effect of dietary copper sulfate on enteritis in fryer rabbits. *Journal of Applied Rabbit Research* 5, 78–82.

Peeters, J.E., Janssens-Geroms, R. and Halen, P.H. (1980) Activité anticoccidienne de la narasine chez le lapin: essays de laboratorie. In: Camps, J. (ed.) *Proceedings of the 2nd World Rabbit Congress*. Asociación Española de Cunicultura, Barcelona, Spain, pp. 325–334.

Peeters, J.E., Maertens, L. and Geeroms, R. (1992) Influence of galacto-oligosaccharides on zootechnical performance, caecal biochemistry and experimental colibacillosis 0103/8+ in weanling rabbits. In: Cheeke, P.R. (ed.) *Proceedings of the 5th World Rabbit Congress*. Oregon State University, Corvallis, Oregon, USA, pp. 1129–1136.

Pettigrew, J.PF., El-Kandelgy, S.M., Johnston, L.J. and Shurson, G.C. (1996) Riboflavin nutrition of sows. *Journal of Animal Science* 74, 2226–2230.

Pinheiro, V. and Almeida, A. (2000) Efeito da adição de pentosanases em dietas para coelhos em crescimento sobre as performances zootécnicas, saúde dos animais, parâmetros fermentativos cecais e composição química do digesta ileal. In: *Proceedings I Jornadas Internacionais de Cunicultura*. Vila Real, Portugal, pp. 209–211.

Pinheiro, V., Mourao, J.L. and Jimenez, G. (2007) Influence of Toyocerin (*Bacillus cereus* var. *toyoi*) on breeding performance of primiparous rabbit does. *World Rabbit Science* 15, 179–188.

Pinheiro, V., Guedes, C.M., Outor-Monteiro, D. and Mourao, J.L. (2009) Effects of fibre level and dietary mannanoligosacharides on digestibility, caecal volatile fatty acids and performances of growing rabbits. *Animal Feed Science and Technology* 148, 288–300.

Plamenac, Z., Djukic-Cosic, D., Malicevic, Z., Bulat, P. And Matovic, V. (2008) Zinc or magnesium supplementation modulates Cd intoxication in blood, spleen, and bone of rabbits. *Biological Trace Element Research* 124, 110–117.

Redrobe, S. (2002) Calcium metabolism in rabbits. *Seminars in Avian and Exotic Pet Medicine* 12, 94–101.

Remois, G., Lafargue-Hauret, P. and Rouillere, H. (1996) Effect of amylases supplementation in rabbit feed on growth performance. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 289–292.

Rey, A.I., Lopez-Bote, C., Castaño, A., Thos, J. and Sanz Arias, R. (1997) Dietary fat rich in mono or di-unsaturated fatty acids reduces lipid oxidation in hepatic tissue of rabbits. *Nutrition Research* 10, 1589–1596.

Ringler, D.H. and Abrams, G.D. (1970) Nutritional muscular dystrophy and neonatal mortality in a rabbit breeding colony. *Journal of the American Veterinary Medicine Association* 157, 1928–1934.

Ringler, D.H. and Abrams, G.D. (1971) Laboratory diagnosis of vitamin E deficiency in rabbits fed a faulty commercial ration. *Laboratory Animal Science* 21, 383–388.

Ritskes-Hoitinga, J., Grooten, H.N.A., Wienk, K.J.H., Peters, M., Lemmens, A.G. and Beynen, A.C. (2004) Lowering dietray phosphorus concentrations reduces kidney calcification, but does not adversely affect growth, mineral metabolism, and bone development in growing rabbits. *British Journal of Nutrition* 91, 367–376.

Rizzi, C., Brecchii, G. and Chiericato, G.M. (2005) A study on the reproductive performance and physiological response of rabbit bucks red on diets with two different mineral contents. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 336–342.

Robledo, D., García, M., Gracia, M.I. and Mateos, G.G. (1999) Estudio comparativo de la composición de los correctores minero-vitamínicos españoles en cunicultura. *ITEA* 20, 478–480.

Salles, M.S., Lombardo de Barros, C.S. and Barros, S.S. (1994) Ionophore antibiotic (narasin) poisoning in rabbits. *Veterinary and Human Toxicology* 36, 437–444.
Schlolaut, W. (1987) Nutritional needs and feeding of German angora rabbits. *Journal of Applied Rabbit Research* 10, 111–121.
Schultz, S.G. and Zalusky, R. (1964) Ion transport in isolated rabbit ileum. II. The interaction between active sodium and active sugar transport. *Journal of General Physiology* 47, 1043–1059.
Schultz, S.G. and Zalusky, R. (1965) Interactions between active sodium transport and active amino acid transport in isolated rabbit ileum. *Nature* 205, 292–294.
Simnett, K.E. and Spray, G.H. (1965a) The effect of a low-cobalt diet in rabbits. *British Journal of Nutrition* 19, 119–123.
Simnett, K.E. and Spray, G.H. (1965b) The absorption and excretion of 58Co cyanocobalamin by rabbits. *British Journal of Nutrition* 19, 593–598.
Simon, O., Vilfried, V. and Scharek, L. (2003) Micro-organisms as feed additives-probiotics. In: *Proceedings of the 9th Internal Symposium On Digestive Physiology in Pigs*. Banff, Alberta, Canada, pp. 295–318.
Skrivanova, V., Skrivan, M., Marounek, M. and Baran, M. (2001) Effect of feeding supplemental copper on performance, fatty acid profile and on cholesterol contents and oxidative stability of meat of rabbits. *Archives Animal Nutrition* 54, 329–399.
Skrivanova, V., Volek, Z., Boezina, P. and Marounek, M. (2002) Concentration of copper in muscles, liver, hair and faeces of growing rabbits fed diet supplemented with copper sulphate. *World Rabbit Science* 10, 167–170.
Steenland, E. (1991) Effekt van verschillende Lichtschema's, bezettingsdichtheid en forforgehalte van het voeder op de gezondheid en produktieresultaten van slachtkonijnen. *NOK-blad* 9, 60–68.
Strucklec, M., Dermelj, M., Stibilij, V. and Rajh, I. (1994) The effect of selenium added to feedstuffs on its content in tissues and on growth of rabbits. *Krmiva* 36, 117–123.
Surdeau, P., Henaff, R. and Perrier, G. (1976) Apport et equilibre alimentaire du sodium, du potassium et du chlore chez le lapin croissance. In: Lebas, F. (ed.) *Proceedings of the 1st World Rabbit Congress*, Communication No. 21. WRSA, Dijon, France.
Swick, R.A., Cheeke, P.R. and Patton, N.M. (1981) The effect of soybean meal and supplementary zinc and copper on mineral balance in rabbits. *Journal of Applied Rabbit Research* 4, 57–65.
Thilsted, J.P., Newton, W.M. and Crandell, R.A. (1981) Fatal diarrhea in rabbits resulting from the feeding of antibiotic contaminated feed. *Journal of the American Veterinary Medicine Association* 179, 360–362.
Tor-Aghidye, Y., Cheeke, P.R., Nakaue, H.S., Froseth, J.A. and Patton, N.M. (1992) Effects of β-glucanase (Allzyme B6) on comparative performance of growing rabbits and broiler chicks fed rye, triticale and high-and low-glucan barley. *Journal of Applied Rabbit Research* 15, 1144–1152.
Trocino, A., Xicatto, G., Carraro, L. and Jimenez, G. (2005) Effect of diets supplementation with Toyocerin (*Bacillus cereus* var *toyoi*) on performance and health of growing rabbits. *World Rabbit Science* 13, 17–28.
Underwood, E.J. (1977) *Trace Elements in Human and Animal Nutrition*, 4th edn. Academic Press, New York, USA.
Vandelli, A. (1995) Attenti a calico e fosforo. *Rivista di Coniglicoltura* 12, 36–37.
Van Meirhaeghe, P., Rochette, F. and Homedes, J. (1996) Anticoccidial efficacy of diclazuril in fattening rabbits. Comparative field trials in Spain and Belgium. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 119–124.
Vanparis, O., Hermans, L., Van der Flaes, L. and Marsboom, R. (1989) Efficacy of diclazuril in the prevention and cure of intestinal and hepatic coccidiosis in rabbits. *Veterinary Parasitology* 32, 109–117.
Verde, M.T. and Piquer, J.G. (1986) Effect of stress on the corticosterone and ascorbic acid (vitamin C) content of the blood plasma of rabbits. *Journal of Applied Rabbit Research* 9, 181–185.
Wang, J.Y., Larson, L.L. and Owen, F.G. (1982) Effect of β-carotene supplementation on reproductive performance of dairy heifers. *Theriogenology* 18, 461–467.
Wang, J.Y., Owen, F.G. and Larson, L.L. (1988) Effect of β-carotene supplementation on reproductive performance of lactating Holstein cows. *Journal of Dairy Science* 71, 181–186.
Warren, H.B., Lausen, N.C. and Segre, G.V. (1989) Regulation of calciotropic hormones *in vivo* in the New Zealand White rabbit. *Endocrinology* 125, 2683–2690.
Widdowson, E.M. (1974) Feeding the newborn: comparative problems in man and animals. *Proceedings of the Nutrition Society* 33, 97–102.

Wilburn, E.E., Mahan, D.C., Hill, D., Ship, T. and Yang, H. (2008) An evaluation of natural (*RRR*-α-tocopheryl acetate) or synthetic (*all-rac* α-tocopheryl acetate) vitamin E fortification in the diet or drinking water of weanling pigs. *Journal of Animal Science* 86, 584–591.

Xiccato, G. (1996) Nutrition of lactating does. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 175–180.

Yamini, B. and Stein, S. (1989) Abortion, stillbirth, neonatal death and nutritional myodegeneration in a rabbit breeding colony. *Journal of the American Veterinary Medicine Association* 194, 561–562.

Zakaria, A.H. and Al-Anezi, M.A. (1996) Effect of ascorbic acid and cooling during egg incubation on hatchability, culling, mortality, and the body weights of broiler chickens. *Poultry Science* 75, 1204–1209.

Zimmerman, T., Giddens, W., Di Giacomo, R. and Ladiges, W. (1990) Soft tissue mineralization in rabbits fed a diet containing excess vitamin D. *Laboratory Animal Science* 40, 212–215.

8 Feed Evaluation

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8.1 Units for Feed Evaluation

8.1.1 Energy

The most commonly used unit for expressing energy value in rabbit diets is digestible energy (DE). However, the use of DE leads to some systematic errors when used for raw materials in diet formulation, especially for certain groups of ingredients.

For instance, DE overestimates the energy content of protein concentrates, as it does not take into account either the higher energy losses in urine or the energy cost of urea synthesis in the liver associated with the use of an excess of this type of ingredient in the diet. Similarly, the DE content of feedstuffs containing significant amounts of digestible fibre (e.g. sugarbeet/citrus pulps or soybean hulls) also overvalues their relative energy concentration, as the use of DE does not consider energy losses (methane and heat of fermentation) linked to the microbial digestion of fibre in the caecum. On the other hand, the relative energy content of fats and feeds with a high fat content is underestimated by DE, because dietary fatty acids are retained in the body more efficiently than other dietary components.

These disadvantages explain the current interest in replacing DE with metabolizable energy (ME) or net energy (NE). However, most of the information available at present has been obtained as DE. As a consequence, DE is still used as the unit of expression of the energy value of diets in rabbits. However, some important points should be taken into account to improve the expression of the energy value of raw materials.

To prevent the use of protein concentrates as energy sources, a maximum protein content of compound feeds should be established. This restriction is also required to control the incidence of diarrhoea (de Blas *et al.*, 1981; Gidenne and García, 2006) and to reduce environmental pollution by animal excreta (Maertens *et al.*, 1997). An alternative is to use ME corrected to zero nitrogen equilibrium (MEn), as in poultry nutrition, instead of DE. Values of MEn can be derived for each ingredient by subtracting 4.8 kJ g^{-1} digestible protein from its DE content (Maertens, 1992). For these reasons, MEn values are introduced in the final feedstuff table of this chapter. A better hierarchy between protein- and carbohydrate-rich feedstuffs is obtained using the MEn system. Consequently, raw materials are more optimally chosen for their nutritional characteristics when using the MEn system in the formulation of compound diets for rabbits (Maertens *et al.*, 2002).

Digestible fibre and the fat content of compound feeds should also be limited to allow for maximal energy intake (García *et al.*, 1993) and for technological reasons (rabbit feed must be pelleted), respectively. These restrictions limit the errors associated with the use of DE to <5% in extreme practical diets (de Blas *et al.*, 1985; Ortiz *et al.*, 1989; de Blas and Carabaño, 1996; Fernández and Fraga, 1996). In any case, the use of correction factors (−2.51 and +5.85 kJ DE kg^{-1}) for ingredients or diets containing a high proportion of digestible neutral detergent fibre (NDF) and ether extract (EE), respectively, might be envisaged.

8.1.2 Protein and amino acids

Total crude protein (CP) and amino acids are still the most common units used to formulate diets for rabbits. However, the ileal digestibility of nitrogen is increasingly used mainly for early weaned rabbits and for preventing intestinal problems or epizootic rabbit enteropathy. Large variations in essential amino acid digestibilities have been found for lucerne hays harvested at different stages of maturity (García *et al.*, 1995) and between average lysine, methionine or threonine digestibility in a basal diet compared to synthetic forms of these amino acids (Taboada *et al.*, 1994, 1996; de Blas *et al.*, 1998). These results have been confirmed by work on protein evaluation (Llorente *et al.*, 2006, 2007a, b; Carabaño *et al.*, 2009). Furthermore, as ileal and faecal digesta contains important amounts of protein of endogenous origin (3.8 and 2.5 g 100 g^{-1} dry matter (DM) intake, respectively; García *et al.*, 2004; Llorente *et al.*, 2006) the use of true instead of apparent units is advisable (Carabaño *et al.*, 2009). These results indicate that the use of digestible, instead of crude, protein and amino acid units would considerably improve the accuracy of feed evaluation and decrease nitrogen excretion. However, more information is needed on animal requirements and amino acid digestibility prediction for the most commonly used ingredients.

8.1.3 Fibre

Fibre is one of the main components of rabbit diets and NDF accounts for about one-third of matter. In a recent paper on compound feeds, hemicelluloses, cellulose and soluble fibre plus sugars were at similar levels (172, 140 and 164 g kg^{-1} DM, respectively; Villamide *et al.*, 2009), but with high variation (about 20% coefficient of variation (CV)) and negative correlation between them. Units used to express the fibre content of feed ingredients include NDF, acid detergent fibre (ADF), acid detergent lignin (ADL), crude fibre (CF) and soluble fibre estimated by difference from the organic matter, CP, EE, NDF, starch and sugars. These are described and discussed in detail in Chapter 5.

8.2 Methodology of Feed Evaluation

8.2.1 Complete diets

The evaluation of complete diets is usually undertaken by digestibility assays. The standardization of the procedures used in these assays is the first step in reducing the variability of the results. In this way, a European reference method for the *in vivo* determination of diet digestibility in rabbits has been proposed by the European Group on Rabbit Nutrition (EGRAN) (Pérez *et al.*, 1995a). The most relevant variables to control in a digestibility assay are the length of the experimental period and the number of animals used. The recommended values are at least 7 days of adaptation period and 4 days of collection period (which implies 5 days of control) using ten rabbits per treatment. No advantage in accuracy was found by Pérez *et al.* (1996a) when increasing the adaptation period from 7 to 14 days. The number of replicates can be decreased when the length of the collection period increases. Thus, Villamide and Ramos (1994) found the same variability for DM digestibility using ten rabbits and 4 days, eight rabbits and 7 days and seven rabbits and 10 days of collection period. When using growing rabbits, however, a longer collection period is necessary because of greater differences between intake at the beginning and at the end. Lebas *et al.* (1994) obtained the same accuracy in digestibility determinations with ten cages of one rabbit and four cages of four rabbits each, although in the latter case there is a greater risk of missing data if any of the animals in the group have health problems.

Other sources of variation in digestibility assays are rabbit breed, sex, litter, age and physiological state. No differences have been found for meat breeds of rabbit of the same size (Maertens and De Groote, 1982; Dessimoni, 1984). However, Pascual *et al.* (2008) found greater DM and organic matter digestibility values (about 0.01 point) and ADF digestibility (+0.03 points) in growing rabbits from a genetic line selected for litter size compared to another selected for reproductive longevity. Because of the low sexual dimorphism of this species, the effect of sex is not relevant (Xiccato *et al.*, 1992; Pérez *et al.*, 1995b). Litter has shown an effect on DM intake and excretion in rabbits from 25 to 40 days of age, but does not affect DM digestibility (Gómez-Conde *et al.*, 2004). In any case, to eliminate a possible effect of litter, rabbits of the same litter should be distributed evenly across the treatments.

Digestibility is usually measured in growing rabbits (from 42 days onwards) when their digestive system is fully adapted to non-maternal feed. However, the effects of age during this period and the validity of extrapolation of the results obtained with young animals to reproductive females is not clear. It seems that the effect of age on energy and protein digestibility from 7 weeks to slaughter is limited (Maertens and De Groote, 1982; Xiccato and Cinetto, 1988). In work on nitrogen contamination, a decrease in CP digestibility from 0.790 to 0.703 has been observed with age (Calvet *et al.*, 2008). However, as the caecal content weight increases during the first weeks after weaning (by 70% from 30 to 40 days of age; Peeters *et al.*, 1992) a significant part of the ingested feed remains in the digestive tract, leading to an overestimation of digestibility in young rabbits (Blas *et al.*, 1991; Fernández *et al.*, 1994). Thus, Gómez-Conde *et al.* (2004) obtained a linear decrease of DM digestibility from weaning (25 days) to 32 days of age (0.0217 points each day), whereas digestibility remained constant from 32 to 40 days of age. Comparisons between the digestibility of growing rabbits and breeding does are contradictory. Maertens and De Groote (1982) and Pérez *et al.* (1996b) found higher digestibility values in growing rabbits, whereas de Blas *et al.* (1998) found higher digestibility in breeding does, especially for NDF.

Digestibility determinations in rabbits have a high variability. This is shown in Table 8.1, for which 23 papers on digestibility assays were reviewed (García *et al.*, 2001). The mean standard deviation varied from 0.026 to 0.076 for DM and CF digestibility, respectively (which means a CV of about 3.8% and 40%). When the digestibility and analytical methodology is harmonized, however, the variability decreases. Thus, in a ring-test study on the chemical analysis of feed and faeces and its influence on the calculation of digestibility, Xiccato *et al.* (1996) obtained CVs within laboratories (repeatability) of 0.96%, 1.1% and 6.2% for energy, CP and NDF digestibility, respectively. Furthermore the reproducibility (CV among laboratories) was improved when all of the laboratories used not only the same reference method for digestibility assays, but also harmonized analysis (from 1.6% to 1.0%, from 2.7% to 1.5% and from 21.3% to 7.4%, for gross energy, CP and NDF digestibility, respectively, Pérez *et al.*, 1995b; Xiccato *et al.*, 1996). Therefore, the expected variability in digestibility assays has to be taken into account in the experiment design for calculating the minimum number of rabbits (replicates) to obtain significant differences among treatments (Table 8.1).

8.2.2 Feedstuffs

The nutritive value of feedstuffs can be directly determined (fed as the sole feed) when they are relatively balanced and palatable. Feedstuffs such as lucerne hay and other hays, wheat bran or sunflower meal can be used as sole feeds in a digestibility assay and evaluated directly. Thus, Maertens and De Groote (1981) did not find

Table 8.1. Number of rabbits required to detect a significant difference (P = 0.05) between two means for digestibility traits varying in standard deviation (SD) (Garcia *et al.*, 2001).

		Difference in digestibility				
	SD	0.02	0.03	0.04	0.05	0.06
Dry matter digestibility (20)[a]	0.0258	16	9	7	5	4
Gross energy digestibility (20)	0.0273	17	9	6	5	4
Crude protein digestibility (23)	0.0332	24	13	8	6	5
Neutral detergent fibre digestibility (15)	0.0467	45	22	13	10	8
Acid detergent fibre digestibility (12)	0.0608	71	35	21	15	11
Crude fibre digestibility (7)	0.0763	112	50	31	21	5

[a]The numbers in parentheses indicate the number of trials considered for each trait.

differences between lucerne hay evaluated by substitution or directly, and nor did Villamide *et al.* (2003) comparing direct determination with multiple regression. Similarly, García *et al.* (1995) determined the energy, NDF, CP and amino acid digestibility of five samples of lucerne hay by using the direct method and obtained good accuracy.

Most feedstuffs are not nutritionally balanced in relation to the requirements of rabbits. When they are fed as a sole diet, the digestive transit time and intake can be changed, which, as a consequence, can alter the nutritive value (Fernández-Carmona *et al.*, 1996). Thus, these authors observed an inverse relationship between feed intake and the nutritive value of feedstuffs, as determined directly. For the majority of feedstuffs, therefore, nutritive value is estimated by difference or regression from the nutritive value of diets including the test feedstuff at different inclusion rates.

de Blas and Carabaño (1996) observed a 30% overestimation of the energy value of sugarbeet pulp when determined directly, related to a longer retention time of digesta in the caecum of diets with high levels of soluble fibre. This problem is partially corrected using the substitution method, where a basal diet or a reference feedstuff of known nutritive value is substituted by the feedstuff tested. The evaluation of the feedstuff is undertaken by difference or by regression if more than one substitution rate is used. Figure 8.1 shows the DE of grape pulp determined directly or by difference from a basal diet or a reference feedstuff at an inclusion rate of 300 g kg^{-1}, or by extrapolation using four substitution rates (Villamide *et al.*, 2003). No differences were observed among the values obtained by difference or extrapolation, but an overestimated energy value was shown when DE was determined directly.

The use of substitution methods implies that there is no interaction between the basal diet (or the reference ingredient) and the test ingredient. However, an effect of basal diet concentration has been detected for citrus and sugarbeet pulp evaluation (Table 8.2). The nutritive value of both feedstuffs was significantly lower when estimated from the lowest-energy basal diet, probably because the high levels of indigestible fibre in this diet produced a lower entry rate of the potentially digestible fibre of pulps into the caecum (de Blas and Villamide, 1990). The opposite effect occurs with basal diets with a high proportion of wheat straw or when feedstuffs with high levels of indigestible fibre are evaluated at high substitution rates (>200 g kg^{-1}). Thus, Villamide *et al.* (1991) observed an underestimation of soybean meal when evaluated at a low substitution rate (150 g kg^{-1}) with a basal diet that included 580 g wheat straw kg^{-1}. Dietary interaction has also been observed with fats, showing tallow higher DE values

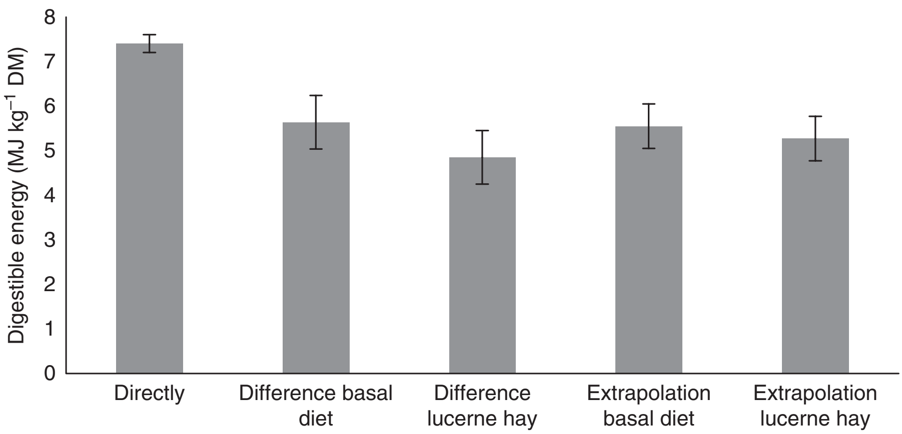

Fig. 8.1. Energy value of grape pulp determined by different methods: directly; by substitution of a basal diet or reference feedstuff (lucerne hay) at 0.3 substitution rate; by extrapolation of a regression with a basal diet and 0.1, 0.2 and 0.3 substitution rate of grape pulp; and 0.1, 0.2 and 0.3 substitution of lucerne hay by grape pulp. Bars represent the standard error (Villamide *et al.*, 2003).

Table 8.2. Effect of type of basal diet on the nutritive value of citrus and sugarbeet pulps (de Blas and Villamide, 1990).

	Citrus pulp				Sugarbeet pulp			
Basal diet DE	LOW[1]	HIGH[2]	SE	*P*	LOW[1]	HIGH[2]	SE	*P*
Digestible energy (MJ kg^{-1} DM)	11.27	13.09	0.73	*	9.97	12.37	0.54	*
Protein digestibility	0.173	0.885	0.15	*	0.178	0.764	0.07	*
ADF digestibility	0.677	0.827	0.04	*	0.385	0.717	0.05	*

ADF, acid detergent fibre; DE, digestible energy; DM, dry matter; SE, standard error.
*Significant at P <0.01.
[1]Low energy (10.01 MJ kg^{-1} DM), [2] High energy (12.32 MJ kg^{-1} DM).

at a 6 g kg^{-1} than at a 12 g kg^{-1} rate of inclusion (Maertens *et al.*, 1986). Similarly, Santomá *et al.* (1987) and Fraga *et al.* (1989) found an increase in the digestibility coefficient of 0.058 of all nutrients when 30–60 g fat kg^{-1} was added to the diets.

Therefore, both the substitution rate of the test ingredient and the basal diet should be designed to prevent interactions and to obtain accurate estimates of the test ingredient's nutritive value, taking into account that the higher the substitution rate the lower the error, but also the greater the probability of interaction between the test ingredient and the basal diet.

When interactions between feedstuffs are expected, or lower rates of inclusion (<200 g kg^{-1}) have to be used (because of technological or nutritional problems), substitution of the basal diet at several rates is recommended (Villamide, 1996). The linearity between the dietary nutritive value and the substitution rate is analysed and, if it is established, estimation of the nutritive value is undertaken by regression and extrapolation to total substitution. When the relationship between the dietary nutritive value and the substitution rate is non-linear, a nutritive value of the test feedstuff can be assigned for a recommended range of inclusion. Outside this range the nutritive value should be calculated from second- or third-degree equations.

No differences between the energy value estimations of grape pulp have been obtained using the substitution or extrapolation methods (Fig. 8.1) and the standard error of estimation only decreased by 0.1 MJ kg^{-1} DM (Villamide *et al.*, 2003). Similarly no advantage has been observed by using lucerne as a reference feedstuff instead of a basal diet in the evaluation of grape pulp despite the highly lignified fibre of this feedstuff, and therefore the lower imbalance among extreme diets (Villamide *et al.*, 2003).

The multiple regression equation method involves a simultaneous evaluation of several diets containing test ingredients in varying proportions. Table 8.3 shows the nutritive value (energy and CP digestibility) of six common ingredients in rabbit diets by the substitution or multiple regression equation method using two data sets. There are no significant differences among the different methodologies in their estimations of nutritive value, except for the CP digestibility of grape pulp. This was overestimated in multiple regression equations with independent data with respect to those obtained with all data or by difference. Nevertheless, the multiple regression method implies a diet design with no correlation among ingredients. This is very difficult to fulfil taking account of rabbit requirements, not only in energy, protein and amino acids, but also in the different components of fibrous content.

The above methodology has been extensively applied to DE and protein determinations; however, its use for amino acids evaluation or fibre digestibility is not recommended because of its high variability. The differences in amino acid content among balanced diets are very low, and therefore attributing a digestibility value to a test ingredient by the differences observed among diets is not accurate. A possible method for overcoming these problems is the use of semi-purified diets, where all the protein comes from the test ingredient or from casein, assuming its complete digestibility (García *et al.*, 2005). In this case, the amino acids found in excreta or ileal digesta can come only from the indigestible test ingredient or from endogenous protein (including caecotrophs). A more detailed description is found in Chapter 3. The question now is whether amino acid digestibility obtained this way can be extrapolated to practical diets. In an assay carried out in our laboratory (Llorente *et al.*, unpublished data), trying to test the additivity of the apparent faecal and ileal and true ileal digestible amino acid of four ingredients (sunflower meal, wheat, wheat bran and lucerne hay), much lower amino

Table 8.3. Comparison of nutritive value (means ± standard error) of ingredients by substitution or multiple regression method (Villamide *et al.*, 2003).

Ingredient	Measure	Substitution	Multiple regression[a] All diets (n = 179)	Multiple regression Independent data[b] (n = 86–169)
Grape pulp	DE, MJ kg^{-1}	6 ± 0.6	5.7 ± 0.33	6.4 ± 0.70
	CPd	0.4 ± 0.60[c]	−0.8 ± 0.57[c]	0.6 ± 0.10
Lucerne meal	DE, MJ kg^{-1}	7 ± 0.1	8.4 ± 0.35	7.4 ± 0.64
	CPd	0.5 ± 0.04	0.7 ± 0.04	0.55 ± 0.06
Wheat bran	DE, MJ kg^{-1}	12.20 ± 0.61	12.6 ± 0.42	12.8 ± 0.44
	CPd	0.778 ± 0.07	0.7 ± 0.05	0.7 ± 0.05
Wheat	DE, MJ kg^{-1}	16.1 ± 0.33	15.6 ± 0.36	14.8 ± 0.47
	CPd	0.71 ± 0.064	0.64 ± 0.067	0.46 ± 0.086
Full-fat soya	DE, MJ kg^{-1}	18.7 ± 0.32	18.6 ± 0.65	18.6 ± 0.92
	CPd	0.80 ± 0.015	0.84 ± 0.031	0.80 ± 0.043
Sunflower seed	DE, MJ kg^{-1}	10.0 ± 0.73	10.0 ± 0.70	8.4 ± 1.11
meal	CPd	0.82 ± 0.041	0.81 ± 0.041	0.6 ± 0.06

CPd, crude protein digestibility; DE, digestible energy.
[a]Including diets that determine the nutritive value by difference.
[b]Independent diets.
[c]Not significant.

acid digestibility was observed in the mixed diet (with a lower proportion of purified components) than in the purified one.

In the case of fibre, there are two problems: (i) the high variability in its digestion (reproducibility from 7% to 34% for NDF, ADF or CF digestibility, whereas that of energy is from 1% to 1.6%; Xiccato *et al.*, 1996); and (ii) the influence of the different fibrous components on transit time and therefore on the digestibility of fibre of the ingredient and of the other components of the basal diet, which implies nutritive interactions. The use of semi-purified diets where all the fibre comes from the test feedstuff increases the precision of estimates, although sometimes the results differ from those obtained by substitution (García *et al.*, 1996). The question is whether these fibrous sources maintain their value when they are combined with another kind of fibre or when they are included at lower levels in commercial diets.

The effect of errors in DE determination of experimental diets on the DE estimate of ingredients is shown in Table 8.4. Small differences in the nutritive value of experimental diets result in large differences (proportionally to substitution rate) in the nutritive value of test feedstuffs, so very careful determinations (large numbers of animals and replicates in the chemical analyses of diets) of the nutritive value of diets must be performed.

8.3 Composition and Nutritive Value of Feedstuffs for Rabbits

Table 8.5 shows the chemical composition and nutritive value of 55 feedstuffs commonly used in rabbit nutrition. Data are expressed on an as-feed basis, with a common DM content for each group of feedstuffs, in view of practical utilization. Chemical composition includes DM, ash, CP, EE, CF, NDF, ADF, ADL, soluble fibre, starch, lysine, methionine, methionine plus cystine, threonine, calcium, phosphorus, sodium, chlorine, magnesium and potassium. The chemical composition is based on the tables from the first edition of this book and partly modified according to data from FEDNA (2003), INRA tables (Sauvant *et al.*, 2004) and CVB (2007).

The nutritive value is based on a literature compilation. The tables from the first edition of this book have been revised, taking into account feedstuffs tables published in 2002 (Maertens *et al.*, 2002) and some more recent experimental data (Falcão e Cunha *et al.*, 2004; Martinez *et al.*, 2006; Gidenne *et al.*, 2007; Michelan *et al.*, 2007). The values proposed in Table 8.5 were retained after assessing the methodology used and were considered as the most accurate at practical levels of dietary inclusion. Amino acid digestibility has been estimated from the data of García *et al.* (2005) and Llorente *et al.*, (2006, 2007a,b) and corrected according to CP digestibility.

Table 8.4. Error in the estimation of mean digestible energy (DE) of an ingredient (12.55 MJ kg^{-1} dry matter (DM), DE of basal diet 11.3 MJ kg^{-1}) when the variables were measured with ±1% error using substitution rates of 200 and 400 g kg^{-1}. Figures express the percentage of error and the difference between the actual and measured DE of ingredients (kJ kg^{-1} DM) (Villamide, 1996).

	200 g kg^{-1}		400 g kg^{-1}	
	%	kJ kg^{-1}	%	kJ kg^{-1}
Error in basal diet				
DM intake or excreted, GE faeces	2.25	284	0.85	109
GE basal diet	5.85	736	2.20	276
Error in substituted diets				
DM intake or excreted, GE faeces	2.73	343	1.35	171
GE test diets	7.33	920	3.67	460

GE, gross energy.

Table 8.5. Composition and nutritional value of raw materials commonly used for rabbits (data in g kg^{-1}, as-fed basis).

	DM	Ash	CP	EE	CF	NDF	ADF	ADL	Soluble fibre	ST	Sugar	Lys	Met	SAA	Thr	Ca
Cereals																
Barley	880	22	103	20	46	175	55	9	25	510	25	3.9	1.7	4.2	3.6	0.6
Maize	880	12	82	35	19	95	25	5	1	640	15	2.3	1.7	3.5	2.9	0.2
Oats	880	26	106	51	111	280	135	22	32	370	15	4.4	1.9	5.3	3.7	1
Triticale	880	18	110	16	23	125	31	9	11	570	30	3.9	1.9	4.6	3.6	0.5
Wheat	880	16	108	18	22	110	31	9	3	600	25	3.3	1.8	4.5	3.4	0.4
Cereal by-products																
Maize gluten feed	900	67	215	43	78	312	94	12	63	180	20	7.1	4.1	9	8	1.7
DDGS	900	60	253	90	81	316	89	12	66	105	10	6.6	5.1	8.9	8.9	1.4
Malt sprouts	900	61	232	19	126	378	139	18	30	110	70	10.8	3.1	6	8.1	2.1
Rice bran	900	90	135	153	81	211	101	36	11	270	30	5.9	2.1	4.4	5.3	1.2
Wheat bran	880	50	150	34	95	405	118	35	1	190	50	5.9	2.4	5.5	4.8	1.5
Wheat feed	880	40	140	40	50	271	77	24	29	270	90	5	2.5	7	5	1
Wheat shorts	880	36	158	36	70	326	100	27	34	240	50	6.3	2.6	5.7	5	1.4
Other energy concentrates																
Beet molasses	750	86	105	–	–	–	–	–	109	–	450	0.4	0.5	1	0.6	2.2
Cane molasses	750	98	45	–	–	–	–	–	137	–	470	0.2	0.2	0.4	0.5	7.4
Cassava 60	880	57	26	7	48	124	77	21	48	600	18	1	0.3	0.7	0.8	3
Cassava 65	880	57	26	7	44	95	68	20	24	650	21	1	0.3	0.7	0.8	2.5
Cassava 70	880	35	26	7	31	80	50	14	7	700	25	1	0.3	0.7	0.8	2
Glycerine	900	45	–	4	–	–	–	–	–	–	853	–	–	–	–	0.4
Legume and oil seeds																
Faba bean	880	33	257	13	77	123	89	8	29	390	35	16.8	1.8	5	9.2	1.2
Lupin	880	35	326	70	128	210	155	15	179	–	60	15.9	2.5	7.3	11.6	2.3
Peas	880	30	220	12	57	120	70	4	18	435	45	16.3	2.2	5.4	8.4	1
Rapeseed	900	41	189	396	81	181	124	49	43	–	50	11.5	4.2	9.2	8.7	4
Soybean	900	47	369	193	56	117	73	8	99	–	75	23.3	5.2	11.4	14.4	2.5
Oil meals																
Coconut cake	900	60	202	74	125	447	235	55	24	–	93	5	3	6.1	6.6	1.4
Palm cake	900	40	147	84	178	605	372	110	4	–	20	4.8	2.8	5	4.6	2.1
Rapeseed meal	900	68	361	25	121	277	189	86	79	–	90	19.4	7.6	16.2	15.7	7
Soybean meal 44	900	68	432	18	77	161	100	8	141	–	80	27.2	6	12.5	16.8	2.9
Soybean meal 46	900	63	450	18	63	132	82	6	157	–	80	28.4	6.3	13.1	17.6	2.9
Soybean meal 48	900	61	468	18	50	124	65	5	149	–	80	29.5	6.6	13.6	18.3	2.9
Sunflower meal 28	900	68	279	27	252	428	302	101	48	–	50	10	6.7	12	10.3	3.5
Sunflower meal 32	900	68	306	23	225	383	270	90	70	–	50	11.2	7.4	13.1	11.3	3
Sunflower meal 36	900	68	342	19	180	306	216	72	115	–	50	12.5	8.2	14.7	12.7	2.5
Oils and fats																
Animal fat	995	–	–	990	–	–	–	–	–	–	–	–	–	–	–	–
Olein	995	–	–	990	–	–	–	–	–	–	–	–	–	–	–	–
Rapeseed oil	995	–	–	990	–	–	–	–	–	–	–	–	–	–	–	–
Soybean oil	995	–	–	990	–	–	–	–	–	–	–	–	–	–	–	–
Sunflower oil	995	–	–	990	–	–	–	–	–	–	–	–	–	–	–	–
Fibrous feedstuffs																
Lucerne meal 12	900	90	126	23	297	475	371	83	156	–	30	5.4	1.9	3.4	5.2	14
Lucerne meal 15	900	99	153	32	261	418	326	73	168	–	30	6.6	2.3	4.1	6.3	15
Lucerne meal 18	900	99	180	36	216	346	270	60	209	–	30	7.7	2.7	4.9	7.4	16
Beet pulp	900	72	90	10	180	428	212	18	240	–	60	5.3	1.9	3.1	4.4	7.6
Cacao hulls	900	80	164	50	183	390	300	140	216	–	–	7.5	1.5	3.5	6	3
Carob meal	900	32	47	5	78	289	248	138	96	7	424	1.6	0.9	1.5	1.7	4.5
Citus pulp	900	67	59	27	133	220	155	16	297	–	230	2	0.7	1.5	2	15.9
Flax chaff	900	76	102	35	315	455	310	110	232	–	–	3	0.5	1	1.5	18
Grape pomace	900	81	117	54	280	560	480	300	68	–	20	4.9	1.7	3.5	3.7	7
Grape seed meal	900	36	99	14	441	730	650	550	21	–	–	4	1.5	3.5	2	6
Grass meal	900	80	150	30	225	460	260	50	100	–	80	6	2	3.5	5.5	7
Olive leaves	900	72	90	40	200	455	318	177	153	–	90	–	–	–	–	11
Rice straw	900	162	60	5	295	585	340	22	88	–	–	–	–	–	–	–
Soybean hulls	900	46	122	20	355	588	426	21	114	–	10	7	1.4	3.4	4.6	5
Sunflower hulls	900	34	54	40	468	693	562	202	69	–	10	2.3	1.2	2.5	2.3	4
Wheat straw	900	61	36	12	395	750	474	80	36	5	–	–	–	–	–	3.8
Wheat straw treated	900	73	32	8	365	694	444	75	88	5	–	–	–	–	–	4.3
Whole maize plant (dehydrated)	900	36	72	25	126	360	153	10	57	330	20	2.5	0.9	1.7	2.6	3

– no analytical data available; AFD, apparent faecal digestibility; AID, apparent ileal digestibility; Ca, calcium; Cl, chlorine; nitrogen retention; Met, methionine; Mg, magnesium; Na, sodium; P, phosphorus; SAA, methionine plus cystine; ST, starch; (NaOH or KOH).

P	Na	Cl	Mg	K	CPd	DE (MJ kg⁻¹)	MEn (MJ kg⁻¹)	AFD lys	AID lys	TID lys	AFD met	AID met	TID met	AFD thr	AID thr	TID thr
3.6	0.2	1.4	1.3	5.1	0.67	12.90	12.50	0.66	0.62	0.80	0.75	0.74	0.87	0.55	0.41	0.70
2.5	0.1	0.5	1.1	3.2	0.65	13.10	12.75	0.64	0.63	0.80	0.73	0.71	0.86	0.53	0.38	0.69
3	0.2	0.7	1.3	4	0.73	10.90	10.45	0.72	0.70	0.82	0.80	0.78	0.90	0.63	0.50	0.75
3.4	0.1	0.5	1.2	4.2	0.75	12.90	12.40	0.74	0.72	0.87	0.82	0.80	0.91	0.65	0.53	0.76
3.5	0.2	0.6	1.2	4.1	0.77	13.10	12.65	0.76	0.75	0.89	0.86	0.83	0.92	0.68	0.56	0.78
8.6	2.2	2.2	3.8	9.7	0.70	11.40	10.65	0.72	0.71	0.84	0.77	0.69	0.89	0.59	0.45	0.73
7.3	0.5	2	2.9	9.7	0.70	12.70	11.75	0.70	0.69	0.84	0.77	0.69	0.89	0.59	0.45	0.73
6.6	0.6	4	1.5	11	0.75	10.80	9.90	0.75	0.74	0.87	0.82	0.73	0.91	0.65	0.53	0.76
16	0.6	0.8	10	16	0.65	12.45	11.95	0.64	0.63	0.80	0.73	0.66	0.86	0.53	0.38	0.69
10.9	0.3	0.8	4.4	11	0.74	10.30	9.75	0.74	0.73	0.86	0.81	0.72	0.91	0.64	0.51	0.76
9	0.2	0.9	4	10.2	0.79	12.35	11.75	0.80	0.79	0.89	0.85	0.76	0.93	0.70	0.59	0.79
10.5	0.3	0.8	4.2	13	0.77	11.20	10.55	0.78	0.77	0.89	0.83	0.74	0.92	0.68	0.56	0.78
0.2	8	10.8	0.5	39.1	0.70	10.65	10.25	0.70	0.69	0.84	0.77	0.69	0.89	0.59	0.45	0.73
0.9	2	20	4.2	45	0.60	10.10	9.90	0.59	0.57	0.77	0.68	0.62	0.84	0.47	0.30	0.65
1.2	0.4	1.1	1.4	12	0.50	12.05	11.95	0.47	0.46	0.70	0.60	0.55	0.79	0.35	0.15	0.58
1.1	0.3	0.7	1.1	7.5	0.50	12.50	12.40	0.47	0.46	0.70	0.60	0.55	0.79	0.35	0.15	0.58
1	0.3	0.7	0.9	4.4	0.50	13.10	12.95	0.47	0.46	0.70	0.60	0.55	0.79	0.35	0.15	0.58
2.4	16	29	0.1	*	–	14.98	14.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.3	0.2	0.7	1.5	12.4	0.80	13.05	12.00	0.81	0.80	0.91	0.86	0.77	0.94	0.71	0.61	0.80
3.2	0.5	0.4	1.7	8.5	0.80	12.70	11.40	0.81	0.80	0.91	0.86	0.77	0.94	0.71	0.61	0.80
4	0.2	0.4	1.2	10.5	0.85	13.20	12.25	0.86	0.86	0.94	0.90	0.80	0.96	0.77	0.68	0.84
6	0.3	0.6	2.4	7.9	0.78	20.90	20.10	0.79	0.78	0.89	0.84	0.75	0.93	0.69	0.58	0.78
5.6	0.1	0.3	3	17	0.85	17.35	15.80	0.88	0.86	0.94	0.90	0.80	0.96	0.77	0.68	0.84
5.4	0.6	6.3	3	18.1	0.65	12.15	11.45	0.64	0.63	0.80	0.73	0.66	0.86	0.53	0.38	0.69
5.8	0.2	1.6	2.6	6.4	0.60	10.45	10.00	0.59	0.57	0.77	0.68	0.62	0.84	0.47	0.30	0.65
10	0.7	0.3	4.5	12.5	0.76	11.35	9.95	0.76	0.76	0.88	0.83	0.74	0.92	0.66	0.55	0.77
6	0.2	0.4	2.5	18	0.82	13.35	11.60	0.83	0.82	0.92	0.88	0.78	0.95	0.74	0.64	0.86
6.1	0.2	0.4	2.7	19.5	0.83	13.95	12.05	0.84	0.84	0.93	0.89	0.79	0.95	0.75	0.65	0.87
6.4	0.2	0.4	2.8	20.5	0.84	14.70	12.70	0.85	0.85	0.93	0.90	0.80	0.95	0.76	0.67	0.87
10	0.3	1.5	5	11	0.73	9.60	8.55	0.73	0.72	0.86	0.80	0.72	0.90	0.63	0.74	0.78
9.5	0.3	1.5	5	10	0.76	10.25	9.00	0.76	0.76	0.88	0.83	0.74	0.92	0.66	0.75	0.79
9	0.3	1.6	5	11	0.80	11.10	9.65	0.81	0.80	0.91	0.86	0.77	0.94	0.71	0.76	0.80
–	–	–	–	–	–	33.45	33.45	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	31.40	31.40	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	35.15	35.15	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	35.55	35.55	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	35.55	35.55	–	–	–	–	–	–	–	–	–
2.6	0.6	3.5	2	19	0.56	6.75	6.35	0.54	0.53	0.74	0.65	0.59	0.82	0.42	0.24	0.62
2.6	0.7	4.8	2.7	21	0.60	7.4	6.95	0.59	0.57	0.77	0.68	0.62	0.84	0.47	0.30	0.65
2.7	0.8	4.9	3	25	0.64	8.3	7.7	0.63	0.62	0.80	0.72	0.65	0.86	0.52	0.36	0.68
1	2	1	2.3	4.9	0.50	10.4	10.1	0.47	0.46	0.70	0.60	0.55	0.79	0.35	0.15	0.58
3.5	0.8	1.5	4	25	0.50	5.45	5.2	0.47	0.46	0.70	0.60	0.55	0.79	0.35	0.15	0.58
1	0.2	1.5	0.5	0	0.20	9	8.9	0.14	0.12	0.49	0.33	0.34	0.65	0.00	0.00	0.36
1.2	1	0.6	1.4	7.1	0.60	11.3	11.05	0.59	0.57	0.77	0.68	0.62	0.84	0.47	0.30	0.65
3	0.6	0.9	1	9	0.40	4.4	4.15	0.36	0.34	0.63	0.51	0.48	0.75	0.23	0.05	0.51
2	0.1	0.1	1.2	16	0.15	5	5	–	–	–	–	–	–	–	–	–
1.2	0.1	0.1	1	6	0.10	3.35	3.35	–	–	–	–	–	–	–	–	–
4	1	0.8	2	25	0.55	8.1	7.65	0.53	0.52	0.73	0.64	0.59	0.82	0.41	0.00	0.62
0.8	1.7	4.5	1.9	–	–	5.35	5.3	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	2.5	2.3	–	–	–	-	–	–	–	–	–
1.6	0.2	0.3	2	12.6	0.50	7.2	6.85	0.47	0.46	0.70	0.60	0.55	0.79	0.00	0.15	0.58
2	1	1	1.7	10.5	0.15	4.3	4.25	–	–	–	–	–	–	–	–	–
0.8	1.6	4.6	0.9	9.5	0.20	2.7	2.7	–	–	–	–	–	–	–	–	–
0.6	8.6	4.3	0.7	8.9	0.25	3.7	3.65	–	–	–	–	–	–	–	–	–
2.8	–	–	1.8	–	0.46	8.52	8.32	0.43	0.41	0.67	0.56	0.52	0.77	0.30	0.09	0.55

CPd , apparent faecal crude protein digestibility; K, potassium; Lys, lysine; MEn, metabolizable energy corrected for zero
Thr, threonine; TID, true ileal digestibility; DDGS, dry distillers grains and solubles; *, depends on the neutralizer used

References

Blas, E., Fando, J.C., Cervera, C., Gidenne, T. and Perez, J.M. (1991) Effet de la nature de l'amidon sur l'utilisation digestive de la ration chez le lapin au cours de la croissance. In: *5émes Journées de la Recherche Cunicole*, Communication No. 50. Paris, France.

Calvet, S., Estellés, F., Hermida, B., Blumetto, O. and Torres, Ag. (2008) Experimental balance to estimate efficiency in the use of nitrogen in rabbit breeding. *World Rabbit Science* 16, 205–211.

Carabaño, R., Villamide, M.J., García, J., Nicodemus, N., Llorente, A., Chamorro, S., Menoyo, D., García-Rebollar, P., García-Ruiz, A.I. and de Blas, J.C. (2009) New concepts and objectives for protein–amino acid nutrition in rabbits: a review. *World Rabbit Science* 17, 1–14.

CVB (2007) *CVB Table: Rabbits, 2007. Chemical Composition and Nutritional Values of Feedstuffs and Feeding Advices*. CVB series no. 42, The Hague, The Netherlands.

de Blas, C. and Carabaño, R. (1996) A review on the energy value of sugar beet pulp for rabbits. *World Rabbit Science* 4, 33–36.

de Blas, C. and Villamide, M.J. (1990) Nutritive value of beet and citrus pulps for rabbits. *Animal Feed Science and Technology* 31, 239–246.

de Blas, C., Pérez, E., Fraga, M.J., Rodríguez, M. and Gálvez, J. (1981) Effect of diet on feed intake and growth of rabbits from weaning to slaughter at different ages and weights. *Journal of Animal Science* 52, 1225–1232.

de Blas, C., Fraga, M.J. and Rodríguez, M. (1985) Units for feed evaluation and requirements for commercially grown rabbits. *Journal of Animal Science* 60, 1021–1028.

de Blas, C., Taboada, E., Nicodemus, N., Campos, R., Piquer, J. and Méndez, J. (1998) Performance response of lactating and growing rabbits to dietary threonine content. *Animal Feed Science and Technology* 70, 151–160.

de Blas, C., Taboada, E., Mateos, G.G., Nicodemus, H. and Méndez, J. (1995) Effect of substitution of starch for fibre and fat in isoenergetic diets on nutrient digestibility and reproductive performance of rabbits. *Journal of Animal Science* 73, 1131–1137.

Dessimoni, R. (1984) Efecto de razas y de diferentes niveles de proteína y fibra bruta sobre la digestibilidad de nturientes en racones de conejos. In: Finzi, A. (ed.) *Proceedings of the 3rd World Rabbit Congress*, Vol. 1. Rome, Italy, pp. 314–322.

Falcão e Cunha, L., Peres, H., Freire, J.P.B. and Castro-Solla, L. (2004) Effects of alfalfa, wheat bran or beet pulp, with or without sunflower oil, on caecal fermentation and on digestibility in the rabbit. *Animal Feed Science and Technology* 117, 131–149.

FEDNA (2003) Tablas FEDNA de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos. Fundación Española para el Desarrollo de la Nutrición Animal, Madrid, Spain.

Fernández, C. and Fraga, M.J. (1996) Effect of fat inclusion in diets for rabbits on the efficiency of digestible energy and protein utilization. *World Rabbit Science* 4, 19–23.

Fernández, C., Cobos, A. and Fraga, M.J. (1994) The effect of fat inclusion on diet digestibility in growing rabbits. *Journal of Animal Science* 72, 1508–1515.

Fernández-Carmona, J., Cervera, C. and Blas, E. (1996) Prediction of the energy value of rabbit feeds varying widely in fibre content. *Animal Feed Science and Technology* 64, 61–75.

Fraga, M.J., Llorente, M., Carabaño, R. and de Blas, C. (1989) Effect of diet and of remating interval on milk production and milk composition of the doe rabbit. *Animal Production* 48, 459–466.

García, A.I., de Blas, J.C. and Carabaño, R. (2004) Effect of diet (casein-based or protein-free) and caecotrophy on ileal endogenous nitrogen and amino acid flow in rabbits. *Animal Science* 79, 231–240.

García, A.I., de Blas, J.C. and Carabaño, R. (2005) Comparison of different methods for nitrogen and amino acid evaluation in rabbit diets. *Animal Science* 80, 169–178.

García, G., Gálvez, J.F. and de Blas, J.C. (1993) Effect of substitution of sugarbeet pulp for barley in diets for finishing rabbits on growth performance and on energy and nitrogen efficiency. *Journal of Animal Science* 71, 1823–1830.

García, J., Pérez, L., Alvarez, C., Rocha, R., Ramos, M. and de Blas, C. (1995) Prediction of the nutritive value of lucerne hay in diets for growing rabbits. *Animal Feed Science and Technology* 54, 33–44.

García, J., Villamide, M.J. and de Blas, C. (1996) Nutritive value of sunflower hulls, olive leaves and NaOH-treated barley straw for rabbits. *World Rabbit Science* 4, 205–209.

García, J., Nicodemus, N., Carabaño, R., Villamide, M.J. and de Blas, J.C. (2001) Determination of the number of replicates required to detect a significant difference between two means in rabbit traits. *World Rabbit Science* 9, 27–32.

Gidenne, T. and García, J. (2006) Nutritional strategies improving the digestive health of the weaned rabbit. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Sciences*. COST and ILVO Publication, Melle, Belgium, pp. 229–238.

Gidenne, T., Aymard, P., Bannelier, C., Coulmier, D. and Lapanouse, A. (2007) Valeur nutritive de la pulpe de betterave déshydratée chez le lapin en croissance. In: *Proceedings 12emes Journées de la Recherche Cunicole*. ITAVI and INRA, Le Mans, France, pp. 106–108.

Gómez-Conde, M.S., Chamorro, S. Nicodemus, N., García, J., Carabaño, R. and de Blas, C. (2004) Effect of the level of soluble fibre on ileal apparent digestibility at different ages. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 130.

Lebas, F., Perez, J.M., Juin, H. and Lamboley, B. (1994) Incidence du nombre d'individus par cage sur la rpecision des coefficients de digestiblité mesurés chez le lapin en croissance. In: *6èmes Journées de la Recherche Cunicole*. ITAVI, Paris, France, pp. 317–323.

Llorente, A., García, A.I., Nicodemus, N., Villamide, M.J. and Carabaño, R. (2006) Digestibilidad ileal aparente y verdadera de aminoácidos de harinas de girasol, productos de soja y guisante en conejos. In: *Proceedings of the XXXI Symposium de Cunicultura de ASESCU*. Lorca, Spain, pp. 117–124.

Llorente, A., Villamide M.J., García, A.I. and Carabaño, R. (2007a) Digestibilidad de la proteína de los aminoácidos de cereales y sus subproductos en conejos. In: *Proceedings of XXXII Symposium de Cunicultura de ASESCU*. Vila-Real, Portugal, pp. 87–90.

Llorente, A., García, A.I., Villamide, M.J. and Carabaño, R. (2007b) Prédiction de la digestibilité iléale azotée pour méthodes *in vitro*. In: *Proceedings of the 12èmes Journées de la Recherche Cunicole*. Le Mans, France, pp. 93–96.

Maertens, L. (1992) Rabbit nutrition and feeding: a review of some recent developments. In: Cheeke, P.R. (ed.) *Proceedings of the 5th World Rabbit Congress*. Oregon State University, Corvallis, Oregon, USA, pp. 889–913.

Maertens, L. and De Groote, G. (1981) L'énergie digestible de la farine de luzerne déterminée par des essais de digestiblité avec des lapins de chair. *Revue de l'Agriculture* 34, 79–92.

Maertens, L. and De Groote, G. (1982) Etude de la variablité des coefficients de digestiblité des lapins suite aux differences d'âge, de sexe, de race et d'origine. *Revue de l'Agriculture* 35, 79–92.

Maertens, L., Huyghebaert, G. and De Groote, G. (1986) Digestibility and digestible energy content of various fats for growing rabbits. *Cuni-Sciences* 3, 7–14.

Maertens, L., Luzi, F. and De Groote, G. (1997) Effect of dietary protein and amino acids on the performance, carcass composition and N-excretion of growing rabbits. *Annales de Zootechnie* 46, 255–268.

Maertens, L., Perez, J.M., Villamide, M.J., Cervera, C., Gidenne, T. and Xiccato, G. (2002) Nutritive value of raw materials for rabbits: EGRAN tables. *World Rabbit Science* 10, 157–166.

Martinez, M., Biglia, S., Moya, V.J., Blas, E. and Cervera, C. (2006) Nutritive value of dehydrated whole maize plant and its effect on performance and carcass characteristics of rabbits. *World Rabbit Science* 14, 15–21.

Michelan, A.C., Scapinello, C., Furlan, A.C., Martins, E.N., de Faria, Hg. and Andreazzi, M.A. (2007) Use of cassava root scrapings in the feeding of rabbits. *Revista Brasileira De Zootecnia* 36, 1347–1353.

Ortiz, V., de Blas, C. and Sanz, E. (1989) Effect of dietary fiber and fat content on energy balance in fattening rabbits. *Journal of Applied Rabbit Research* 12, 159–162.

Pascual, J.J., Ródenas, L., Martínez, E., Cervera, C., Blas, E. and Baselga, M. (2008) Genetic selection of maternal lines and digestive efficiency in rabbits: long term selection for litter size at weaning versus hyper selection for reproductive longevity. *World Rabbit Science* 16, 165–171.

Peeters, J.E., Maertens, L. and Geeroms, R. (1992) Influence of galacto-oligosaccharides on zootechnical performance, caecal biochemistry and experimental colibacillosis 0103/8+ in weanling rabbits. In: Cheeke, P.R. (ed.) *Proceedings of the 5th World Rabbit Congress*. Oregon State University, Corvallis, Oregon, USA, pp. 1129–1136.

Pérez, J.M., Lebas, F., Gidenne, T., Maertens, L., Xiccato, G., Parigi Bini, R., Dalle Zotte, A., Cossu, M.E., Carazzolo, A., Villamide, M.J., Carabaño, R., Fraga, M.J., Ramos, M.A., Cervera, C., Blas, E., Fernádnez, J., Falcão e Cunha, L. and Bengala Freire, J. (1995a) European reference method for *in vivo* determination of diet digestbility in rabbits. *World Rabbit Science* 3, 41–43.

Pérez, J.M., Cervera, C., Falcão e Cunha, L., Maertens, L., Villamide, M.J. and Xiccato, G. (1995b) European ring-test on *in vivo* determination of digestibility in rabbits: reproducibility of a reference method in comparison with domestic laboratory procedures. *World Rabbit Science* 3, 171–178.

Pérez, J.M., Bourdillon, A., Lamboley, B. and Naour, J. (1996a) Length of adaptation period: influence on digestive efficacy in rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, pp. 263–266.

Pérez, J.M., Fortun-Lamothe, L. and Lebas, F. (1996b) Comparative digestibility of nutrients in growing rabbits and breeding does. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, pp. 267–270.

Santomá, G. de Blas, C., Carabaño, R. and Fraga, M.J. (1987) The effects of different fats and their inclusion level in diets for growing rabbits. *Animal Production* 45, 291–300.

Sauvant, D., Perez, J.-M. and Tran, G. (2004). Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage: porcs, volailles, bovins, ovins, caprins, lapins, chevaux, poissons. *2éme Edition revue et corrigée*. INRA Editions, Paris, France.

Taboada, E., Méndez, J., Maeos, Gg. and de Blas, C. (1994) The response of highly productive rabbits to dietary lysine content. *Livestock Production Science* 40, 329–337.

Taboada, E., Méndez, J. and Blas, C. (1996) The response of highly productive rabbits to dietary sulphur amino acid content for reproduction and growth. *Reproduction Nutrition Development* 36, 191–203.

Villamide, M.J. (1996) Methods of energy evaluation of feed ingredients for rabbits and their accuracy. *Animal Feed Science and Technology* 57, 211–223.

Villamide, M.J. and Ramos, M.A. (1994) Length of collection period and number of rabbits in digestibility assays. *World Rabbit Science* 2, 29–35.

Villamide, M.J., Fraga, M.J. and de Blas, C. (1991) Effect of type of basal diet and rate of inclusion on the evaluation of protein concentrates with rabbits. *Animal Production* 52, 215–224.

Villamide, M.J., García, J., Cervera, C., Blas, E., Maertens, L. and Pérez, J.M. (2003) Comparison among methods of nutritional evaluation of dietary ingredients for rabbits. *Animal Feed Science and Technology* 109, 195–207.

Villamide, M.J., Carabaño, R., Maertens, L., Pascual, J., Gidenne, T., Falcão e Cunha, L. and Xiccato, G. (2009) Prediction of the nutritional value of European compound feeds for rabbits by chemical components and *in vitro* analysis. *Animal Feed Science and Technology* 150, 283–294.

Xiccato, G. and Cinetto, M. (1988) Effect of nutritive level and of age on feed digestibility and nitrogen balance in rabbit. In: *Proceedings of the 4th World Rabbit Congress, Budapest,* Vol. 3. Sandor Holdas, Hercegalom, Budapest, Hungary pp. 96–104.

Xiccato, G., Cinetto, M. and Dalle Zotte, A. (1992) Effeto del livello nutritive e della categoria di conigli sull'eficienza digestiva e sul bilancio azotato. *Zootechnia Nutritione Animale* 18, 35–43.

Xiccato, G., Carazzolo, A., Cervera, C., Falcão e Cunha, L., Gidenne, T., Maertens, L., Pérez, J.M. and Villamide, M.J. (1996). European ring-test on the chemical analyses of feed and faeces: influence on the calculation of nutrient digestibility in rabbits. In: *Proceedings of the 6th World Rabbit Congress*, *Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 293–297.

9 Influence of Diet on Rabbit Meat Quality

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The continued demand for high standards of quality in meat production call for the development of new tools capable of meeting such demands. The quality of rabbit meat largely depends upon the rabbit's nutrition. Most research conducted in recent years on rabbit meat quality has focused on incorporating bioactive compounds in meat using different feeding strategies. The influence of different dietary factors on meat quality and safety are discussed in this chapter.

9.1 Rabbit Meat Quality

9.1.1 Definition of meat quality

Meat quality consists of: (i) nutritional properties, such as appropriate proportions of bioactive compounds, proteins, lipids and their essential sub-constituents; (ii) sensory characteristics, such as appearance, texture and flavour; (iii) health, which depends on fat and saturated fatty acid (SFA) content; and (iv) technological factors, such as processing. It also includes the consumer's perception of animal rearing conditions in relation to animal welfare, the impact of animal production on the environment and food safety.

Rabbit meat consumption depends heavily on cultural, traditional and religious beliefs. Rabbit meat production is strongly developed in Mediterranean countries of the European Union. Meat sensory properties are crucial in the consumer's choice. Traditional consumers consider rabbit meat to have positive sensory properties, being tender, lean and with a delicate flavour (Dalle Zotte, 2002). Hedonistic quality differs from standard aspects and depends on the various types of meat presentation. In traditional markets rabbits are sold as a whole carcass and retail cuts. The current market, however, and younger consumers in particular, is more concerned with the way a product is presented. Rabbit meat could gain commercial acceptance if sold fresh and in ready-to-cook- and-serve retail packs, and if packaging systems were competitive.

Consumer demand is now more influenced by growing concerns with the healthiness of the meat. Other issues more widespread in the developed world include residues and contaminants. Because the wholesomeness of rabbit meat is becoming an indispensable requirement for consumers, rabbit meat quality chain managers must become more willing to certify their product and guarantee traceability 'from farm to fork'.

Although all of the above factors are very important for the consumer, cost is perhaps the most critical. The lack of increased meat consumption in developed countries now creates fierce competition between several meats. In this sense, because rabbit meat is more expensive than other 'white meats', its consumption may increase if efforts are made to inform the public of its high nutritional and dietetic properties while increasing its serviceability and preservability.

9.1.2 Nutritive value

Rabbit meat offers excellent nutritive and dietetic properties (see reviews by Combes, 2004; Dalle Zotte, 2004; Combes and Dalle Zotte, 2005; Hernández and Gondret, 2006). It is a lean meat that is rich in protein. The leanest portion is the loin and the fattest is the foreleg, with average lipid contents of 1.8 and 8.8 g 100 g^{-1}, respectively. Rabbit meat offers a moderately high energy value, even if this depends primarily on its high protein content, which accounts for 0.80 of the energy value (Table 9.1).

Along with high protein content, rabbit meat also contains high levels of essential amino acids (EAAs). Compared to other meats, it is richer in lysine (2.12 g 100 g^{-1}), sulphur-containing amino acids (1.10 g 100 g^{-1}), threonine (2.01 g 100 g^{-1}), leucine (1.73 g 100 g^{-1}) and phenylalanine (1.04 g 100 g^{-1}). This elevated and balanced EAA content together with easy digestibility give rabbit meat proteins a high biological value. Furthermore, rabbit meat does not contain uric acid and has a low purine content (Hernández, 2007).

The meat is an important source of B vitamins. The consumption of 100 g of rabbit meat provides around 0.21 of vitamin B_6 and 0.77 of daily vitamin B_3 requirements. With respect to vitamin B_{12}, ruminants and rabbits are a much richer source than other meats, and the consumption of 100 g of rabbit meat provides three times the daily recommendation of vitamin B_{12} (Combes and Dalle Zotte, 2005). The vitamin E content of rabbit meat depends on the rabbit's diet; it can be increased by >50% with extra dietary supplements (Castellini *et al.*, 2000).

Like other white meats, rabbit meat contains low levels of iron (1.3 and 1.1 mg 100 g^{-1} for the hind leg and loin, respectively; Dalle Zotte, 2004) and zinc (0.55 and 1.1 mg 100 g^{-1} in the carcass and hind leg, respectively; Lombardi-Boccia *et al.*, 2005; Hermida *et al.*, 2006). Because the haem iron in meat is easily absorbed, rabbit meat can also contribute to meeting human requirements.

Rabbit meat is characterized by its low sodium content (37 and 49.5 mg 100 g^{-1} for the loin and hind leg, respectively), which makes it particularly appropriate for those with hypertension. Conversely, rabbit meat is rather rich in phosphorus (222 and 234 mg 100 g^{-1} for the loin and hind leg, respectively; Dalle Zotte, 2004). Poultry, pig and lamb meat have lower phosphorus contents, at

Table 9.1. Chemical composition and energy value of rabbit meat portions (g 100 g^{-1}, unless otherwise stated) (adapted from Combes and Dalle Zotte, 2005).

	Fore leg		Loin (*m. longissimus dorsi*)		Hind leg		Carcass	
	Average ± SD	No.[a]	Average ± SD	No.[a]	Average ± SD	No.[a]	Average ± SD	No.[a]
Water	70 ± 1.3	4	75 ± 1.4	24	74 ± 0.8	33	70 ± 2.6	6
Protein	19 ± 0.4	3	22 ± 1.3	21	22 ± 0.7	31	20 ± 1.6	6
Lipid	9 ± 2.5	4	2 ± 1.5	24	3 ± 1.1	36	8 ± 2.3	6
Ash	–	–	1 ± 0.1	14	1 ± 0.5	20	2 ± 1.3	4
Energy (kJ 100 g^{-1})	899 ± 47	2	603	1	658 ± 17	7	789 ± 106	3

SD, standard deviation.
[a] Number of studies considered.

200, 174 and 147–194 mg 100 g^{-1}, respectively (Williams, 2007). The selenium levels of rabbit meat vary widely according to dietary selenium supplementation, ranging from 9.6 µg 100 g^{-1} in non-supplemented diets to about 39.5 µg 100 g^{-1} with a supplementation of 0.50 mg kg^{-1} feed (Dokoupilová *et al.*, 2007). According to Rayman (2004), 140 g of meat of selenium-fed rabbits would meet the recommended selenium daily intake for adults.

Meat is a major source of SFAs and cholesterol and its consumption can have a negative influence on health (Valsta *et al.*, 2005). Nutritionists recommend not only limiting fat intake, but also consuming large amounts of polyunsaturated fatty acids (PUFA), especially n-3 rather than n-6 PUFA. Current recommendations state that the n-6:n-3 ratio in human diets should be <4.0. Rabbit meat has a higher PUFA content (0.27–0.33 of total fatty acid) than other meats (Table 9.2). C18:2 n-6 is a major fatty acid in rabbit meat, derived entirely from the diet. It represents 0.22 of total fatty acids. C18:3 n-3 is also an essential fatty acid and it is very abundant in rabbit meat, accounting for 0.03 ± 0.015 total fatty acids (this compares with 0.014 in lamb, 0.010 in pork and 0.001–0.023 in beef; Enser *et al.*, 1996). Rabbit meat contains significant proportions of long-chain (C20–22) PUFA. Important PUFA are C20:4 n-6, C20:5 n-3 (eicosapentaenoic acid, EPA) and C22:6 n-3 (docosahexaenoic acid, DHA), which all play various metabolic roles. Rabbit meat possesses a fairly high n-6:n-3 ratio, at 5.1, 10 and 6.6 for the loin, hind leg and carcass, respectively.

Rabbit meat contains the lowest cholesterol levels (47.0 and 61.2 mg 100 g^{-1}, for the loin and hind leg, respectively; Table 9.2) of all the most popular meats (60, 74 and 81 mg 100 g^{-1} in beef, turkey and chicken, respectively; Dalle Zotte 2004).

9.1.3 Sensory properties and processing characteristics

In the sensory map made by Rødbotten *et al.* (2004) comparing meat from 15 commercial animal species, rabbit meat was ranked among the most tender, together with lamb, roe deer, moose, hare and chicken. The sensory map assigned rabbit meat coarseness similar to that of the hare, lamb and roe deer. Rabbit meat was considered the meat with the least colour, odour intensity and odour attributes (sweet, metallic, liver, gamy), flavour intensity and flavour attributes such as sweet, liver and gamy (just after chicken, turkey and pork). Rabbit meat was also ranked with the lowest fatty feeling in the mouth and its juiciness was ranked medium-low as a result.

In addition to the general descriptors listed above, the sensory analysis of rabbit meat considers other specific descriptors

Table 9.2. Relative proportions of different types of fatty acids (proportion of total fatty acid) and cholesterol content (mg 100 g^{-1}) of rabbit meat portions (adapted from Combes and Dalle Zotte, 2005).

	Loin (*m. longissimus dorsi*)		Hind leg		Carcass	
	Average ± SD	No.[a]	Average ± SD	No.[a]	Average ± SD	No.[a]
SFA	0.39 ± 0.048	17	0.39 ± 0.055	18	0.41 ± 0.016	4
MUFA	0.28 ± 0.044	17	0.28 ± 0.036	17	0.32 ± 0.24	4
PUFA	0.33 ± 0.067	17	0.32 ± 0.084	17	0.27 ± 0.020	5
EPA	0.002 ± 0.0013	10	0.001 ± 0.0002	11	0.0001 ± 0.00003	2
DHA	0.004 ± 0.0034	10	0.002 ± 0.0027	10	0.0001 ± 0.00001	2
n-6:n-3	5 ± 2.2	10	10 ± 3.7	13	7 ± 1.3	4
Cholesterol	47.0 ± 7.9	5	61 ± 5.2	17	55 ± 18.5	3

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
[a]Number of studies considered.

such as fibrousness, sticky, intensity of rabbit odour and flavour, aniseed odour and flavour, intensity of grass odour, overall appraisal (Carrilho *et al.*, 2009) and off-flavour perception (rancid, freeze-burned) (Dalle Zotte *et al.*, 2008). The latter study showed that the high intensity of rabbit flavour was considered positive by 0.72 of the panellists, and the off-flavour evaluation did not affect the rabbit flavour judgment.

The main sensory properties of meat are colour, juiciness, tenderness and flavour. Muscle pH and water-holding capacity (WHC) exert a high influence on the technological and eating quality of meat. The post-mortem evolution of pH and the pHu (pH measured at 24 h post-mortem) affects the brightness of meat, its WHC and toughness (Lawrie, 1998). In rabbits, the important factors affecting muscle pHu are identified as muscle type, age, slaughter method and carcass treatment post-mortem whereas, animal diet has a small effect (Dalle Zotte, 2002). In rabbits, pHu ranges between 5.4 and 6.4 depending on muscle location (Hulot and Ouhayoun, 1999). To date, the literature has not reported any abnormal post-mortem acidification kinetics characteristic of pale, soft and exudative or acidic meat in the rabbit. However, dark, firm, dry meat has been reported (Rodríguez-Calleja *et al.*, 2005).

Meat colour is the most important factor affecting consumer acceptance and purchasing decisions. Rabbit meat colour has primarily been instrumentally measured using the CIE colour system (CIE, 1976). The three fundamental colour coordinates are L* (lightness), a* (redness) and b* (yellowness). Mean L*a*b* colour values of the rabbit longissimus dorsi (LD) muscle are L*= 56–60, a*= 2.6–3.4 and b*= 4.0–5.0 (Dalle Zotte, 2004). The main colour variability factors are the type of muscle (muscle energy metabolism and contractile properties), muscle pHu and myoglobin content (Ouhayoun and Dalle Zotte, 1993), age and diet of the rabbits (Dalle Zotte *et al.*, 1996) and even the activity undertaken by the animal (Gondret *et al.*, 2009). Compared to white meat species (pork, turkey and chicken) and when evaluating similar muscles (breast white muscle in poultry), rabbit meat rates first in lightness and third in redness after pork and turkey (Dalle Zotte, 2004; Molette *et al.*, 2005).

The WHC determines the juiciness of meat, and is defined as the ability of meat to retain its water during the application of external forces such as cutting, heating, grinding or pressing. As previously mentioned, meat WHC is greatly affected by pH. Drip loss is defined as the loss of fluid from meat cuts by the shrinkage of contractile muscle proteins in the form of drip. A rapid pH fall or a lower pHu tends to cause protein denaturation and greater drip loss. Karakaya *et al.* (2006) compared the WHC and the cook loss of mutton, goat, beef and rabbit ground meat, and observed a lower WHC in rabbit and beef meat (about 0.22) than in mutton and goat meat (0.41–0.46). Cook loss, however, did not vary much among the four species compared (around 0.34). Several other studies on rabbit LD muscle have indicated a higher WHC (0.33) (Pla and Cervera, 1997; Martinez *et al.*, 2005) than that found by Karakaya *et al.* This is still lower, however, than that of chicken meat fillet (about 0.44). Cook loss is the most widely used variable for estimating WHC in rabbit meat. According to the meat cuts considered, cook loss varies from 0.205 in the hind leg to 0.271 in the loin (Dalle Zotte, 2007; Combes *et al.*, 2008; Dalle Zotte *et al.*, 2009).

Tenderness can be measured instrumentally by Warner-Bratzler shear force testing (WBSF) and texture profile analysis testing. Meat tenderness depends mainly on the post-mortem changes affecting myofibrillar proteins and the connective tissue (collagen content and solubility), which represents the ‘background’ toughness. Although information on collagen content and its solubility in rabbit meat is scarce, high collagen solubility has in any case been documented (0.75 ± 0.081 as a proportion; Combes, 2004) because of the young slaughter age of rabbits (10–11 weeks). Therefore, the myofibrillar component represents a more important factor than connective tissue characteristics in influencing rabbit meat tenderness. WBSF values of rabbit meat are quite low at 2.4 ± 1.6 kg cm^{-2}, and the meat of the loin (2.9 kg cm^{-2}) is tougher than that of the hind leg (0.94 kg cm^{-2}) (Dalle Zotte, 2007; Dalle Zotte *et al.*, 2009). In addition, an improvement in meat tenderness in rabbit loins after 7 days of aging has been observed by several authors (Gil *et al.,* 2006; Hernández and Pla, 2008).

9.1.4 Rabbit meat and its role as a functional food

In recent years, much attention has been paid to the influence of diet on human health and well-being. The principal function of the diet is to provide the nutrients required to satisfy the nutritional needs of an individual. Some food and food components have physiological and psychological effects beyond the contribution of basic nutrients (Jones and Jew, 2007). These are called functional foods.

In the case of meat, the object of including functional ingredients is not only concerned with providing meat with certain desirable properties, but also attempts to change its image in these health-conscious days. Indeed, meat is a major source of SFAs and cholesterol and its consumption could be related to cardiovascular disease, hypertension, obesity and diabetes (Valsta *et al.*, 2005). However, different strategies can be effectively used to increase or reduce bioactive compounds in order to produce functional meat and meat products (for a review, see Jiménez-Colmenero *et al.*, 2006).

Rabbit meat, as has been previously discussed, is a lean meat rich in proteins of high biological values, with highly unsaturated lipids and low cholesterol content. Moreover, rabbit meat consumption could become a good way to provide these bioactive compounds to human consumers, since manipulation of the rabbit diet is very effective in increasing the levels of n-3 PUFA (Kouba *et al.*, 2008; Tres *et al.*, 2008), conjugated linoleic acid (CLA) (Corino *et al.*, 2002, 2003), or vitamin E (Castellini *et al.*, 1999) as will be discussed later.

9.2 Influence of Dietary Factors on Meat Quality

9.2.1 Effect of dietary energy and feed restriction

When feeding modifies growth potential it also modifies carcass and meat quality. An efficient chemostatic appetite regulation mechanism makes daily energy intake constant, but this only occurs at >9.2 MJ digestible energy (DE) kg^{-1}. The best meat production performance is obtained by feeding rabbits *ad libitum* with a DE concentration >10.45 MJ kg^{-1}. Rabbits also require a certain fibre quantity, however, and this limits high DE intake.

Nutrient requirements change as rabbits age, and feeding plans have been developed accordingly. The influence of feeding plans on carcass and meat quality has not, however, been shown to have a great effect. Diets with a high energy content from postweaning to slaughter have been found to lower the feed conversion ratio (FCR) significantly, enhance dissectible fat content and decrease pHu in the LD muscle, without noticeable variations in meat proximate composition or lightness (Dalle Zotte *et al.*, 1996; Xiccato, 1999). Furthermore, muscle fibre type distribution and fibre cross-sectional area were not affected by the dietary energy level.

Although starch is the main dietary energy source in rabbit feed, it is hard to separate its effect on carcass and meat quality from that of other dietary components, particularly fibre. Increased starch levels usually decrease crude fibre and fibre fraction concentration. Dietary starch levels alone do not appear to affect rabbit carcass and meat quality significantly, apart from their role in increasing the diet's energy concentration and thereby improving FCR. However, Xiccato *et al.* (2002) found that a starch level of 206 g kg^{-1} dry matter (DM) resulted in a higher slaughter yield, but also increased meat cook loss and WBSF. More recently, Carraro *et al.* (2007) did not observe any differences in carcass and meat quality after raising the starch level from 120 to 180 g kg^{-1}.

Sartori *et al.* (2003) found that phase feeding programmes with an increasing dietary starch to acid detergent fibre (ADF) ratio from 0.8 in the post-weaning period to ≥1 during the fattening period, implemented for the purpose of reducing post-weaning digestive disorders, did not affect slaughter yield, carcass adiposity, meatiness or *m. biceps femoris* lightness. For the same purpose, two digestible fibre to ADF ratios were tested (1.0 and 1.3). Once again, growth performance and slaughter traits were unaffected (Carraro *et al.*, 2007).

Feed restriction is becoming a common practice on rabbit farms, and is performed to reduce post-weaning digestive disorders and improve the FCR. Feed restriction can be quantitative in terms of level (proportion of restriction of the *ad libitum* intake) and length or qualitative (DE concentration <9.2 MJ DE kg^{-1}).

Regarding restriction levels, some studies have shown that when rabbits ingest <0.85 of the *ad libitum* diet, growth, feed efficiency, slaughter yield, carcass adiposity and lipid content can be seriously compromised. This intake level is therefore unsuitable for meat production (Dalle Zotte, 2002). Moreover, an 0.85–0.90 feed restriction from 4 weeks to slaughter increases lightness and cook loss and reduces redness in LD muscle (Metzger *et al.*, 2008).

Feed restriction affects live performance, carcass yield and muscle to bone ratio variously according to restriction plans. When moderately feed-rationed post weaning, for example, rabbits show a large compensatory growth rate during the following more-liberal fattening period correlated with good global feed efficiency (Xiccato, 1999; Dalle Zotte, 2002). Moreover, the lowering of the pHu of the hind leg muscles of re-fed rabbits suggests an enhancement of the muscular glycolytic metabolic pathway. This feature was confirmed in a study by Dalle Zotte *et al.* (2005). Specifically, muscular glycolytic metabolism, slowed by feed rationing of 0.70 *ad libitum* from 5 to 8 weeks of age, was compensated for by subsequent re-feeding (0.90 *ad libitum*) from 8 to 11 weeks of age. Tůmová *et al.* (2006) tested different restriction plans from weaning to 3 weeks before slaughtering. Although restriction plans did not affect slaughter yield or meat pHu, late restriction (after 56 days of age) reduced rabbit loin and perirenal fat incidence. Not even 0.80 feed restriction from weaning to 60 days of age and 0.90 up to slaughter during the summer had a negative effect on carcass traits, meat pHu, WHC or proximate composition (Bovera *et al.*, 2008). Together, these results show that the best performance is achieved by early feed restriction followed by *ad libitum* feed intake.

With current attempts to produce certified quality label rabbits characterized by slow growth and higher slaughter age, some producers feed rabbits with moderate restriction. Poorer live performance, carcass weight, yield and adiposity and less intramuscular fat content and proportion of oxidative fibres in LD muscle have been observed when food intake is restricted, even if these rabbits were 3 weeks older than the *ad libitum* rabbits when slaughtered (see the review of Dalle Zotte, 2002). A study by Larzul *et al.* (2004) showed that although carcass traits and meat composition are largely compromised by feed restriction, the sensory quality of the rabbit meat remains unaffected.

9.2.2 Effect of dietary fibre content

The relationship between energy content and digestible protein (DP) to DE ratios makes the fibre level a fundamental variable in rabbit diets. Because the rabbit's feed intake capacity is a limiting factor, increasing dietary fibre content may lead to energy deficiency. The dilution of DE and feed restriction have common effects on both overall body growth rate and the relative growth of tissues and organs and body composition. Diets with high fibre levels invariably decrease growth rates, but when such a rate is unimpaired by fibre increase, the slaughter yield remains the same (Ouhayoun, 1989). When a high dietary fibre level decreases the growth rate, slaughter yield falls due to increased digestive tract proportions. Carcass adiposity and meat lipid content decrease, but water and protein contents rise. Comparing three diets with increasing crude fibre content (138, 163 and 198 g kg^{-1}) and decreasing energy level (10.2, 9.3 and 8.6 MJ DE kg^{-1}), Parigi Bini *et al.* (1994) observed no differences in slaughter yield, carcass meatiness or fatness; only the hind leg meat from rabbits fed a more fibrous diet was leaner and richer in water. Carrilho *et al.* (2009) performed a similar study, using three increasing levels of dietary crude fibre (143, 180 and 205 g kg^{-1} DM) with decreasing energy levels (9.3, 9.1 and 8.0 MJ DE kg^{-1} DM). These three diets were fed to rabbits from 5 to 8 weeks of age, followed by a finishing diet until slaughter. No significant differences ascribed to diet were observed in instrumental (pHu, L*a*b* colour, WHC, toughness) or sensory meat traits. Neither crude fibre dietary content nor the digestible fibre to ADF ratio appear to directly compromise carcass or meat quality. Even increasing dietary fibre levels during the last week of the fattening period does not seem to significantly impair slaughter traits or meat pHu (Margüenda *et al.*, 2008; Villena *et al.*, 2008).

9.2.3 Effect of dietary protein

The effects of dietary protein content on live performance, carcass and meat quality have been studied by modifying dietary protein concentrations (iso-energetic diets) or simultaneously varying protein and energy content. The former complicates extrapolating the real protein effect because changes in the DP to DE ratio reveal different protein intakes. Because the optimum level of protein with balanced EAAs increases with the dietary energy level, the latter simplifies the dietary protein effect calculation.

DP to DE ratios below optimum values of 10.5–11.0 g MJ^{-1} are insufficient to cover the daily protein requirements, and might therefore compromise the growth rate because muscular protein accretion is suboptimal. Animals might show low dissectible fat deposits due to delays in tissue development or elevated intracellular lipid accumulation caused by high energy levels. The decreased growth rate obtained in this way seems to enhance meat quality by limiting muscle glycolytic metabolism, and produces less lean meat with better WHC (see the reviews of Xiccato, 1999; Dalle Zotte, 2002).

Effects on carcass and meat quality with DP to DE ratios above the optimum value of 10.5–11.0 g MJ^{-1} have not been precisely established. Some authors have observed no variation in live performance or carcass and meat quality; others, however, have observed significant reductions in dissectible fat deposit only at very high DP to DE ratios (>12 g MJ^{-1}), together with worse live performance and meatiness with ratios >14 g MJ^{-1}. In a 10.5–11.0 g MJ^{-1} DP to DE range, growth performance is high and remains in the range because protein intake permits the maximum expression of muscular protein synthesis. Meat water and nitrogen content tend to increase at the expense of fat content. Other meat qualities are unaffected at higher dietary DP to DE ratios (see reviews of Xiccato, 1999; Dalle Zotte, 2002).

The effect of lower DP to DE ratios (11.5 versus 12.5) on nitrogen output has been demonstrated by Maertens *et al.* (1998). In this DP to DE ratio range, whenever EAAs (lysine, sulphur amino acids and threonine) cover daily requirements, decreasing dietary protein content appears possible without compromising the zootechnical performance or carcass and meat quality. Reviewing protein-amino acid nutrition in rabbits, Carabaño *et al.* (2008) found that crude protein levels in commercial feeds currently exceed recommendations, especially in final growth phases. The authors suggested adopting protein levels of 140g kg^{-1} from weaning to slaughter. If the DP to DE ratio is around 9.5 and the amino acid supply is correct, this level does not appear to impair growth performance.

Protein requirements change during growth, and growth can be compromised by an unbalanced dietary protein supply. Low-protein diets in early post weaning can, in fact, cause low slaughter yield as a consequence of impaired growth. Compensative growth may produce leaner carcasses, however. In contrast, a high DP to DE ratio during early post weaning and onwards may increase carcass fat deposition and lipid content (see the review of Xiccato, 1999). The effects of dietary protein levels or specific dietary EAAs on the rheological and sensory properties and fatty acid profiles of rabbit meat have not yet been assessed.

9.2.4 Effect of dietary fat

Increasing the fat content of the diet improves its energy level and results in a higher DE intake and improved growth and feed efficiency (Maertens, 1998; Xiccato, 1999; Dalle Zotte, 2002). The level and source of fat in the diet can have different impacts on carcass and meat quality. A low or moderate addition of fat (20–60 g kg^{-1}) increases carcass yield (Castellini and Battaglini, 1992) and the amount of dissectible fat (Fernández and Fraga, 1996). An increase in dietary fat over these values (>90 g kg^{-1}) may impair carcass quality due to an excess of carcass adiposity (Pla and Cervera, 1997). However, higher dietary fat inclusion may also increase the meat lipid content (Pla and Cervera, 1997), increasing meat quality as a consequence of the influence of muscular fat content on sensory characteristics such as juiciness and tenderness.

The composition of dietary fat, as a result of the use of various fat sources, can modify the fatty acid composition of different rabbit tissues. It is well known that rabbits, and other non-ruminants, are able to incorporate dietary fatty acids into adipose and muscle tissue lipids. The effect of various dietary fat sources has been the subject of many experiments, which are discussed in this review.

Changes in the n-3 and n-6 fatty acid profile

The lipid fraction has considerable implications on health. The recommendation to increase consumption of PUFA, particularly n-3 PUFA, is based on its role on the development and prevention of cardiovascular disease, atherosclerosis and other diseases (Goodnight, 1993; Simopoulos, 2002). Much research has been carried out on modifying the nutritional value of meat through animal diets.

The addition of vegetable fat compared to animal fat sources in the diet leads to differences in rabbit meat quality, especially regarding the fatty acid composition of the tissues and meat flavour. For instance, sensory test panels attribute a higher 'liver' taste to animals fed with an animal fat diet, while meats of animals fed with a vegetable diet have a higher 'aniseed' or 'grass' flavour. However, no differences between groups have been found for texture parameters (Oliver *et al.*, 1997; Hernández *et al.*, 2000).

The dietary use of linseed in its different forms (oil, extruded, whole) has been proposed by many authors as a way to raise the content of n-3 PUFA and reduce the ratio n-6 to n-3 PUFA. Dal Bosco *et al.* (2004) studied the synergistic effect of dietary α-linolenic acid and vitamin E on the oxidative stability and nutritional and eating characteristics of fresh and stored rabbit meat. The ability of rabbits to synthesize long-chain PUFA (EPA and DHA) from the dietary precursor, leading to an increase in the n-3 PUFA content of the meat of rabbits consuming the n-3 diet, without any alteration of oxidative stability and sensory quality of the meat was confirmed. Tres *et al.* (2008) evaluated the effects of replacing beef tallow added to rabbit feeds with different levels (0, 15 and 30 g kg^{-1}) of n-6- or n-3-rich vegetable sources (sunflower and linseed oil, respectively). The level and source of the fat added influenced meat fatty acid composition, modifying the n-6 to n-3 PUFA ratio, which was more nutritionally favourable when linseed oil was used (Table 9.3). However, carcass and meat quality may be affected as a consequence of the PUFA increase, although diets enriched with 30 g kg^{-1} of sunflower or linseed oil and 100 ppm of

Table 9.3. Effect of the source and dose of unsaturated fat used to replace beef tallow in feeds on the content of n-6 and n-3 fatty acids in raw meat (adapted from Tres *et al*., 2008).

	30 g BT kg^{-1}	15 g SO + 15 g BT kg^{-1}	30 g SO kg^{-1}	15 g LO + 15 g BT kg^{-1}	30 g LO kg^{-1}
Ratio PUFA:SFA	0.6	1	1.5	1.0	1.5
Ratio n-6:n-3	7.4	12.0	16.9	1.8	1.1

BT: beef tallow; LO: linseed oil; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; SO: sunflower oil.

α-tocopherol acetate have been found to have small effects on rabbit carcass characteristics. Retail cuts, lightness and yellowness were the most affected traits (Pla *et al.*, 2008). A small effect on instrumental texture properties was also found, but there was no negative effect on sensory characteristics (Hernández and Pla, 2008).

Extruded linseed has also been used to improve the nutritional value of rabbit meat (Gigaud and Combes, 2008; Kouba *et al.*, 2008; Maertens *et al.*, 2008), leading to a decrease in the n-6 to n-3 ratio. Bianchi *et al.* (2006) studied the influence of dietary use of whole linseed at different proportions (from 0 to 80 g kg^{-1}) and supplemented with α-tocopherol acetate (200 mg kg^{-1} feed) on rabbit meat quality, also finding a decrease in the n-6 to n-3 PUFA ratio with the linseed diet supplementation. The increase of the PUFA content produced by the dietary use of linseed could lead to oxidation and a reduction in the shelf life of the meat (Monahan, 2000). Therefore, supplementation with antioxidants such as α-tocopherol acetate is required in these experiments.

Alternative dietary sources of n-3 PUFA for rabbit production have been studied. Supplementation of rabbit diets with false flax (*Camelina sativa* L.) seeds (Peiretti *et al.*, 2007) or chia (*Salvia hispanica* L.) (Peiretti and Meineri, 2008) has been successful in increasing PUFA content and reducing the n-6 to n-3 PUFA ratio, without significant adverse effects on growth performance and carcass characteristics. Grass-based diets can also modify the fatty acid composition of rabbit meat, enhancing the n-3 fatty acid content (Forrester-Anderson *et al.*, 2006).

Other strategies for specifically increasing long-chain fatty acids such as EPA and DHA are based on the dietary use of fish oils or algae. A high increase of specifically long-chain PUFA can be achieved by feeding rabbits diets enriched with fish sources, such as herring meal (Castellini and Dal Bosco, 1997) or fish oil (Bernardini *et al.*, 1999; Kowalska, 2008). However, high levels of lipid oxidation, lower growth and impaired carcass and meat quality may result, depending on the fish oil used (Navarrete *et al.*, 2007).

Conjugated linoleic fatty acid

CLA has also received a great deal of attention as a supplement in rabbit feed. CLA is a mixture of positional and geometric isomers of linoleic acid (18:2, n-6) with conjugated double bonds. It has been reported to have a wide range of beneficial effects, including anticarcinogenic (Kelley *et al.*, 2007), antiatherogenic (McLeod *et al.*, 2004) and antiobesity (Whigham *et al.*, 2007) activities.

Food sources that originate from ruminants are known to have markedly higher CLA concentrations than those from monogastric animals (Schmid *et al.*, 2006). Non-ruminants are unable to synthesize CLA, and the CLA present in their meat therefore comes from the diet. In addition, rabbits are able to recycle part of their end microbial fermentation products through caecotrophy, so that the amount of CLA retained in their meat might be higher than in other non-ruminant species (Gómez-Conde *et al.*, 2006).

Dietary CLA inclusion is an effective tool for increasing, in a dose-dependent manner, the amount of CLA in the intramuscular lipids of rabbits, with *cis*-9, *trans*-11 being the predominant isomer (Lo Fiego *et al.*, 2005; Petacchi *et al.*, 2005). It is possible to increase the CLA content in rabbit loin from 1.3 to 10.4 mg 100 g^{-1} meat with a 5 g kg^{-1} supplementation of CLA in the diet (Corino *et al.*, 2007).

In addition to the beneficial effects of CLA on human health, CLA can favourably modify the rabbit's body composition (Corino *et al.*, 2002, 2003) due to its potential to increase lean tissue deposition. The effect of dietary CLA inclusion depends not only on the extent and dose of CLA supplementation, but also on the animal's age (Corino *et al.*, 2002, 2003). Rabbit growth performance and carcass characteristics at standard slaughter weight (2.5 kg, 76 days) were not affected by diets supplemented with 2.5 or 5 g CLA kg^{-1}. However, CLA supplementation reduced perirenal fat weight at a heavy slaughter weight (3.1 kg) and lowered the concentration of serum triglycerides and total cholesterol (Corino *et al.*, 2002). Regarding the chemical composition of rabbit meat, a significant decrease in meat lipid content was evident only when rabbits were fed with a high supplementation level of CLA (5 g kg^{-1}) and at heavy slaughter weight (3.1 kg; Corino *et al.*, 2003).

9.2.5 Antioxidants

Rabbit meat has a high content of PUFA, which may lead to oxidation problems. In addition, there has been increasing interest in the use of antioxidants in rabbit feed formulae because the dietary manipulation of tissue lipid composition to produce meat with a high PUFA content may decrease meat oxidative stability. Lipid oxidation is a major non-microbial factor responsible for the quality deterioration of muscle foods. It leads to discoloration, higher drip loss, the development of off-odours and off-flavours and loss of nutritional value (Monahan, 2000).

Vitamin E is commonly used in animal feed for their antioxidant activity. Hernández and Gondret (2006) reviewed the use of vitamin E in the rabbit diet. In recent years various studies have examined the influence of the addition of α-tocopherol acetate to the diet on the deposition of α-tocopherol in tissues, meat quality characteristics, oxidative stability and the shelf life of rabbit meat. Several authors have shown that the deposition of α-tocopherol in rabbit muscle is very efficient and has a strong relationship with the dose supplemented in the diet (Dal Bosco *et al.*, 2001; Lo Fiego *et al.*, 2004). For instance, the addition of 100 mg α-tocopherol acetate kg^{-1} of feed increases the content of α-tocopherol in rabbit meat by threefold (Tres *et al.*, 2008). These authors also found that cooking reduces α-tocopherol in rabbit meat by 9%. However, the α-tocopherol level of cooked meat depends on the cooking method, with the resistance of vitamin E higher for fried and roasted meat than for boiled meat (Dal Bosco *et al.*, 2001).

Vitamin E is effective in reducing lipid oxidation of rabbit meat during refrigerated or frozen storage (Castellini *et al.*, 1999; Lo Fiego *et al.*, 2004) and also after cooking (Castellini *et al.*, 1999; Tres *et al.*, 2008). Dietary α-tocopherol acetate supplementation has been found to stabilize the colour of raw meat (Corino *et al.*, 1999). In addition, a high α-tocopherol level improves some physical traits of meat, reducing shear values and increasing WHC (Castellini *et al.*, 1998). The effect of synergetic supplementation of the diet with vitamins C and E has been found to increase vitamin content and reduce lipid oxidation (Castellini *et al.*, 2000; Dalle Zotte *et al.*, 2000; Lo Fiego *et al.*, 2004).

Various natural ways of improving the oxidative stability of rabbit meat have also been studied. For example, lipid oxidative stability has been improved by increasing the level of oats in the rabbit diet (López-Bote *et al.*, 1998). Coni *et al.* (2000) verified the antioxidant efficiency of extra-virgin olive oil and oleuropein, an olive oil biophenol, in rabbit plasma and isolated low-density lipoproteins. However, oleuropein does not appear to reduce meat susceptibility to oxidation (Paci *et al.*, 2001). Supplementation of the rabbit diet with essential oil of oregano has been found to improve the oxidative stability of muscle tissues (Botsoglou *et al.*, 2004).

9.3 Influence of Diet on Rabbit Meat Safety

Food safety is an important issue for consumers, especially in the meat sector. Major meat safety issues and related challenges include microbial pathogens, food additives and chemical residues.

Safety and the shelf life of meat are limited by microbial growth. Dominant contaminants on carcasses and packed rabbit meat are *Pseudomonas*, lactic acid bacteria, yeasts and *Brochothrix thermosphacta* (Rodríguez-Calleja *et al.*, 2004) with total bacteria counts between 4.01 and 4.96 log cfu g^{-1}. However, components of the feed may play a specific role in the growth rate of some microbial groups, affecting the microbiological characteristics of carcass and rabbit meat. Vannini *et al.* (2003) showed that a dietary supplementation of whole linseeds limited the growth rate of several microbial groups (except psychrotrophic bacteria), with a consequent increase in meat shelf life. In addition, high percentages of dehydrated lucerne meal in the diet seem to have an inhibiting effect on microbial growth in rabbit meat products (Vannini *et al.*, 2002).

Dietary fibre can affect the microbial ecology of rabbit meat. The source and level of dietary fibre has a major impact in controlling the digestive content. The rabbit gastrointestinal content represents a main concern at the slaughterhouse because of its impact on carcass yield, potential microbial contamination of meat and the cost of offal withdrawal (Villena *et al.*, 2008). Margüenda *et al.* (2008) studied the effect of dietary type and level of fibre on carcass yield and its microbiological quality. They showed that a decrease in dietary fibre (from 350 to 320 g neutral detergent fibre kg^{-1}), when sources of insoluble fibre are included, enhances carcass yield and improves microbiological quality. For the same level of fibre, including 100 g beet pulp kg^{-1}, there was an improvement in the microbiological characteristics of the rabbit carcass without affecting the carcass yield. In addition, an increased level of dietary fibre may reduce muscle glycogen content in rabbits by increasing the pH of the meat, which could have an impact on the meat shelf life (Gierus and Teixeira, 1997). However, Villena *et al.* (2008) found no relationship between the level of fibre in the diet and the final pH of the meat.

The effect of dietary oregano essential oil supplementation on microbial growth of rabbit carcasses has now been studied (Soultos *et al.*, 2009). The incorporation of oregano essential oil in the diet at the level of 100–200 mg kg^{-1} had no detrimental effects on rabbit performance and had an inhibitory effect on the microbial growth of carcasses during refrigerated storage.

The composition, quality and contamination of fat materials used in animal feed are of considerable importance in assessing the quality and safety of meat production. The level of polycyclic aromatic hydrocarbons (PAHs) in rabbit tissues and their rate of transfer from feed have been studied by Devier and Budzinski (2007). PAHs were not detected in meat or liver, even when extremely high total concentrations, from 1 to 4 mg g^{-1}, were present in the feeds, confirming the high capacity of animals to rapidly metabolize these contaminants. However, PAH metabolites are more toxic than the corresponding PAH. When the contents of PAH corresponding to those usually found in fat by- and co-products were assayed, no PAH metabolites were found.

Ábalos *et al.* (2007) studied the presence of dioxins (polychlorodibenzo-p-dioxins and dibenzofurans, PCDD/Fs) and ‘dioxin-like’ polychlorinated biphenyls (DL-PCBs) in rabbit and chicken meat samples from animals fed with fish oil spiked with different levels of contaminants. Three different levels of contaminants under the maximum quantity allowed by the European Union Directive (Commission Directive 2006/13/EC of 3 February, 2006) were tested. The profile of PCDD/Fs in chicken samples from the three different treatments resembled the profile previously observed in the corresponding feeds. Generally, the levels of the different compounds increased when increasing their amount in the feed. In rabbit meat samples, however, different bioaccumulation behaviour was observed. The profile of PCDD/Fs in rabbit meat did not correspond to that present in feeds. In fact, there were no significant differences in PCDD/F toxicity among rabbit samples from the three different treatments. For DL-PCBs, the profile was similar between feeds and meat samples, both in chickens and rabbits.

References

Ábalos, M., Abad, E., Parera, J., Martrat, M.G., Sauló, J. and Rivera, J. (2007) PCDD/Fs and DL-PCBs in meat samples from chickens and rabbits fed with fish oil spiked feed at different levels of contamination. *Organohalogen Compounds* 69, 106–109.

Bernardini, M., Dal Bosco, A. and Castellini, C. (1999) Effect of dietary n-3/n-6 ratio on fatty acid composition of liver, meat and perirenal fat in rabbit. *Animal Science* 68, 647–654.

Bianchi, M., Petracci, M. and Cavani, C. (2006) Effects of dietary inclusion of dehydrated lucerne and whole linseed on rabbit meat quality. *World Rabbit Science* 14, 247–258.

Botsoglou, N.A., Florou-Paneri, P., Christaki, E., Giannenas, I. and Spais, A.B. (2004) Performance of rabbits and oxidative stability of muscle tissues as affected by dietary supplementation with oregano essential oil. *Archives of Animal Nutrition* 58, 209–218.

Bovera, F., Piccolo, G., D'Urso, S., Nizza, S. and Cutrignelli, M.I. (2008) Feed restriction during summer: effect on rabbit carcass traits and meat quality. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1325–1329.

Carabaño, R., Villamide, M.J., García, J., Nicodemus, N., Llorente, A., Chamorro, S., Menoyo, D., García-Rebollar, P., García-Ruiz, A.I. and De Blas, J.C. (2008) New concepts and objectives for protein-amino acid nutrition in rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 135–155.

Carraro, L., Trocino, A., Fragkiadakis, M., Xiccato, G. and Radaelli, G. (2007) Digestible fibre to ADF ratio and starch level in diets for growing rabbits. *Italian Journal of Animal Science* 6, 752–754.

Carrilho, M.C., Campo, M.M., Olleta, J.L., Beltrán, J.A. and López, M. (2009) Effect of diet, slaughter weight and sex on instrumental and sensory meat characteristics in rabbits. *Meat Science* 82, 37–43.

Castellini, C. and Battaglini, M. (1992) Prestazione productive e qualità delle carni di coniglio: influenza della concentrazione energetica della dieta e del seso. *Zootecnia e Nutrizione Animale* 18, 251–258.

Castellini, C. and Dal Bosco, A. (1997) Effects of dietary herring meal on the omega-3 fatty acid content of rabbit meat. In: *Proceedings of Symposium Food and Health: Role of Animal Products*. Elsevier, Milano, Italy, pp. 67–71.

Castellini, C., Dal Bosco, A., Bernardini, M. and Cyril, H.W. (1998) Effect of dietary vitamin E on the oxidative stability of raw and cooked rabbit meat. *Meat Science* 50, 153–161.

Castellini, C., Dal Bosco, A. and Bernardini, M. (1999) Effect of dietary vitamin E supplementation on the characteristics of refrigerated and frozen rabbit meat. *Italian Journal of Food Science* 11, 151–160.

Castellini, C., Dal Bosco, A. and Bernardini, M. (2000) Improvement of lipid stability of rabbit meat by vitamin E and C administration. *Journal of the Science and Food and Agricultural* 81, 46–53.

CIE (1976) Official recommendations on uniform colour spaces, colour differences equations and metric colour terms. Supplement nº 2 to CIE publications nº 15. Colorymetry, Paris, France.

Combes, S. (2004) Valeur nutritionnelle de la viande de lapin. *INRA Production Animales* 17, 373–383.

Combes, S. and Dalle Zotte, A. (2005) La viande de lapin: valeur nutritionnelle et particularités technologiques. In: *Proceedings of 11èmes Journées de la Recherche Cunicole*. Paris, France, pp. 167–180.

Combes, S., González, I., Déjean, S., Baccini, A., Jehl, N., Juin, H., Cauquil, L., Gabinaud, B., Lebas, F. and Larzul, C. (2008) Relationships between sensory and physicochemical measurements in meat of rabbit from three different breeding systems using canonical correlation analysis. *Meat Science* 80, 835–841.

Coni, E., Benedetto, R., Pasquale, M., Masella, R., Modesti, D., Mattei, R. and Carlini, E.A. (2000) Protective effect of oleuropein, an olive oil biophenol, on low lipoprotein oxidizability in rabbits. *Lipids* 35, 45–54.

Corino, C., Pastorelli, G., Pantaleo, L., Oriani, G. and Salvatori, G. (1999) Improvement of color and lipid stability of rabbit meat by dietary supplementation with vitamin E. *Meat Science* 52, 285–289.

Corino, C., Mourot, J., Magni, S., Pastorelli, G. and Rosi, F. (2002) Influence of dietary conjugated linoleic acid on growth, meat quality, lipogenesis, plasma leptin and physiological variables of lipid metabolism in rabbits. *Journal of Animal Science* 80, 1020–1028.

Corino, C., Filetti, F., Gambacorta, M., Manchisi, A., Magni, S., Pastorelli, G., Rossi, R. and Maiorano, G. (2003) Influence of dietary conjugated linoleic acids (CLA) and age at slaughtering on meat quality and intramuscular collagen in rabbits. *Meat Science* 66, 97–103.

Corino, C., Lo Fiego, D.P., Macchioni, P., Pastorelli, G., Di Giancamillo, A., Domeneghini, C. and Rossi, R. (2007) Influence of dietary conjugated linoleic acids and vitamin E on meat quality, and adipose tissue in rabbits. *Meat Science* 76, 19–28.

Dal Bosco, A., Castellini, C. and Bernardini, M. (2001) Nutritional quality of rabbit meat as affected by cooking procedure and dietary vitamin E. *Journal of Food Science* 66, 1047–1051.

Dal Bosco, A., Castellini, C., Bianchi, L. and Mugnai, C. (2004) Effect of dietary α-linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. *Meat Science* 66, 407–413.

Dalle Zotte, A. (2002) Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livestock Production Science* 75, 11–32.

Dalle Zotte, A. (2004) Avantage diététiques. Le lapin doit apprivoiser le consommateur. *Viandes Produits Carnés* 23, 1–7.

Dalle Zotte, A. (2007) Meat quality of rabbits reared under organic production system. In: *Proceedings of 53rd ICoMST*. Beijing, China, pp. 87–88.

Dalle Zotte, A., Ouhayoun, J., Parigi Bini, R. and Xiccato, G. (1996) Effect of age, diet and sex on muscle energy metabolism and on related physicochemical traits in the rabbit. *Meat Science* 43, 15–24.

Dalle Zotte, A., Cossu, M.E. and Parigi Bini, R. (2000) Effect of the dietary enrichment with animal fat and vitamin E on rabbit meat shelf-life and sensory properties. In: *Proceedings of 46th ICoMST*. Buenos Aires, Argentina, pp. 4.II–P8.

Dalle Zotte, A., Rémignon, H. and Ouhayoun, J. (2005) Effect of feed rationing during post-weaning growth on meat quality, muscle energy metabolism and fibre properties of biceps femoris muscle in the rabbit. *Meat Science* 70, 301–306.

Dalle Zotte, A., Masoero, G., Brugiapaglia, A., Contiero, B., Jekkel, G. and Gàbor, M. (2008) Sensory and rheological evaluation of meat from rabbits reared at different floor type and stocking density. In: *Proceedings of 54th ICoMST*. Cape Town, South Africa, pp. 112–113.

Dalle Zotte, A., Princz, Z., Metzger, Sz., Szabó, A., Radnai, I., Biró-Németh, E., Orova, Z. and Szendrő, Zs. (2009) Response of fattening rabbits reared under different housing conditions. 2. Carcass and meat quality. *Livestock Science* 122, 39–47.

Devier, M.H. and Budzinski, H. (2007) Levels of PAHs and PBDEs in animal tissues and rate of transfer from feed. Available from: http://www.ub.es/feedfat/UBORD%20Present%20Florence%20defin.pdf (accessed 18 January 2010).

Dokoupilová, A., Maorunek, M., Skřivanová,V. and Březina, P. (2007) Selenium content in tissues and meat quality in rabbits fed selenium yeast. *Czech Journal Animal Science* 52, 165–169.

Enser, M., Hallett, K., Hewitt, B., Fursey, G.A.J. and Wood, J.D. (1996) Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science* 42, 443–456.

Fernández, C. and Fraga, M.J. (1996) The effect of dietary fat inclusion on growth, carcass characteristics, and chemical composition of rabbit. *Journal of Animal Science* 74, 2088–2094.

Forrester-Anderson, I.T., McNitt, J., Way, R. and Way, M. (2006) Fatty acid content of pasture-reared fryer rabbit meat. *Journal of Food Composition* 19, 715–719.

Gierus, M. and Teixeira, J.B. (1997) Forage substitution in a grain-based diet affects pH and glycogen content of semimembranosus and semitendinosus rabbit muscle. *Journal of Animal Science* 75, 2920–2923.

Gigaud, V. and Combes, S. (2008) The effect of decreasing the omega 6/omega 3 ratio in feed on fatty acid content of rabbit meat to meet human dietary recommendations In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1353–1358.

Gil, M., Ramírez, A., Pla, M., Ariño, B., Hernández, P., Pascual, M., Blasco, A., Guerrero, L., Hajós, G., Szerdahelyi, E.N. and Oliver, M.A. (2006) Effect of selection for growth rate on the ageing of myofibrils, the meat texture properties and the muscle proteolytic potential of longissimus from two groups of rabbits. *Meat Science* 72, 121–129.

Gómez-Conde, M.S., Menoyo, D., Chamorro, S., López-Bote, C.J., García-Rebollar, P. and De Blas, J.C. (2006) Conjugated linoleic acid content in cecotrophes, suprarenal and intramuscular fat in rabbits fed commercial diets. *World Rabbit Science* 14, 95–99.

Gondret, F., Hernández, P., Rémignon, H. and Combes, S. (2009) Skeletal muscle adaptations and biomechanical properties of tendons in response to jump exercise in rabbits. *Journal of Animal Science* 87, 544–553.

Goodnight, S.H. (1993) The effects of n-3 fatty acids on atherosclerosis and the vascular response to injury. *Archives of Pathology and Laboratory Medicine* 117, 102–106.

Hermida, M., Gonzalez, M., Miranda, M. and Rodrìguez-Otero, J.L. (2006) Mineral analysis in rabbit meat from Galicia (NW Spain). *Meat Science* 73, 635–639.

Hernández, P. (2007) Carne de conejo, ideal para dietas bajas en ácido úrico. *Revista Científica de Nutrición. N° 8 Septiembre*. *Boletín de cunicultura*, 154, 33–36.

Hernández, P. and Gondret, F. (2006) Rabbit meat quality. In: Maerterns, L. and Coudert, P. (eds) *Recent Advances in Rabbit Sciences*. ILVO, Melle, Belgium, pp. 269–290.

Hernández, P. and Pla, M. (2008) Effect of the dietary n-3 and n-6 fatty acids on texture properties and sensory characteristics of rabbit meat. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1359–1363.

Hernández, P., Pla, M., Oliver, M.A. and Blasco, A. (2000) Relationships between meat quality measurements in rabbits fed with three diets with different fat type and content. *Meat Science* 55, 379–384.

Hulot, F. and Ouhayoun, J. (1999) Muscular pH and related traits in rabbits: a review. *World Rabbit Science* 7, 15–36.

Jiménez-Colmenero, F., Reig, M. and Toldrá, F. (2006) New approaches for the develpoment of functional meat products. In: Nollet, L.M.L. and Toldrá, F. (eds) *Advanced Technologies for Meat Processing*. CRC Press, Boca Raton, Florida, USA, pp. 275–308.

Jones, P.J. and Jew, S. (2007) Functional food development: concept to reality. *Trends in Food Science and Technology* 18, 387–390.

Karakaya, M., Saricoban, C. and Yilmaz, M.T. (2006) The effect of mutton, goat, beef and rabbit-meat species and state of rigor on some technological parameters. *Journal of Muscle Foods* 17, 56–64.

Kelley, N.S., Hubbard, N.E. and Erickson, K.L. (2007) Conjugated linoleic acid isomers and cancer. *Journal of Nutrition* 137, 2599–2607.

Kouba, M., Benatmane, F., Blochet, J.E. and Mourot, J. (2008) Effect of a linseed diet on lipid oxidation, fatty acid composition of muscle, perirenal fat, and raw and cooked rabbit meat. *Meat Science* 80, 829–834.

Kowalska, D. (2008) Effect of dietary supplementation with rapeseed and fish oil mixture and antioxidant on rabbit meat quality. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1372–1376.

Larzul, C., Thébault, R.G. and Allain, D. (2004) Effect of feed restriction on rabbit meat quality of the Rex du Poitou®. *Meat Science* 67, 479–484.

Lawrie, R.A. (1998) *Lawrie's Meat Science*, 6th edn. Woodhead Publishing, Cambridge, UK.

Lo Fiego, D.P., Santero, P., Macchioni, P., Mazzoni, D., Piattoni, F., Tassone, F. and De Leonibus, E. (2004) The effect of dietary supplementation of vitamins C and E on the α-tocopherol content of muscles, liver and kidney, on the stability of lipids, and on certain meat quality parameters of the longissimus dorsi of rabbits. *Meat Science* 67, 319–327.

Lo Fiego, D.P., Maccioni, P., Santoro, P., Rossi, R., Pastorelli, G. and Corino, C. (2005) Influence of conjugated linoleic acid (CLA) on intramuscular fatty acid composition in the rabbit. *Italian Journal of Animal Science* 4, 553–555.

Lombardi-Boccia, G., Lanzi, S. and Aguzzi, A. (2005) Aspects of meat quality: trace elements and B vitamins in raw and cooked meats. *Journal of Food Composition and Analysis* 18, 39–46.

López-Bote, C.J., Sanz, M., Rey, A., Castaño, A. and Thos, J. (1998) Lower oxidation in the muscle of rabbits fed diets containing oats. *Animal Feed Science and Technology* 70, 1–9.

Maertens, L. (1998) Fats in rabbit nutrition: a review. *World Rabbit Science* 6, 341–348.

Maertens, L., Cavani, C., Luzi, F. and Capozzi, F. (1998) Influence du rapport protéines/énergie et de la source énergétique de l'aliment sur les performances, l'excrétion azotée et les caractéristiques de la viande des lapins en finition. In: *Proceedings of 7èmes Journées de la Recherche Cunicole en France, Lyon*. ITAVI, Paris, France, pp. 163–166.

Maertens, L., Huyghebaert, G. and Delezie, E. (2008) Fatty acid composition of rabbit meat when fed a linseed based diet during different periods after weaning. In: Xiccato, G., Trocino, A. and Lukefahr, S.D.

(eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1381–1386.
Margüenda, I., Carabaño, R., García-Rebollar, P., Fragkiadakis, M., Sevilla, L., Vadillo, S. and Nicodemus, N. (2008) Effect of dietary type and level of fibre on carcass yield and its microbiological characteristics. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1387–1392.
Martinez, M., Motta, W., Cervera, C. and Pla, M. (2005) Feeding mulberry leaves to fattening rabbits: effects on growth, carcass characteristics and meat quality. *Animal Science* 80, 275–281.
McLeod, R.S., LeBlanc, A.M., Langille, M.A., Mitchell, P.L. and Currie, D.L. (2004) Conjugated linoleic acids, atherosclerosis, and hepatic very-low-density lipoprotein metabolism. *American Journal of Clinical Nutrition* 79, 1169S–1174S.
Metzger, Sz., Bianchi, M., Cavani, C., Petracci, M., Gyovai,M., Biró-Németh, E., Radnai, I. and Szendrő, Zs. (2008) Effect of nutritional status of kits on carcass traits and meat quality (preliminary results). In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1399–1403.
Molette, C., Rémignon, H. and Babilé, R. (2005) Modification of glycolyzing enzymes lowers meat quality of turkey. *Poultry Science* 84, 119–127.
Monahan, F. (2000) Oxidation of lipids in muscle foods: fundamental and applied concerns. In: Decker E., Faustman F. and López-Bote C. (eds) *Antioxidants in Muscle Foods*. Wiley & Sons, New York, USA, pp. 3–23
Navarrete, C., Martínez, E., Ródenas, L., Moya, V.J., Pascual, J.J., Blas, E. and Cervera, E. (2007) Empleo de destilados de palma y de aceites de pescado en piensos de conejo. In: *Proceedings of II Congreso Ibérico de Cunicultura*. Vila Real, Portugal, pp. 173–181.
Oliver, M.A., Guerrero, L., Diaz, I., Gispert, M., Pla, M. and Blasco, A. (1997) The effect of fat-enriched diets on the perirenal fat quality and sensory characteristics of meat from rabbits. *Meat Science* 47, 95–103.
Ouhayoun, J. (1989) La composition corporelle du lapin. *INRA Productions Animales* 2, 215–226.
Ouhayoun, J. and Dalle Zotte, A. (1993) Muscular energy metabolism and related traits in rabbit. A review. *World Rabbit Science* 1, 97–108.
Paci, G., Schiavone, A. and Marzoni, M. (2001) Influence d'un extrait végétal naturel (oléuropéie) sur les processus oxydatifs de la viande de lapin: premiers résultats. In: *Proceedings of 9èmes Journées de la Recherche Cunicole*. Paris, France, pp. 27–30.
Parigi Bini, R., Xiccato, G., Dalle Zotte, A. and Carazzolo, A. (1994) Effets de differents niveaux de fibre alimentaire sur l'utilisation digestive e la qualité bouchère chez le lapin. In: *Proceedings of 6èmes Journées de la Recherche Cunicole*, Vol. 2. INRA-ITAVI, La Rochelle, France, pp. 347–354.
Peiretti, P. and Meineri, G. (2008) Effects on growth performance, carcass characteristics, and the fat and meat fatty acid profile of rabbits fed diets with chia (*Salvia hispanica* L.) seed supplements. *Meat Science* 80, 1116–1121.
Peiretti, P., Mussa. P.P., Prola, L. and Meineri, G. (2007) Use of different level of false flax (*Camelina sativa* L.) seed in diets for fattening rabbits. *Livestock Science* 107, 192–198.
Petacchi, F., Buccioni, A., Giannetti, F. and Capizzano, G. (2005) Influence of CLA supplementation on the lipid quality of rabbit meat. *Italian Journal of Animal Science* 4, 556–558.
Pla, M. and Cervera, C. (1997) Carcass and meat quality of rabbit given diets having high level of vegetable or animal fat. *Animal Science* 65, 299–303.
Pla, M., Zomeño, C. and Hernández, P. (2008) Effect of the dietary n-3 and n-6 fatty acids on rabbit carcass and meat quality. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1425–1430.
Rayman, M.P. (2004) The use of high-selenium yeast of raise selenium status: how does it measure up? *British Journal Nutrition* 92, 557–573.
Rødbotten, M., Kubberød, E., Lea, P. and Ueland, Ø. (2004) A sensory map of the meat universe. Sensory profile of meat from 15 species. *Meat Science* 68, 137–144.
Rodríguez-Calleja, J.M., Santos, J.A., Otero, A. and García-López, M.L. (2004) Microbiological quality of rabbit meat. *Journal of Food Protection* 67, 966–971.
Rodríguez-Calleja, J.M., García-López, M.L., Santos, J.A. and Otero, A. (2005) Development of the aerobic spoilage flora of chilled rabbit meat. *Meat Science* 70, 389–394.
Sartori, A., Queaque, P.I., Trocino, A. and Xiccato, G. (2003) Increasing dietary starch to ADF ratio in phase feeding programs for early weaned rabbits. *Italian Journal of Animal Science* 2, 432–434.

Schmid, A., Collomb, M., Sieber, R. and Bee, G. (2006) Conjugated linoleic acid in meat and meat products: a review. *Meat Science* 73, 29–41.

Simopoulos, A.P. (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedical Pharmacotherapy* 56, 365–379.

Soultos, N., Tzikas, Z., Christaki, E., Papageorgiou, K. and Steris, V. (2009) The effect of dietary oregano essential oil on microbial growth of rabbit carcasses during refrigerated storage. *Meat Science* 81, 474–478.

Tres, A., Bou, R., Codony, R. and Guardiola, F. (2008) Influence of dietary doses of n-3- or n-6-rich vegetable fats and α-tocopheryl acetate supplementation on raw and cooked rabbit meat composition and oxidative stability. *Journal of Agricultural and Food Chemistry* 56, 7243–7253.

Tůmová, E., Zita, L. and Štolc, L. (2006) Carcass quality in restricted and *ad libitum* fed rabbits. *Czech Journal Animal Science* 51, 214–219.

Valsta, L.M., Tapanainen, H. and Männistö, S. (2005) Meat fats in nutrition. *Meat Science* 70, 525–530.

Vannini, L., Iucci, L. and Guerzonni, M.E. (2002) Risk assessment in rabbit meat products through the slaughtering/processing/storage phases. In: *Proceedings of 2nd meeting of the Working group 5 'Meat quality and safety'*. COST, Athens, Greece, pp. 5.

Vannini, L., Sado, S., Iucci, L., Ndagijimana, M. and Guerzonni, M.E. (2003) The dietary use of linseed in growing rabbits: effects on microbial population and spoilage patterns of meat products. In: *Proceedings of the 3rd meeting of the Working group 4 'Nutrition and pathology' and 5 'Meat quality and safety'*. COST, Prague, Czech Republic, pp. 32.

Villena, P., García-Rebollar, P., Rebollar, P.G., Núñez, N., Nicodemus, N., Margüenda, I. and Carabaño, R. (2008) Effect of a high fibrous diet in the finishing period on carcass yield and meat quality of rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1461–1466.

Whigham, L.D., Watras, A.C. and Schoeller, D.A. (2007) Efficacy of conjugated linoleic acid for reducing fat mass: a meta-analysis in humans. *American Journal of Clinical Nutrition* 85, 1203–1211.

Williams, P. (2007). Nutritional composition of red meat. *Nutrition & Dietetics* 64, S113–S119.

Xiccato, G. (1999) Feeding and meat quality in rabbits: a review. *World Rabbit Science* 7, 75–86.

Xiccato, G., Trocino, A., Sartori, A. and Queaque, P.I. (2002) Effect of dietary starch level and source on performance, caecal fermentation and meat quality in growing rabbits. *World Rabbit Science* 10, 147–156.

10 Nutrition and Feeding Strategy: Interactions with Pathology

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10.1 Introduction

Nutrition and feeding strategies play a key role in rabbit breeding, not only to optimize production itself (e.g. meat, milk, fur), but also to prevent various pathologies through: (i) the presence of toxic compounds in the feeds or utilization of unbalanced diets; and (ii) the presence of pathogenic agents (viruses, bacteria, parasites) in feeds or drinking water. This last aspect is not considered in this chapter since it does not cover nutrition directly, but is rather a question of feeding management and hygiene. Similarly, the presence of undesirable pesticides in feed ingredients can impair rabbit health. Very few specific data are available for rabbits in production conditions, and so this aspect is also not considered here; readers can consult more specialized books devoted to this aspect of animal feeding.

In this chapter, it is assumed that some effort has been made to provide the daily minimum requirements for the main individual components such as energy, protein and amino acids, minerals and vitamins, as recommended in other chapters. Nevertheless it is generally difficult to provide all nutrients and energy exactly at the optimum level and, as a consequence of the composition of available raw materials, it is necessary to accept an excess or imbalance in some components to ensure that the minimum of other nutrients is met.

By itself, an imbalance should only be responsible for low performance, not for health troubles if the breeding conditions are good. For example, in controlled experimental conditions, a diet containing only 60 g fibre kg^{-1} dry matter (DM, as acid detergent fibre, ADF) does not induce digestive trouble (Davidson and Spreadbury, 1975). A similar situation has been observed with diets containing up to 280–300 g crude protein (CP) kg^{-1} (Lebas, 1973). Such imbalances only induce a higher susceptibility of rabbits to problems, mainly digestive disorders, and the above extreme levels must never be recommended for practical feeding. One of the objectives of this chapter is to indicate the rules, when known, that are able to minimize the risk of disorders and some give ideas on 'acceptable' imbalances in everyday feeding practice.

In addition to imbalance problems, absolute excess of ingredients such as some minerals (e.g. phosphorus) or vitamins (e.g. vitamin D), can be toxic independently of the health status of rabbits. The only question is – when does a nutrient supplied above the recommended minimum or optimum become toxic?

The present chapter therefore considers health troubles (mainly digestive) linked to the balance of dietary components and the presence of nutrients in excess, mainly in relation to the initial composition of feed ingredients. The first part will consider feeding strategies, particularly the control of feed intake for the young rabbit to reduce post-weaning digestive troubles. The second part will cover the health consequences of non-nutritional components that are frequently associated with feed ingredients, such as mycotoxins. And the final part will be devoted to water quality, since water is also able to induce nutritional disorders when certain soluble components are too concentrated.

10.2 Methods to Estimate Health Status and Measure the Risk of Digestive Troubles

A common indicator used to evaluate the impact of a disease in breeding is the mortality rate. More recently, a morbidity indicator has been developed for the growing rabbit to more precisely assess the incidence of clinical symptoms (Gidenne, 1995), and it may be combined with mortality to obtain the health risk index (HRi = morbidity + mortality rate). This approach allows a more precise assessment of health status. However, these traits show large variations according to many factors. For instance, the mortality rate of rabbits fed the same diet may range from 0% up to 70% according to various factors, such as litter effect, preventive medication, age at weaning and the sanitary and immune status of the animals. This means that a large number of animals is required to detect a significant difference in mortality between two treatments. For instance, to detect a difference between two mortality rates of 5%, >300 animals are required in each group (Table 10.1).

When the clinical symptoms (e.g. diarrhoea, caecal impaction, borborygmus), are clear, the morbidity rate is relatively easy to measure. However, when only a reduction in growth rate is detectable, a threshold must be defined to class the animal as morbid or not, such as the average −2 × standard deviation (SD, signifying the 2.5% of the animals with a lower growth rate) or up to 3 SDs. However, a large set of rabbits within a group is required to precisely define the mean and its range of variation. Moreover, adequate statistical methods are necessary to treat discrete data (such as mortality or morbidity). For instance, when analysing models with more than one factor or including more than two levels (within a factor) or to test interaction among two factors, a specific categorical analysis based on a weighted least-square analysis must be used instead of a simple chi-squared test.

Table 10.1. Number of rabbits required per treatment to detect a significant difference (P = 0.05) in the mortality rate between two treatments.

Difference to be detected (%)	Number of rabbits required
5	338
10	87
15	40
20	23

10.3 Problems Related to Major Nutrient Imbalances

Among the various health problems related to feeding, intestinal pathology and respiratory diseases are the predominant causes of morbidity and mortality in commercial rabbit husbandry. The first mainly occurs in young rabbits, after weaning (4–10 weeks of age), while the second preferentially affects adults. In France, enteritis in growing rabbits induced a mortality rate of 11–12% before the appearance of epizootic rabbit enteropathy (ERE) (Koehl, 1997). However, with general production cycles, mortality is currently around 8.5% (Lebas, 2008), but with frequent use of preventive antibiotherapy. Nevertheless, it may frequently exceed 15% and even reach up to 50%. Moreover, digestive disorders are responsible for important morbidity characterized by growth depression and poor feed conversion. These economic losses, less obvious than mortality, are often underestimated by rabbit breeders.

Diagnosis of intestinal diseases is difficult because, whatever the cause (nutritional problems or a true specific illness), symptoms and lesions are generally similar. The difficulty in recognizing the aetiology for rabbit intestinal disorders is reinforced by the fact that, as for most diseases in humans or animals, several factors are involved in the development of enteritis and must be considered. The first is the status of the animal itself (age, genetics, immunity). The second concerns the pathogenic agents involved (parasites, bacteria, viruses). The third is represented by environmental factors, including nutritional factors and breeding conditions such as hygiene, stress and so on. Although many factors are able to provoke enteritis, the main and constant clinical sign observed is the diarrhoea that occurs in about 0.90 of enteritis cases (Licois *et al.,* 1992). This may be related to the characteristics of the rabbit intestinal tract and its complex physiology.

The composition of caecal contents as well as caecal function and caecal bacterial community and activity (see Chapter 1) are significantly affected in cases of enteritis (Figs 10.1 and 10.2). The motility of the caecum is stimulated whereas that of the ileum and jejunum is inhibited in experimentally induced diarrhoea with *Coccidia* (Fioramonti *et al.*, 1981). Furthermore, Hodgson (1974) observed increased motility of the proximal colon, which appeared contracted and thickened, in rabbits fed a low-fibre diet, and a higher retention of digesta in the total tract that should be related to lower feed intake. This probably reflects a higher antiperistaltic activity of the proximal colon (see Chapter 1) induced by the high proportion of fine particles in a low-fibre diet. It is thus difficult to postulate that rabbit diarrhoea is characterized by hypomotility of the caeco-colic segment. In parallel, caecal fermentative activity is upset (Fig. 10.2): for a 6-week-old rabbit, the caecal volatile fatty acid (VFA) concentration falls to <50 mM, butyrate is particularly affected (leading to a C3:C4 ratio in the range of 1.5–8 instead of 0.5–0.8) and larger inter-individual variations in the fermentation pattern are observed. Higher pH (+0.5) and ammonia levels may also be observed. The composition of the caeco-colic microbiota might also be affected, but the few results available are inconsistent, with some showing a decrease and others an increase in *Escherichia coli* and/or clostridia.

10.3.1 Fibre and starch intake

Fibre intake should be expressed in terms of quantity or quality (type) of cell wall constituents (see definition in Chapter 5). Similarly, the effect of starch intake may

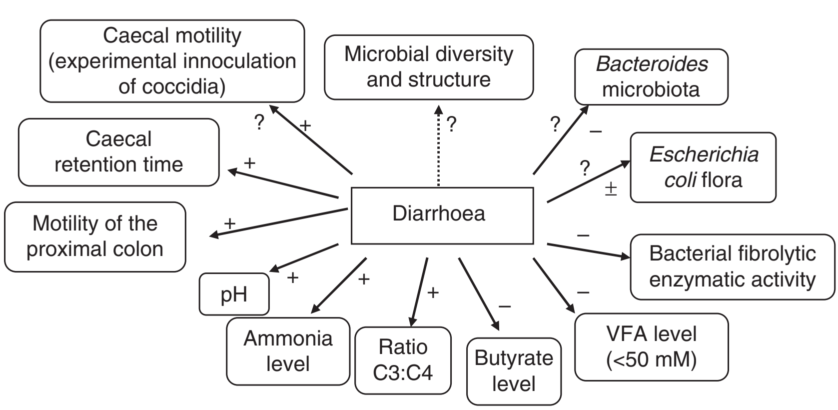

Fig. 10.1. Changes in the caecocolic ecosystem occurring in cases of digestive troubles (diarrhoea) in the growing rabbit.?, further studies recommended; ±, inconsistent results; VFA, volatile fatty acid.

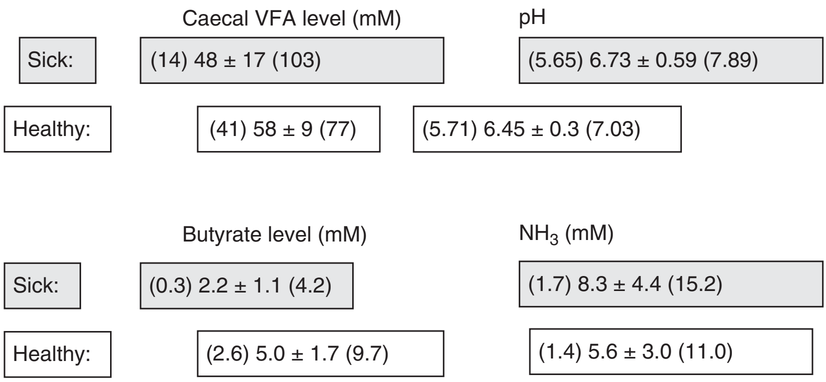

Fig. 10.2. The *in vivo* caecal fermentation pattern (mean ± standard deviation) of healthy[a] and sick[b] growing rabbits. Figures in parentheses are the minimum and maximum values observed from a set of 80 and 21 rabbits, respectively, for healthy and sick animals (data from Bellier, 1994). [a]Cannulated rabbits, 7–11 weeks old. [b]Rabbits having acute digestive troubles or abnormally low intake.

differ according to the origin of the starch (see definition in Chapter 2).

Consequences of a reduction in fibre intake

An increased dietary starch to fibre ratio (associated with <300 g neutral detergent fibre (NDF), <150 g ADF and >200 g starch kg^{-1}), without major changes in the proportions of the cell wall constituents (e.g. hemicelluloses, lignins), could lead both to a lower ileal flow of DM and bacterial biomass production in the caecum of the young rabbit (Figs 10.1 and 10.2). In healthy growing animals, when the fibre intake is too low (<8–11 g ADF kg^{-1} live weight day^{-1}), the caecal fibre level decreases while the starch concentration remains low (around 15–40 g kg^{-1}), and there are no consistent changes in the concentration of the fermentation end products (ammonia, VFA) and caecal pH (Fig. 10.3). Some authors have described lower fermentative activity (Bellier and Gidenne, 1996; Gidenne *et al.*, 2000, 2002, 2004a; Nicodemus *et al.*, 2003a, 2004), but most have not. However, the VFA molar proportion is affected by the fibre level, since the proportion of butyrate generally rises significantly when the fibre to starch ratio decreases.

It remains difficult to explain how these changes in the caecal ecosystem determine the greater incidence of digestive troubles (mainly diarrhoea, but also caecal impaction, mucus excretion and low feed intake) observed with low-fibre diets. It is probable that the microbial community is largely affected; for example, the caecal archaea has been seen to be double with a standard diet compared to a fibre-deficient diet (Bennegadi *et al.*, 2003). Furthermore, when dietary NDF is reduced from 300 to 250 g kg^{-1} microbiota biodiversity increases in the ileum but is reduced in the caecum (Nicodemus *et al.*, 2004). Moreover, the favourable effect of a high fibre intake on rabbit digestive health has been shown using an experimental infection model reproducing colibacillosis (Gidenne and Licois, 2005) or ERE (Gidenne *et al.*, 2001b).

Several hypotheses have been suggested to explain how the dietary supply of starch and fibre affects digestive physiology, but none has been completely validated by experimental results. Prohaszka (1980) put forward the antibacterial effect of caecal VFA originating from fibre fermentation, particularly in the case of *in vitro E. coli* assays. However, numerous studies have not observed a close relationship between the concentration of caecal VFA and pH or between *E. coli* flora and caecal pH. In addition, Padilha *et al.* (1995) showed that, between 29 and 49 days of age, caecal pH decreases while *E. coli* flora remains steady.

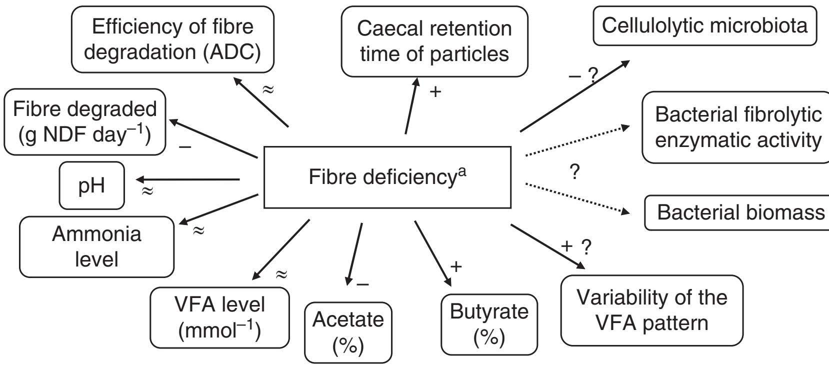

Fig. 10.3. Effect of fibre deficiency[a] on several parameters of the caecal ecosystem in the growing healthy rabbit. [a]Lower than 8–11 g acid detergent fibre (ADF) kg^{-1} live weight day^{-1}, compared with the required >15 g ADF kg^{-1} day^{-1} for a diet balanced in fibre quantity. ?, Further studies needed; ≈, not a significant effect. ADC, apparent digestibility coefficient.

The favourable effect on health of a high level of low-digestible fibre (lignocellulose or ADF) could correspond to control of the rate of passage of digesta, particularly in the caeco-colic segment. Moreover, most results indicate that all of the factors contributing to an increase in retention time (lowering the fibre level, reducing the particle size of the feed, feed restriction) contribute to destabilizing the caecal microbial activity and favour enteritis. It could be speculated that a low caecal turnover of digesta leads to an insufficient supply of substrates available for the fibrolytic flora (Fig. 10.4).

Many experiments have been performed to evaluate the respective effects of fibre and starch on the incidence of diarrhoea in the growing rabbit, particularly just after weaning (Colin *et al.*, 1976; de Blas *et al.*, 1986; Blas *et al.*, 1994; Bennegadi *et al.*, 2001). This period is critical because it is associated with a large incidence of digestive problems, and also because overall digestive physiology actively matures and feed intake rapidly increases. Experiments that have dealt with this question compared diets with varying levels of fibre and simultaneously an inverse variation in the level of starch (since rabbits

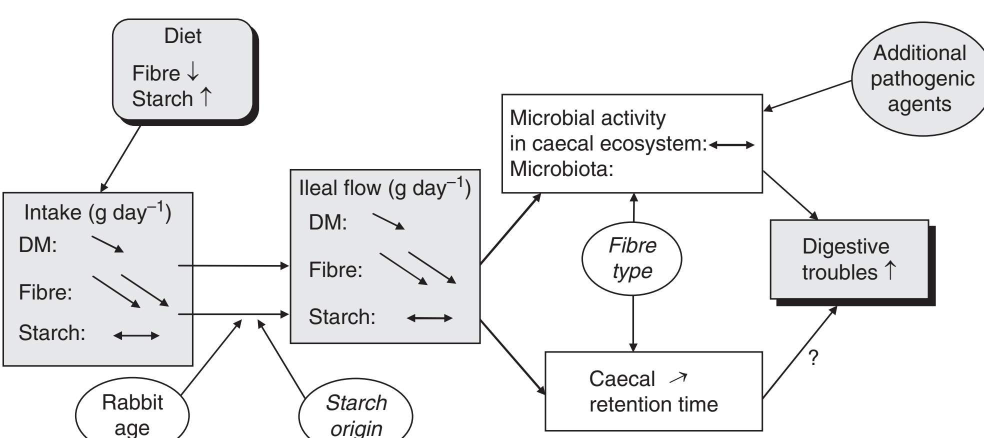

Fig. 10.4. Relationships between feeding the growing rabbit with low-fibre, high-starch diets and the incidence of digestive troubles.

are fed with complete feeds). Consequently, when a study has reported a positive effect of increased dietary fibre intake on digestive health, it has been difficult to exclude the possibility of an effect of reduced starch intake. Thus, two opposing hypotheses can be constructed: are digestive troubles linked to carbohydrate overload in the caecum or to fibre deficiency (or both)? This question has been approached by studying the ileal flow of starch and fibre in the growing rabbit (5–9 weeks old). With high-starch diets (≥300 g starch kg^{-1}, mainly from wheat), ileal starch digestibility was very high (>0.97) and the flow of starch remained <2 g day^{-1} (intake around 30 g day^{-1}) at the ileum, while that of fibre was at least ten times higher (around 20 g NDF day^{-1}) (Gidenne *et al.*, 2000; García *et al.*, 2004; Nicodemus *et al.*, 2004). An overload of starch therefore appears very unlikely, since starch digestion is very efficient already at 5 weeks old. Moreover, a large-scale study using a network of six experimental breeding units (GEC French group) demonstrated through a 2×2 factorial design (two levels of starch: 120 versus 190 g kg^{-1}, combined with two ADF levels: 150 versus 190 g kg^{-1}) that only the fibre level played a role in the occurrence of digestive problems, and not the starch level (Gidenne *et al.*, 2004b).

Furthermore, by comparing iso-fibre diets, but with several starch sources (maize, wheat, barley) varying in their intestinal digestion, Gidenne *et al.* (2005a,b) observed no effect of starch ileal flow on the incidence of diarrhoea in the weaned rabbit. These results support the minor influence of starch on the health status of the animal when fibre requirements are covered. The positive effect of enzyme supplementation (a mixture of β-glucanases, β-xylanases, α-amylases and pectinases) on mortality (Gutiérrez *et al.*, 2002b; Cachaldora *et al.*, 2004) might thus be related to the partial hydrolysis of non-starch polysaccharides that produce complex oligomers, which may modulate the gut microbiota and lead to better digestive health. Moreover, since starch digestion is incomplete in the young rabbit, replacement of some starch by lactose has been studied, as occurs in piglets' diets. However, lactose ileal digestibility was much lower than that recorded for starch (0.74 versus 0.92), which might be due to the severe reduction in lactase activity after weaning. This result led to a higher ileal flux of lactose and higher mortality (Gutiérrez *et al.*, 2002a), possibly explained by a microbiota imbalance in the caecum.

Fibre intake thus plays a major role in the development of digestive problems in the classically weaned rabbit (28–35 days old). With rabbits weaned earlier (at 25 days of age), Gutiérrez *et al.* (2002a) observed that mortality remained low and similar with diets having 360 versus 300 g NDF kg^{-1}, but that the mortality rate tended to increase (P = 0.06) after a change of diet at 39 days of age, from experimental to commercial, for those previously fed with a diet containing 360 g NDF kg^{-1}.

Accordingly, several large-scale studies have aimed to clearly validate the relationship between dietary fibre and starch levels and diarrhoea incidence for the classically weaned rabbit, using an experimental design with a high number of animals per treatment. The relationship between low-fibre diets (<140 g ADF kg^{-1}) and a higher incidence of diarrhoea was clearly established in two studies where the quality of the fibre (e.g. the proportions of fibre fraction as analysed through the Van Soest procedure) was estimated (Blas *et al.*, 1994; Bennegadi *et al.*, 2001). In France, the GEC group has performed several large-scale studies (using at least 300 animals per treatment and five sites) to establish fibre recommendations for the prevention of digestive problems in the growing rabbit (weaned). The relevance of the Van Soest criteria was studied, since the crude fibre method was too imprecise for this purpose. A review of these studies and of new fibre recommendations has been published (Gidenne, 2003). A summary of the fibre requirements for post-weaned and growing rabbits from French (INRA) and Spanish (Technical University of Madrid) research groups is presented in Table 10.2.

The favourable effect of dietary fibre has also been analysed in the young rabbit during the weaning period (3–5 weeks old) in a large-scale study (six sites and three repro-

Table 10.2. Fibre and starch requirements (g kg^{-1})[a] for the young weaned rabbit to prevent digestive troubles.

	INRA		Technical University of Madrid	
	Post weaning (28–42 days old)	Growing (42–70 days old)	Post weaning (25–39 days old)	Growing (39–70 days old)
Neutral detergent fibre (NDF)	≥310	≥270	300≤ NDF <360	320≤ NDF <350
Lignocellulose (ADF)	≥190	≥170	–	160≤ ADF <185
Lignin (ADL)	≥55	≥50	–	≥55
Cellulose (ADF – ADL)	≥130	≥110	–	–
Ratio lignins/cellulose	>0.40	>0.40	–	–
Hemicelluloses (NDF – ADF)	>120	>100	–	–
DgF[b]/ADF	≤1.3	≤1.3	–	–
Neutral detergent soluble fibre[c]	–	–	120	–
Particles >0.3 mm	–	–	–	>210
Starch	–	–	<200	145< starch <175

ADF, acid detergent fibre; ADL, acid detergent lignin; DgF, digestible fibre; NDF, neutral detergent fibre.
[a]As fed basis, corrected to a dry matter content of 900 g kg^{-1}.
[b]Hemicelluloses (NDF – ADF) + water-insoluble pectins.
[c]According to Hall *et al.* (1997).

ductive cycles) by Fortun-Lamothe *et al.* (2005). A lower mortality rate was reported for litters fed on a diet rich in fibre or when fibre and lipid replaced starch. However, in the suckling rabbit (or <5 weeks old) it can be speculated that feed intake regulation is not completely established and neither is pancreatic enzymatic activity (see Chapter 1). The combination of these two factors would lead to a high flow of starch into the caecum (Gidenne *et al.*, 2005a), which may then favour digestive disturbances.

The substitution of starch for fibre has also been studied for rabbit doe diets, using five iso-energetic diets (10.6 MJ digestible energy (DE) kg^{-1}) with increasing levels of NDF (from 278 to 371 g kg^{-1}) and fat (from 20 to 51 g kg^{-1}) at the expense of starch (decreasing from 237 to 117 g kg^{-1}) (de Blas *et al.*, 1995). Some impairment in the performances of does was observed in those fed the highest levels of fibre. This might be explained by higher fermentation losses in the caecum, together with an insufficient uptake of glucose from the gut to meet the requirements for pregnancy and milk lactose synthesis. Conversely, negative effects of high dietary starch concentrations were also mentioned and were related to an increase in diarrhoea mortality for the does.

Effect of the type of cell wall constituents

Apart from the important role of fibre intake, the quality of fibre (see Chapter 5) also interferes with diarrhoea incidence in the growing rabbit (30–70 days old). The favourable effect of lignocellulose on digestive disorders and mortality in fattening rabbits has been established in several studies. For example, the HRi has been found to decrease from 28% to 18% when the dietary ADF content increases from 150 to 190 g kg^{-1} (Gidenne *et al.*, 2004b). But, within lignocellulosic components, the favourable effect of the lignin (acid detergent lignin, ADL) has also been demonstrated, and a strong negative relationship was found with the HRi (Fig. 10.5) (Perez *et al.*, 1994, 1996; Nicodemus *et al.*, 1999; Gidenne *et al.*, 2001a). As discussed previously, these studies confirm that the effects of lignin and cellulose are confounded with NDF level. Increasing the cellulose fraction (ADF – ADL) also favours digestive health (Perez *et al.*, 1996). However, lignin plays a specific role since an increase in the ratio of lignin to cellulose is associated with a lower HRi (Gidenne *et al.*, 2001a). In summary, the lignin requirement (ADL) for the growing rabbit can be assumed to be 5–7 g day^{-1} and the cellulose requirement 11–12 g day^{-1}. Moreover,

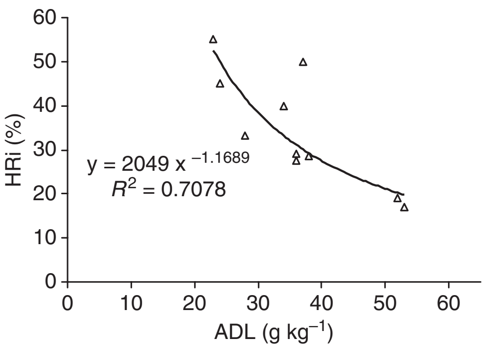

Fig. 10.5. Increasing the dietary lignin concentration reduces the risk of digestive troubles (health risk index, HRi[a]) in the growing rabbit. ADL, acid detergent lignin (Van-Soest sequential procedure; EGRAN, 2001). [a]HRi from digestive trouble = mortality + morbidity rate by diarrhoea, measured from 28 to 70 days of age, on at least 40 rabbits per diet (data for ten diets ranging from 14% to 20% acid detergent fibre level; Gidenne, 2003).

Nicodemus *et al.* (2006) reported better performance (e.g. milk production) for does fed a high-lignin diet (59 g ADL kg^{-1}). To date, however, no accurate and quick analytical method for determining lignin levels is available. Consequently, estimating the amount of lignin in a raw material remains difficult, particularly in tannin-rich ingredients (e.g. grape marc), and caution must be taken in establishing requirements.

Although the digestive health of the classically weaned rabbit depends on the level and quality of lignocellulose, it also varies greatly for the same ADF level (Fig. 10.6) because the level of more digestible fibre (DgF) fractions (i.e. hemicelluloses (NDF – ADF) + water-insoluble pectins) could also vary independently of lignin and cellulose levels. Thus, a dietary recommendation for lignocellulose alone appears to be insufficient to prevent digestive disturbances in the rabbit. For instance the ratio of DgF to ADF ranges from 0.9 to 1.7 in Fig. 10.6. The DgF fraction may play a key role in digestive efficiency and digestive health, since it is more rapidly fermented compared to ADF and compatible with the retention time of the caeco-colic segment (9–13 h, see Chapter 1). Without changes in ADF dietary levels, the frequency of digestive problems decrease when DgF replaces starch (Perez *et al.*, 2000) or protein (Gidenne *et al.*, 2001b). This could originate from the favourable effect of DgF (compared to starch or protein) on caecal fermentative activity (Jehl and Gidenne, 1996; García *et al.*, 2002) and possibly from its moderate effect on rate of passage (Gidenne *et al.*, 2004b). However, too high an incorporation of DgF with respect to lignin and cellulose should be avoided to minimize the HRi (morbidity + mortality) during fattening. It is thus recommended that the ratio DgF to

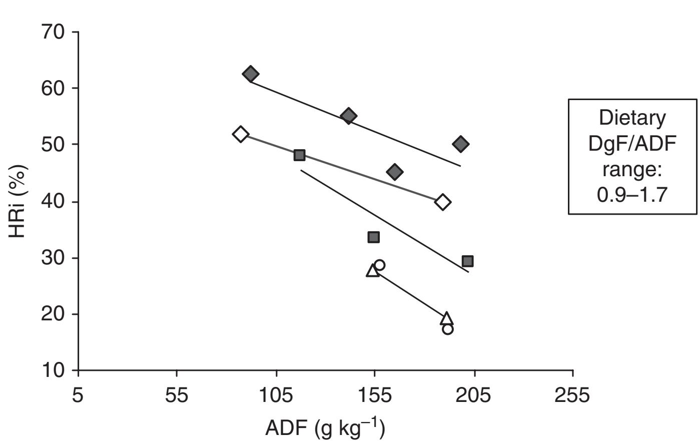

Fig. 10.6. The risk of digestive problems (health risk index, HRi)[a] in the growing rabbit is jointly dependent of low-digested acid detergent fibre (ADF)[b] and digestible fibre (DgF)[c]. [a]HRi from digestive trouble = mortality + morbidity rate by diarrhoea, measured from 28 to 70 days of age, on at least 40 rabbits per diet (one point = one diet, n = 13; Gidenne, 2003). [b]Lignocellulose (Van-Soest sequential procedure; EGRAN, 2001). [c]Water-insoluble pectins plus hemicelluloses (NDF – ADF).

ADF remain <1.3 (when the dietary ADF level is >150 g kg^{-1}, see Table 10.2).

Another way to analyse the role of cell wall polysaccharides that are rapidly fermented is to determine the neutral detergent soluble fibre (NDSF) residue (Hall *et al.*, 1997). This corresponds to the cell wall polysaccharides soluble in neutral detergent solution (= sum of water-soluble and -insoluble pectins + β-glucans + fructans + oligosaccharides (degree of polymerization >15)). Although the NDSF level is moderate in rabbit feeds, a reduction in its level (from 120 to 80 g kg^{-1}) may be detrimental to the digestive health of the early-weaned rabbit. Conversely, a higher level of NDSF improves mucosal morphology, functionality and immune response. Moreover, NDSF reduces the proportion of animals with *Clostridium perfringens* in the caecum and other pathogens such as *Campylobacter* both in the ileum and caecum. Accordingly, mortality due to ERE is reduced with a diet containing 120 g soluble fibre kg^{-1} (Table 10.3) (Gómez-Conde *et al.*, 2007, 2009).

The quality of dietary fibre might also be improved by determining the particle size distribution. It is acknowledged that the particle size distribution of a feed can affect digesta motility and, more importantly, the caeco-colic rate of passage. Fibrous raw materials with a small proportion of large particles (>0.3 mm) due to grinding (screen size 0.5–1 mm) or previous processing are retained for longer (Laplace and Lebas, 1977; Gidenne *et al.*, 1991; García *et al.*, 1999), but are not associated with a negative effect on the digestive health status (Lebas *et al.*, 1986; Gidenne *et al.*, 1991; Nicodemus *et al.*, 2006). Only a very low content of large particles (<0.21 particles of <0.3 mm) would have a negative impact on performance. Nevertheless, a content of coarse particles <0.25 is unusual in practice; in a series of 77 commercial French feeds, the average proportion of coarse particles was 0.388 (minimum 0.227, mean −2 SDs 0.27; Lebas and Lamboley, 1999).

In conclusion, one criterion is not sufficient for fibre recommendation, because the risk of digestive problems in the growing rabbit is jointly dependent on low-digested ADF and the DgF fraction. As for other dietary fibre components, there is a minimum below which a 'fibre deficiency' may occur (see Table 10.2). In summary, it is important to establish the precise role of fibre in the young rabbit, and particularly the effects of the NDSF fraction.

10.3.2 Protein level and quality

Protein requirements are high in the young animal (see Chapters 12 and 3), not only for

Table 10.3. Effect of the dietary neutral detergent soluble fibre (NDSF) level on mucosal morphology and functionality, immune response, frequency of detection of *Clostridium perfringens* at 35 days and mortality of 25-day weaned rabbits during fattening (Gómez-Conde *et al.*, 2007, 2009).

	Dietary NDSF[a] level (g kg^{-1} as fed)			
	120	90	70	*P*
Jejunum morphology and functionality (35 days)				
Villi length (μm)	722[c]	567[d]	493[e]	0.001
Crypt depth (μm)	89[c]	115[d]	113[d]	0.001
Sucrase specific activity (μmol glucose g^{-1} protein)	8671[c]	6495[d]	5202[e]	0.019
Immune response in lamina propria (35 days)				
CD4$^+$ (%)	35.1	33.9	26.2	NS
CD8$^+$ (%)	21.3	26.9	30.3	0.074
Frequency of detection *C. perfringens* (%)[b]				
Ileum	0	22.2	9.1	0.062
Caecum	5.7[c]	2.9[c]	17.6[d]	0.047
Mortality, 25–60 days (%)	5.3[c]	8.5[c,d]	14.4[d]	0.016

NS, not significant.
[a]According to Hall *et al.* (1997).
[b]Terminal restriction fragment length polymorphism approach.
[c,d,e]Means having the same superscript letter are not different (P<0.05).

body growth but also for intestinal mucosa development and renewal. Conversely, an excessive protein supply does not affect growth itself, but will promote the incidence of diarrhoea. For instance, de Blas *et al.* (1981) observed increased mortality during the fattening period with high-protein diets. A level of 1.8–1.9 g CP MJ^{-1} DE seems optimum; even if with an increase of up to 2.6 g, Kjaer and Jensen (1997) observed only a slight non-significant increase in mortality. Similarly, Catala and Bonnafous (1979) showed that a higher ileal flow of protein (obtained through reduced protein digestion by a ligature of the pancreatic duct) leads to increased microbial proliferation in the hindgut. An excess of dietary protein could also favour the proliferation of *clostridia* in the rabbit and slightly increase the prevalence of *E. coli* (Haffar *et al.*, 1988; Cortez *et al.*, 1992), and thus could lead to an increase of enteritic mortality. For instance, in a large-scale study, Gidenne *et al.* (2001b) showed that the replacement of protein by digestible fibre reduced the health risk for diarrhoea (Fig. 10.7). A hypothesis to explain this is a higher availability of substrates for microbial growth, with an increased prevalence of pathogenic species, when animals are fed with high-protein diets. A higher ileal flow of protein is also associated with a lower caecal pH in the young rabbit (Gutiérrez *et al.*, 2003; Nicodemus *et al.*, 2003b, 2004), which may affect the commensal microbiota.

Weaning implies a change from milk to vegetable proteins. The digestion of the latter is worse, and raw materials occasionally contain antinutritive factors such lectins, antitrypsin or antigenic compounds. This may impair apparent ileal digestion or induce changes in the morphology of the intestinal mucosa, as occurs in other species. In rabbits, Scheele and Bolder (1987) observed an increase in mortality before weaning (35 days old) in rabbits fed diets containing a high proportion of soybean meal (200 g kg^{-1}) in comparison with two diets based on animal protein (310 versus 100 g kg^{-1}, respectively). Gutiérrez *et al.* (2000) observed that the substitution of soybean meal with animal plasma had a positive effect on the morphology of intestinal mucosa, feed intake, growth and mortality. In another study, Gutiérrez *et al.* (2003) compared four protein concentrates (sunflower meal, soybean meal 48, soybean concentrate and potato protein) in iso-nutritive starter diets. Animals fed diets with the protein sources with a lower content of antinutritive factors (sunflower meal and soybean concentrate) showed higher apparent ileal protein digestibility and growth performance and a lower mortality rate than those on the other diets. However, the gastric acidity, villus morphology and faecal digestibility values were similar between diets, and

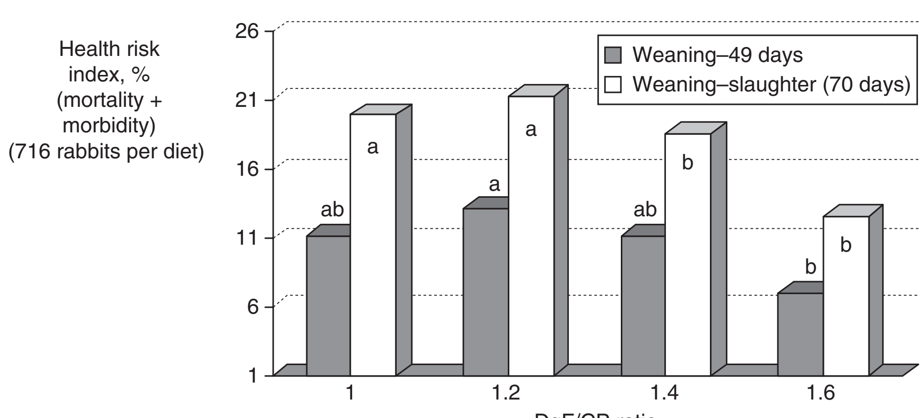

Fig. 10.7. Impact of crude protein (CP) replacement by digestible fibre (DgF), on the health risk index between weaning and slaughter (Gidenne *et al.*, 2001b). a, b: within the same period (weaning–49 days or weaning–slaughter), means having a letter in common and do not differ at the level P = 0.05.

no differences in phenotypic distribution of lymphocytes in the duodenal lamina propria (which might be related to the development of a tolerance mechanism by the animal) were detected (Mézes and Balogh, 2009). The importance of the reduction of the ileal flow of protein (by using digestible sources or reducing the protein level) in reducing the mortality rate has been supported in other experiments (García-Ruiz *et al.*, 2006; Chamorro *et al.*, 2007).

Most feed manufacturers limit the dietary protein level in fattening diets because of the increased mortality rate observed on rabbit farms when protein levels exceed by 20 g kg^{-1} or more the minimum levels recommended for the maximum growth rate. Moreover, an excessive protein supply will probably become increasingly unusual in Europe because of increased dietary cost and, most importantly, because the European animal management strategy favours a reduction in nitrogen excretion to the environment through the use of low-protein diets (Maertens, 1999).

10.3.3 Lipids

Few studies have dealt with the role of dietary lipids on the digestive health of growing rabbits, since dietary levels are usually <30 g kg^{-1} and lipids are well digested in the small intestine. Furthermore, it is difficult to separate the effect of lipids from that of DE intake. However, it has been found that some medium-chain fatty acids (MCFA), such caprylic and capric acid (in triacylglycerol form), exhibit antimicrobial activity for some bacteria of the caecal digestive microbiota (Marounek *et al.*, 2002). Moreover maternal milk, rich in MCFA, protects the young rabbit against colibacillosis (Gallois *et al.*, 2007) and the addition of MCFA to the feed has a favourable impact on the digestive health of the growing rabbit (Skrivanova and Marounek, 2006). However, contrasting results are obtained when rabbits are experimentally infected with pathogenic *E. coli* (Gallois *et al.*, 2008; Skrivanova *et al.*, 2008).

Some fatty acids, such omega (n)-3, have been implicated in the development of an immune response. Fortun-Lamothe and Boullier (2007) and Maertens *et al.* (2005) reported a higher post-weaning viability for young rabbits fed a diet with a low n-6 to n-3 ratio (1.0 versus 4.4). Moreover, the addition of fat to starter diets increases the energy intake of kits and contributes to the maintenance of good body condition. Therefore, this favours harmonious digestive maturation and immune system development, thus reducing weaning risk and improving resistance to digestive problems.

Furthermore, the incorporation of fat in the diets of breeding does may be of interest in terms of increasing their DE intake. However, contradictory results have been obtained indicating either a higher (Lebas and Fortun-Lamothe, 1996) or lower kit mortality (Fraga *et al.,* 1989; Fernandez-Carmona *et al.*, 1996). Despite this, neither the average weight of breeding does nor their fertility or prolificacy were significantly affected by dietary fat incorporation (Fortun-Lamothe, 2006).

10.3.4 Feed intake strategy and digestive pathology of the growing rabbit

Studies on feed intake regulation usually aim to analyse the effects on the carcass quality of the growing rabbit or to analyse digestive efficiency. More recently, however, some studies have dealt with the relationship between intake level and the incidence of digestive problems, including a study with an experimental ERE infection. The effect of a quantitative linear reduction of feed intake level (*ad libitum* to 0.6 of *ad libitum)* on the digestive health and growth of the rabbit was measured in a large-scale study (six experimental units, 2000 rabbits per treatment; Gidenne *et al.*, 2009a). During feed restriction, the mortality and morbidity rates were significantly reduced (from 12% to 3.5% and from 12% to 6% for *ad libitum* + 0.9 *ad libitum* feeding level versus 0.7 + 0.6 *ad libitum*). Feed restriction for 20 days after weaning proportionally reduced the growth rate. Thereafter, returning to *ad libitum* feed intake led to compensatory growth and better feed efficiency. Over the whole fattening period, the live weight loss of the more restricted rabbits (0.6 *ad libitum*) was 7.7%, compared to the control rabbits fed *ad libitum* from weaning. The favourable effect of limiting intake on the digestive health of the young rabbit has been confirmed by another large-scale study (Gidenne *et al.*, 2009b), where there was no major effect of the mode of feed distribution (one or two times a day). Moreover, Boisot *et al.* (2003) also demonstrated a similar positive effect of feed restriction when rabbits were challenged with ERE inoculum. Physiological mechanisms explaining such a favourable effect of reducing feed intake on diarrhoea incidence have yet to be elucidated.

Similar results have been obtained by reducing the intake level through a time restriction for water consumption (Boisot *et al.*, 2004; Verdelhan *et al.*, 2004). Consequently, strategies for controlling the intake of the young after weaning are now widespread in French professional breeders, in parallel with the development of automatic feeding equipment.

10.4 Problems Associated with Dietary Compounds Present at Toxic Levels

10.4.1 Minerals and vitamins

Although recommendations for optimum and maximum levels of mineral and vitamins are described in detail in Chapter 7, it is important for this chapter to consider maximum acceptable levels in diets. In effect it is important that, during diet formulation, there is control over nutrient levels such that, even if analysis is not available, they are well below toxic levels.

The main available information is summarized in Table 10.4. The values are those from Lebas *et al.* (1996), amended according to the most recent data obtained mainly during the last World Rabbit Congresses: Bernardini *et al.* (1996) and Virag *et al.* (2008) for vitamin E in the growing rabbit; Abdel-Khalek *et al.* (2008) for vitamins E and C in breeding does; Abd El-Rahim *et al.* (1996) for iron; and Guimarães and Motta (2000) and Ayyat and Marai (2000) for zinc.

It must be pointed out that the maximum acceptable level is in general far higher than the recommended level, but with some noticeable exceptions such as potassium, phosphorus and vitamin D.

10.4.2 Mycotoxins

Mycotoxins are metabolites produced by certain fungi in the field on standing crops or during the harvesting of feedstuffs. Mould growth can also occur on stored grains or other raw materials because of non-hygienic storage conditions. These toxic substances may be contained within the spore or secreted into the substrate on which the fungi are growing. Most of these substances have a high degree of animal toxicity. Feeding rabbits on naturally moulded diets (mixed toxin contamination) is responsible for many problems such as decreased feed intake, functional alteration of the liver and genital tract and changes in blood constituents (Abdelhamid, 1990). Mycotoxicoses appear in chronic and acute forms. The acute form is caused by the rapid ingestion of large amounts of toxins over a short period. For more details, see the review of Mézes (2008).

Aflatoxins are naturally occurring toxins produced in grains and other feedstuffs both before and after harvest by toxigenic strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 (AFB1) is of primary concern because it is the most abundant and the most toxic. Acute or chronic aflatoxicosis may occur depending on the dietary concentration of toxins. Rabbits are extremely sensitive to aflatoxin. The acute, oral, single-dose median lethal dose is about 0.3 mg kg^{-1} body weight (Newberne and Butler, 1969), among the lowest of any animal species. Moderate to severe death losses can be encountered with diets containing even low concentrations of toxin (<100 ppb) (Krishna *et al.*, 1991). Signs of toxicity include hepatic lesions (Abdelhamid *et al.*, 2002), anorexia,

Table 10.4. Maximum levels of minerals or vitamins that can be given without problems and levels known to induce signs of toxicity in the rabbit.

	Maximum level observed without problems	Concentration with signs of toxicity	Period of life
Minerals			
Calcium (g kg^{-1})	25	40	Growth
	19	25	Reproduction
Phosphorus (g kg^{-1})	8	–	Growth
	8	10	Reproduction
Magnesium (g kg^{-1})	3.5	4.2	Growth
Sodium (g kg^{-1})	6	7	Growth
Potassium (g kg^{-1})	16	15–20	Growth
	16	20	Reproduction
Chlorine (g kg^{-1})	4.2	–	Growth
Copper (ppm)	150–200	200–300	Growth
Fluorine (ppm)	–	400	Growth
Iodine (ppm)	10,000	–	Growth
	–	100	Reproduction
Iron (ppm)	400	500	Growth
Manganese (ppm)	–	50	Growth
Selenium (ppm)	0.32	–	Growth
Zinc (ppm)	200	400	Growth
Vitamins			
Vitamin A (IU kg^{-1})	100,000	–	Growth
	40,000	75,000	Reproduction
Vitamin D (IU kg^{-1})	2,000	3,000	Reproduction
Vitamin E (mg kg^{-1})	300	–	Growth
	160	–	Reproduction
Vitamin C (g kg^{-1})	2	–	Growth
Vitamin C (mg kg^{-1})	400	–	Reproduction

weight loss and emaciation, followed by icterus in the terminal stages (Morisse *et al.*, 1981). Acute aflatoxin poisoning (AFB1 daily doses >0.04 mg kg^{-1} body weight) causes a prolonged blood-clotting time, extensive liver damage and death from liver failure (Clark *et al.*, 1980, 1982, 1986).

Zearalenone (F-2 toxin) is an oestrogenic substance that is frequently recovered from maize and other grains contaminated by *Fusarium graminearum* (Perez and Leuillet, 1986). Zearalenone causes hypertrophic development of the genital tract of the female rabbit (Pompa *et al.*, 1986; Abdelhamid *et al.*, 1992). It can also affect components of the uterine tubal fluid known to be of critical importance during the early preimplantation period (Osborn *et al.*, 1988). Zearalenone induces changes in blood serum enzyme activities. Low doses (10 μg kg^{-1}) result in significant increases in alkaline phosphatase (ALP) activity, while higher doses (100 μg kg^{-1}) lead to significant increases in the activity of aspartate aminotransferase, alanine aminotransferase, ALP, γ-glutamyl transpeptidase and lactate dehydrogenase, indicating possible liver toxicity due to chronic effects of the toxin (Čonková *et al.*, 2001). Levels of zearalenone in feed as low as 1–2 ppm can interfere with the normal reproductive activity of rabbits when fed for only a few days (1–2 weeks). This high sensitivity of rabbits to this mycotoxin could be related to the slow hepatic transformation of zearalenone mainly into α-zearalenol, a more uterotrophic metabolite (Pompa *et al.*, 1986).

Another group of toxins produced by *Fusarium* species is the trichothecenes: T-2 toxin and vomitoxin. T-2 toxin is produced by some strains of the fungus *Fusarium tricinctum*. It is relatively common in fibrous raw materials that have been harvested or stored in poor conditions. In affected rabbits, T-2 toxin causes marked feed refusal, lesions of the digestive tract and impairment of blood-clotting mechanisms (Gentry, 1982; Fekete *et al.*, 1989). Long-term (4–7 weeks) feeding of sub-lethal quantities of T-2 toxin (0.19 ppm) have been found to alter the ovarian activity of sexually mature female rabbits (Fekete and Huszenicza, 1993). Administration *per os* of 4 mg kg^{-1} body weight of T-2 toxin causes death within 24 h (Vanyi *et al.*, 1989).

Vomitoxin (4-deoxynivalenol) may be found in cereal grains. Contamination of rabbit feeds with this toxin results in feed refusal and vomiting. Adverse effects on fetal development have also been encountered in does. Khera *et al.* (1986) observed that a level of 0.00024 mg vomitoxin g^{-1} diet caused a 100% incidence of fetal resorption.

The nephrotoxins (ochratoxin and citrinin) have been also implicated in rabbit mycotoxicosis. Ochratoxin is produced by toxigenic strains of *Aspergillus ochraceus*. Galtier *et al.* (1977) examined the excretion of ochratoxin A in rabbit females after a single intravenous administration (1–4 mg kg^{-1} body weight) and demonstrated transfer of the toxin into the milk: the level in milk reached 1 ppm for the highest dose of administration. The actual toxicity for rabbits is unknown, but it can be pointed out that, in the above-mentioned experiment, lactating does accommodated a single dose of 4 mg kg^{-1} body weight.

Citrinin is found in mouldy cereals contaminated by various fungal species of *Aspergillus* and *Penicillium*. Ingestion of this toxin induces acute erosive gastritis and fluid diarrhoea, with some rabbits dying less than 24 h after oral administration of a single 100–130 mg kg^{-1} body weight dose (Hanika *et al.*, 1983). In the rabbit, citrinin also causes renal damage with tubular dysfunction and necrosis similar to that found in other animal species (Hanika *et al.*, 1984).

10.5 Water Quality and Pathology

In most texts on animal nutrition, the part devoted to water quality is very short. A common comment is that 'the water provided for animals must be drinkable' and the recommended values given are those for human consumption, without further comment.

If these values are effectively obtained at the watering point available to the animals, there is effectively no health problem linked to water quality. Nevertheless, the bacterial and chemical composition of the water destined for animal drinking does not always respect all of the recommended criteria.

In no way should water polluted with bacteria be recommended for rabbits, even if it is known that animals are more tolerant than humans. As very simple low-cost systems are available, the solution is disinfection. The classic criteria for the bacterial quality of drinkable water are presented in Table 10.5.

For minerals, removing the excess is technically possible in most cases, but the cost is very high and the constant question is: is it necessary for the health of rabbits? Different experiments have been conducted to establish the real tolerance of rabbits to mineral concentrations in drinking water, mainly in hot sub-Saharan regions or in intensive animal production areas. The results are summarized in Table 10.6. Values are given for each mineral, but it does not mean that water with all of criteria at maximum will be accepted by rabbits.

It can be pointed out that, when known, the tolerance limits of rabbits are very wide compared to the maximum 'officially' acceptable values for human consumption. One of the most significant is the tolerance of rabbits to high levels of nitrates or nitrites

Table 10.5. Recommended bacteriological status of drinkable water for human consumption.[a]

Microorganisms	Maximum count
Salmonella spp.	0 in 5,000 ml
Staphylococcus spp.	0 in 100 ml
Enteroviruses	0 in 10,000 ml
Faecal *Streptococcus* spp.	0 in 100 ml
Thermo-tolerant coliforms	0 in 100 ml
Clostridium spp.	1 in 20 ml

[a]*Official Journal of the European Communities, 1998*; Council Directive 98/83/EC of 3 November, 1998, on the quality of water intended for human consumption.

Table 10.6. Chemical composition of drinkable water for rabbits.

Physical parameter (units)	Official recommendations for human consumption[a] Recommended maximum	Maximum tolerable	Maximum experimented on rabbits without problems Value	Reference
pH	7–8.5	6.5– 9.2	3.5–9.0	Porter *et al.* (1988)
Chemical parameters (in ppm)				
Total soluble salts	500	1500	3000	Abdel-Samee and El-Masry (1992)
Sodium	100	150	900	Ayyat *et al.* (1991)
Potassium	10	12	140	Ayyat *et al.* (1991)
Phosphorus	2	5	–	
Calcium	75	200	400	Porter *et al.* (1988)
Magnesium	30	150	–	
Iron	0.2	1.0	–	
Copper	0.1	1.5	60	Abo El-Ezz *et al.* (1996)
Manganese	0.05	0.5	12	Abdel-Samee and El-Masry (1992)
Zinc	5	15	55	Abdel-Samee and El-Masry (1992)
Aluminium	0.2	–	250	Rémois and Rouillière (1998)
Antimony	0.01	–	–	
Arsenic	0.05	0.20	–	
Cadmium	0.005	0.05	–	
Chromium	0.05	1.0	–	
Cobalt	–	1.0	–	
Fluoride	1.5	2.0	–	
Lead	0.05	0.10	0.40	Habeeb *et al.* (1997)
Mercury	0.001	0.01	–	
Nickel	0.05	1.00	–	
Selenium	0.01	–	–	
Silver	0.01	–	–	
Vanadium	–	0.10	–	
Chloride (Cl)	250	600	1100	Habeeb *et al.* (1997)
Sulfate (SO_4)	200	400	1340	Rémois and Rouillière (1998)
Nitrate (NO_3)	45	50	600	Kammerer and Pinault (1998)
Nitrite (NO_2)	0.05	0.10	11	Morisse *et al.* (1989)
Ammonium (NH_4)	0.05	0.50	–	
H_2S	0.05	0.10	–	
Bicarbonate	–	–	400	Ayyat *et al.* (1991)
Nitrogen (N from NO_2 and NO_3 excluded)	2	–	–	
Cyanide (CN)	0.05	–	–	

[a] *Official Journal of the European Communities, 1998*; Council Directive 98/83/EC of 3 November, 1998, on the quality of water intended for human consumption.

(tenfold the maximum accepted for human consumption), which has led to considerable debate in intensive animal production regions such as the Netherlands or Brittany in France. None of the maxima for rabbits is lower than that recommended for human consumption. Therefore, no specific chemical control is necessary if the water provided for rabbits is the same as that provided for human consumption by a controlled public system. Conversely, alteration of water quality by increasing some minerals can be illegal for human consumption, but is not necessarily injurious to rabbit health (Table 10.6).

References

Abdelhamid, A.M. (1990) Effect of feeding rabbits on naturally moulded and mycotoxin-contaminated diet. *Archives of Animal Nutrition* 40, 55–63.

Abdelhamid, A.M., Kelada, I.P., Ali, M.M. and El-Ayouty, S.A. (1992) Influence of zearalenone on some metabolic physiological and pathological aspects of female rabbits at two different ages. *Archives of Animal Nutrition* 42, 63–70.

Abdelhamid, A.M., Ragab, M.A. and El-Shaieb, A.F. (2002) The use of tafla or aluminosilicate for alleviating toxic effects of aflatoxin-contaminated diets of growing rabbits. In: *Proceedings of the 1st Conference of Animal and Fish Production*. Mansoura, Egypt, pp. 389–413.

Abdel-Khalek, A.M., Selim, N.A., El-Medany, Sh.A. and Nada, S.A. (2008) Response of doe rabbits to dietary antioxidant vitamins E and C during pregnancy and lactation. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 519–523.

Abd El-Rahim, M.I., El-Kerdawy Dawlat, A., El-Kerdawy, H.M. and Abdallah Fatma, R. (1996) Bioavailability of iron in growing rabbits fed excess levels of dietary iron, under Egyptian conditions. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 51–57.

Abel-Samee, A.M. and El-Masry, K.A. (1992) Effect of drinking natural saline well water on some productive and reproductive performance of California and New-Zealand White rabbits maintained under north Sinai conditions. *Egyptian Journal of Rabbit Science* 2, 1–11.

Abo El-Ezz, Z.R., Salem, M.H., Hassan, G.A., El-Komy, A.G. and Abd El-Moula, E. (1996) Effect of different levels of copper sulphate supplementation on some physical traits of rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 59–65.

Ayyat, M.S. and Marai, I.F.M. (2000) Growth performance and carcass traits as affected by breed and dietary supplementation with different zinc levels, under Egyptian conditions. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 83–88.

Ayyat, M.S., Habeeb, A.A. and Bassuny, S.M. (1991) Effects of water salinity on growth performance, carcass traits and some physiological aspects of growing rabbits in summer season. *Egyptian Journal of Rabbit Science* 1, 21–34.

Bellier, R. (1994) *Contrôle Nutritionnel de l'activité Fermentaire Caecale Chez le Lapin*. Thèse doctorat, Institut National Polytechnique de Toulouse, France.

Bellier, R. and Gidenne, T. (1996) Consequences of reduced fibre intake on digestion, rate of passage and caecal microbial activity in the young rabbit. *British Journal of Nutrition* 75, 353–363.

Bennegadi, N., Gidenne, T. and Licois, L. (2001) Impact of fibre deficiency and health status on non-specific enteropathy of the growing rabbit. *Animal Research* 50, 401–413.

Bennegadi, N., Fonty, G., Millet, L., Gidenne, T. and Licois, D. (2003) Effects of age and dietary fibre level on caecal microbial communities of conventional and specific pathogen-free rabbits. *Microbial Ecology in Health and Disease* 15, 23–32.

Bernardini, M., Dal Bosco, A., Castellini, C. and Miggiano, G. (1996) Dietary vitamin E supplement in rabbit: antioxidant capacity and meat quality. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 3. Association Française de Cuniculture, Lempdes, France, pp. 137–140.

Blas, E., Cervera, C. and Fernandez Carmona, J. (1994) Effect of two diets with varied starch and fibre levels on the performances of 4–7 weeks old rabbits. *World Rabbit Science* 2, 117–121.

Boisot, P., Licois, D. and Gidenne, T. (2003) Feed restriction reduces the sanitary impact of an experimental reproduction of epizootic rabbit enteropathy syndrome (ERE), in the growing rabbit. In: Bolet, G. (ed.) *Proceedings of 10ème J. Rech. Cunicoles, 19–20 Nov., Paris, France*. ITAVI Paris, France, pp. 267–270.

Boisot, P., Duperray, J., Dugenétais, X. and Guyonvarch, A. (2004) Interest of hydric restriction times of 2 and 3 hours per day to induce feed restriction in growing rabbits. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 759–764.

Cachaldora, P., Nicodemus, N., García, J., Carabaño, R. and de Blas, J.C. (2004) Efficacy of Amylofeed® in growing rabbit diets. *World Rabbit Science* 12, 23–31.

Catala, J. and Bonnafous, R. (1979) Modifications de la microflore quantitative, de l'excrétion fécale et du transit intestinal chez le lapin, après ligature du canal pancréatique. *Annales de Zootechnie* 28, 128.

Chamorro, S., Gomez Conde, M.S., Perez De Rozas, A.M., Badiola, I., Carabaño, R. and de Blas, J.C. (2007) Effect on digestion and performance of dietary protein content and increased substitution of lucerne hay with soya-bean protein concentrate in starter diets for young rabbits. *Animal* 1, 651–659.

Clark, J.D., Jain, A.V., Hatch, R.C. and Mahaffey, E.A. (1980) Experimentally induced chronic aflatoxicosis in rabbits. *American Journal of Veterinary Research* 41, 1841–1845.

Clark, J.D., Jain, A.V. and Hatch, R.C. (1982) Effects of various treatments on induced chronic aflatoxicosis in rabbits. *American Journal of Veterinary Research* 4, 106–110.

Clark, J.D., Greene, C.E., Calpin, J.P., Hatch, R.C. and Jain, A.V. (1986) Induced aflatoxicosis in rabbits: blood coagulation defects. *Toxicology and Applied Pharmacology* 86, 353–361.

Colin, M., Maire, C., Vaissaire, J. and Renault, L. (1976) Experimental study on replacement of crude fibre by mineral ballasts (sand and mica) in rabbit diets. *Recherches Médicales Vétérinaires* 152, 457–465.

Čonková, E., Laciaková, A., Pástorová, B., Seidel, H. and Kovác, G. (2001) The effect of zearalenone on some enzymatic parameters in rabbits. *Toxicology Letters* 121, 145–149.

Cortez, S., Brandeburger, H., Greuel, E. and Sundrum, A. (1992) Investigations of the relationships between feed and health status on the intestinal flora of rabbits. *Tierärztlische Umschau* 47, 544–549.

Davidson, J. and Spreadbury, D. (1975) Nutrition of the New Zealand White rabbit. *Proceedings of the Nutrition Society* 34, 75–83.

de Blas, J.C., Pérez, E., Fraga, M.J., Rodriguez, M. and Galvez, J.F. (1981) Effect of diet on feed intake and growth of rabbits from weaning to slaughter at different ages and weights. *Journal of Animal Science* 52, 1225–1232.

de Blas, J.C., Santoma, G., Carabaño, R. and Fraga, M.J. (1986) Fiber and starch level in fattening rabbit diets. *Journal Animal Science* 63, 1897–1904.

de Blas, J.C., Taboada, E., Mateos, G.G., Nicodemus, N. and Méndez, J. (1995) Effect of substitution of starch for fiber and fat in isoenergetic diets on nutrient digestibility and reproductive performance of rabbits. *Journal of Animal Science* 73, 1131–1137.

EGRAN (2001) Technical note: attempts to harmonise chemical analyses of feeds and faeces, for rabbit feed evaluation. *World Rabbit Science* 9, 57–64.

Fekete, S. and Huszenicza, G. (1993) Effect of T-2 toxin on ovarian activity and some metabolic variables of rabbits. *Laboratory Animal Science* 43, 646–649.

Fekete, S., Tamas, J., Vanyi, A., Glavits, R. and Bata, A. (1989) Effect of T-2 toxin on feed intake, digestion and pathology of rabbits. *Laboratory Animal Science* 39, 603–606.

Fernandez-Carmona, J., Cervera, C. and Blas, E. (1996) High fat for rabbits breeding does housed at 30°C. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 167–169.

Fioramonti, J., Sorraing, J.M., Licois, D. and Bueno, L. (1981) Intestinal motor and transit disturbances associated with experimental coccidiosis (*Eimeria magna*) in the rabbit. *Annales de Recherches Vétérinaires* 12, 413–420.

Fortun-Lamothe, L. (2006) Energy balance and reproductive performance in rabbit does. *Animal Reproduction Science* 93, 1–15.

Fortun-Lamothe, L. and Boullier, S. (2007) A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. *Livestock Science,* 107, 1–18.

Fortun-Lamothe, L., Lacanal, L., Boisot, P., Jehl, N., Arveux, A., Hurtaud, J. and Perrin, G. (2005) Effects of level and origin of dietary energy on reproduction performance of the does and health status of the young. In: Bolet, G. (ed.) *Proceedings of 11ème J. Rech. Cunicoles, 29 and 30 Nov. 2005, Paris*. ITAVI, Paris, France, pp. 129–132.

Fraga, M.J., Lorente, M., Carabaño, R. and de Blas, J.C. (1989) Effect of diet and remating interval on milk production and milk composition of the doe rabbit. *Animal Production* 48, 459–466.

Gallois, M., Gidenne, T., Tasca, C., Caubet, C., Coudert, C., Milon, A. and Boullier, S. (2007) Maternal milk contains antimicrobial factors that protect young rabbits from enteropathogenic *Escherichia coli* infection. *Clinical and Vaccine Immunology* 14, 585–592.

Gallois, M., Gidenne, T., Orengo, J., Caubet, C., Tasca, C., Milon, A. and Boullier, S. (2008) Testing the efficacy of medium chain fatty acids against rabbit colibacillosis. *Veterinary Microbiology* 131, 192–198.

Galtier, P., Baradat, C. and Alvinerie, M. (1977) Etude de l'élimination d'ochratoxine A par le lait chez la lapine. *Annales de la Nutrition et de l'Alimentation* 31, 911–918.

García, J., Carabaño, R. and de Blas, C. (1999) Effect of fiber source on cell wall digestibility and rate of passage in rabbits. *Journal of Animal Science* 77, 898–905.

García, J., Gidenne, T., Falcão e Cunha, L. and de Blas, C. (2002) Identification of the main factors that influence caecal fermentation traits in growing rabbits. *Animal Research* 51, 165–173.

García, J., Gómez, M.S., Chamorro, S., Nicodemus, N., de Blas, C. and Carabaño, R. (2004) Nuevas herramientas para la valoración nutritiva de los piensos de conejos recién destetados: implicaciones prácticas. *Cunicultura* 170, 223–228.

García-Ruiz, A.I., García-Palomares, J., García-Rebollar, P., Chamorro, S., Carabaño, R. and de Blas, C. (2006) Effect of protein source and enzyme supplementation on ileal protein digestibility and fattening performance in rabbits. *Spanish Journal of Agricultural Research* 4, 297–303.

Gentry, P.A. (1982) The effect of administration of a single dose of T-2 toxin on blood coagulation in the rabbit. *Canadian Journal of Comparative Medicine* 46, 414–419.

Gidenne, T. (1995) Effect of fibre level reduction and gluco-oligosaccharides addition on the growth performance and caecal fermentation in the growing rabbit. *Animal Feed Science and Technology* 56, 253–263.

Gidenne, T. (2003) Fibres in rabbit feeding for digestive troubles prevention: respective role of low-digested and digestible fibre. *Livestock Production Science* 81, 105–117.

Gidenne, T. and Licois, D. (2005) Effect of a high fibre intake on the resistance of the growing rabbit to an experimental inoculation with an enteropathogenic strain of *Escherichia coli*. *Animal Science* 80, 281–288.

Gidenne, T., Carré, B., Segura, M., Lapanouse, A. and Gomez, J. (1991) Fibre digestion and rate of passage in the rabbit: effect of particle size and level of lucerne meal. *Animal Feed Science and Technology* 32, 215–221.

Gidenne, T., Pinheiro, V. and Falcão e Cunha, L. (2000) A comprehensive approach of the rabbit digestion: consequences of a reduction in dietary fibre supply. *Livestock Production Science* 64, 225–237.

Gidenne, T., Arveux, P. and Madec, O. (2001a) The effect of the quality of dietary lignocellulose on digestion, zootechnical performance and health of the growing rabbit. *Animal Science* 73, 97–104.

Gidenne, T., Kerdiles, V., Jehl, N., Arveux, P., Briens, C., Eckenfelder, B., Fortune, H., Montessuy, S., Muraz, G. and Stephan, S. (2001b) An increase of dietary ratio 'digestible fibre/crude protein' does not affect the performances of the growing rabbit but reduce enteritis incidence: preliminary results of a multi-site study. In: Bolet, G. (ed.) *Proceedings of the 9th J. Rech. Cunicoles, 28 and 29 Nov., Paris*. ITAVI, Paris, France, pp. 65–68.

Gidenne, T., Jehl, N., Segura, M. and Michalet-Doreau, B. (2002) Microbial activity in the caecum of the rabbit around weaning: impact of a dietary fibre deficiency and of intake level. *Animal Feed Science and Technology* 99, 107–118.

Gidenne, T., Jehl, N., Lapanouse, A. and Segura, M. (2004a) Inter-relationship of microbial activity, digestion and gut health in the rabbit: effect of substituting fibre by starch in diets having a high proportion of rapidly fermentable polysaccharides. *British Journal of Nutrition* 92, 95–104.

Gidenne, T., Mirabito, L., Jehl, N., Perez, J.M., Arveux, P., Bourdillon, A., Briens, C., Duperray, J. and Corrent, E. (2004b) Impact of replacing starch by digestible fibre, at two levels of lignocellulose, on digestion, growth and digestive health of the rabbit. *Animal Science* 78, 389–398.

Gidenne, T., Segura, M. and Lapanouse, A. (2005a) Effect of cereal sources and processing in diets for the growing rabbit. I. Effects on digestion and fermentative activity in the caecum. *Animal Research* 54, 55–64.

Gidenne, T., Jehl, N., Perez, J.M., Arveux, P., Bourdillon, A., Mousset, J.L., Duperray, J., Stephan, S. and Lamboley, B. (2005b) Effect of cereal sources and processing in diets for the growing rabbit. II. Effects on performances and mortality by enteropathy. *Animal Research* 54, 65–72.

Gidenne, T., Combes, S., Feugier, A., Jehl, N., Arveux, P., Boisot, P., Briens, C., Corrent, E., Fortune, H., Montessuy, S. and Verdelhan, S. (2009a) Feed restriction strategy in the growing rabbit. 2. Impact on digestive health, growth and carcass characteristics. *Animal* 3, 509–515.

Gidenne, T., Murr, S., Travel, A., Corrent, E., Foubert, C., Bebin, K., Mevel, L., Rebours, G. and Renouf, B. (2009b) Effets du niveau de rationnement et du mode de distribution de l'aliment sur les performances et les troubles digestifs post-sevrage du lapereau – premiers resultats d'une étude concertée du reseau GEC. *Cuniculture Magazine* 36, 65–72.

Gómez-Conde, M.S., García, J., Chamorro, S., Eiras, P., Rebollar, P.G., Pérez de Rozas, A., Badiola, I., de Blas, C. and Carabaño, R. (2007) Neutral detergent-soluble fibre improves gut barrier function in 25 d old weaned rabbits. *Journal of Animal Science* 85, 3313–3321.

Gómez-Conde, M.S., Pérez de Rozas, A., Badiola, I., Pérez-Alba, L., de Blas, J.C., Carabaño, R. and García, J. (2009) Effect of neutral detergent soluble fibre on digestion, intestinal microbiota and performance in twenty five day old weaned rabbits. *Livestock Science* 125, 192–198.

Guimarães, C.S. and Motta, F.W. (2000) Bioavailibility of dietary zinc sources for fattening rabbits. In: Blasco A. (ed.) *Proceedings of the 7th World Rabbit Congress, Valencia*, Vol. 3. Valencia University Publications, Valencia, Spain, pp. 255–261.

Gutiérrez, I., García, P., Carabaño, R. and de Blas, J.C. (2000) Effect of suplementation with animal plasma and antibiotics on jejunal morphology of early-weaned rabbits. *World Rabbit Science* 8, 263–267.

Gutiérrez, I., Espinosa, A., García, J., Carabano, R. and de Blas, J.C. (2002a) Effect of levels of starch, fiber, and lactose on digestion and growth performance of early-weaned rabbits. *Journal of Animal Science* 80, 1029–1037.

Gutiérrez, I., Espinosa, A., García, J., Carabano, R. and de Blas, J.C. (2002b) Effects of starch and protein sources, heat processing, and exogenous enzymes in starter diets for early weaned rabbits. *Animal Feed Science and Technology* 98, 175–186.

Gutiérrez, I., Espinosa, A., García, J., Carabano, R. and de Blas, C. (2003) Effect of protein source on digestion and growth performance of early-weaned rabbits. *Animal Research* 52, 461–471.

Habeeb, A.A.M., Marai, I.F.M., El-Maghawry, A.M. and Gad, A.E. (1997) Physiological response of growing rabbit to different concentrations of salinity in drinking water under winter and hot summer conditions. *Egyptian Journal of Rabbit Science* 7, 81–94.

Haffar, A., Laval, A. and Guillou, J.P. (1988) Entérotoxémie à *Clostridium spiroforme* chez des lapins adultes. *Le Point Vétérinaire* 20, 99–102.

Hall, M.B., Lewis, B.A., Van Soest, P.J. and Chase, L.E. (1997) A simple method for estimation of neutral detergent-soluble fibre. *Journal of the Science of Food and Agriculture* 74, 441–449.

Hanika, C., Carlton, W.W. and Tuite, J. (1983) Citrinin mycotoxicosis in the rabbit. *Food and Chemical Toxicology* 21, 487–496.

Hanika, C., Carlton, W.W., Boon, G.D. and Tuite, J. (1984) Citrinin mycotoxicosis in the rabbit: clinicopathological alterations. *Food and Chemical Toxicology* 22, 999–1008.

Hodgson, J. (1974) Diverticular disease. Possible correlation between low residue diet and raised intracolonic pressures in the rabbit model. *American Journal of Gastroenterology* 62, 116–123.

Jehl, N. and Gidenne, T. (1996) Replacement of starch by digestible fibre in the feed for the growing rabbit. 2) Consequences on microbial activity in the caecum and on incidence on digestive disorders. *Animal Feed Science and Technology* 61, 193–204.

Kammerer, M. and Pinault, L. (1998) Pollution de l'eau d'abreuvement par les nitrates. Tolérance générale et influence sur la croissance pondérale chez le lapin. *7e Journées de la Recherche Cunicole en France, Lyon*. ITAVI, Paris, France, pp. 191–197.

Khera, K.S., Whalen, C. and Angers, G. (1986) A teratology study on vomitoxin (4-deoxynivalenol) in rabbits. *Food and Chemical Toxicology* 24, 421–424.

Kjaer, K.B. and Jensen, J.A. (1997) Perirenal fat, carcass conformation, gain and feed efficiency of growing rabbits as affected by dietary protein and energy content. *World Rabbit Science* 5, 93–97.

Koehl, P.F (1997) GTE Renalap 96: une lapine produit 118 kg de viande par an. *Cuniculture* 24, 247–252.

Krihsna, L., Dawra, R.K., Vaid, J. and Gupta, V.K. (1991) An outbreak of aflatoxicosis in Angora rabbits. *Veterinary and Human Toxicology* 33, 159–161.

Laplace, J.P. and Lebas, F. (1977) Le transit digestif chez le lapin. 7) Influence de la finesse de broyage des constituants d'un aliment granulé. *Annales de Zootechnie* 26, 413–420.

Lebas, F. (1973) Effet de la teneur en proteines de rations à base de soja ou de sésame sur la croisssance du lapin. *Annales de Zootechnie* 22, 83–92.

Lebas, F. (2008) Performances moyennes des élevages cunicoles en 2007 Présentation rapide des résultats RENACEB et RENALAP d'après Annick Jentzer Service économique de l'ITAVI. *Cuniculture Magazine* 35, 39–44.

Lebas, F. and Fortun-Lamothe, L. (1996) Effects of dietary energy level and origin (starch vs oil) on performance of rabbit does and their litters: average situation after 4 weanlings. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Française de Cuniculture, Lempdes, France, pp. 217–222.

Lebas, F. and Lamboley, B. (1999) Méthode de détermination par tamisage en phase liquide de la taille des particules contenues dans un aliment granulé pour lapins. *World Rabbit Science* 7, 229–235.

Lebas, F., Maître, I., Seroux, M. and Franck, T. (1986) Influence du broyage des matières premières avant agglomération de 2 aliments pour lapins différant par leur taux de constituants membranaires: digestibilité et performances de croissance. In: *Proceedings of the 4th J. Rech. Cunicole Fr., Paris*, Vol. 1. ITAVI. Paris, France, pp. 9.1–9.13.

Lebas, F., Coudert, P., De Rochambeau, H. and Thébault, R.G. (1996) *Le lapin Elevage et Pathologie*. FAO, Rome, Italy.

Licois, D., Guillot, J.F., Mouline, C. and Reynaud, A. (1992) Suceptibility of the rabbit to an enteropathogenic strain of *Escherichia coli* O-103: effect of animal's age. *Annals of Veterinary Research* 23, 225–232.

Maertens, L. (1999) Towards reduced feeding costs, dietary safety and minimal mineral excretion in rabbits: a review. *World Rabbit Science* 7, 65–74.

Maertens, L., Aerts, J.M. and De Brabander, D.L. (2005) Effect of a diet rich in n-3 fatty acids on the performances and milk composition of does and the viability of their progeny. In: Bolet, G. (ed.) *Proc. 11ème J. Rech. Cunicoles*. ITAVI, Paris, France pp. 205–208.

Marounek, M., Skrivanova, V. and Savka, O. (2002) Effect of caprylic, capric and oleic acid on growth of rumen and rabbit caecal bacteria. *Journal of Animal and Feed Science* 11, 507–516.

Mézes, M. and Balogh, K. (2009) Mycotoxins in rabbit feed: a review. *World Rabbit Science* 17, 53–62.

Morisse, J.P., Wyers, M. and Drouin, P. (1981) Aflatoxicose chronique chez le lapin. Essai de reproduction expérimentale. *Recueil de Médecine Vétérinaire de l'Ecole d'Alfort* 157, 363–368.

Morisse, J.P., Boilletot, E. and Maurice, R. (1989) Incidence des nitrites chez les lapins. *Cuniculture* 16, 197–199.

Newberne, P.M. and Butler, W.H. (1969) Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: a review. *Cancer Research* 29, 236–250.

Nicodemus, N., Carabaño, R., García, J., Mendez, J. and de Blas, J.C. (1999) Performance response of lactating and growing rabbits to dietary lignin content. *Animal Feed Science and Technology* 80, 43–54.

Nicodemus, N., Redondo, R., Carabaño, R., de Blas, C. and García, J. (2003a) Effect of level of fibre and type of ground of fibre sources on digestion and performance of growing rabbits. In: Gidenne, T. and Blasco, A. (eds) *Proceedings of the 3rd Meeting of Nutrition and Pathology and Meat Quality Working Groups of COST ACTION*. Prague, Czech Republic, p. 22.

Nicodemus, N., Gómez Conde, M.S., Espinosa, A., García, J., Carabaño, R. and de Blas, C. (2003b) Efecto de la utilización de bacitracina de zinc y sulfato de apramicina sobre la digestión en gazapos destetados precozmente. In: *Proceedings of the XXVIII Symposium de Cunicultura. Alcañiz*. ASESCU and Diputación General de Aragón, Spain, pp. 163–170.

Nicodemus, N., Pérez-Alba, L., Carabaño, R., de Blas, C., Badiola, I. and Pérez de Rozas, A.J.G. (2004) Effect of fibre and level of ground of fibre sources on digestión and ileal and caecal characterization of microbiota of early weaned rabbits. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 143.

Nicodemus, N., García, J., Carabaño, R. and de Blas, J.C. (2006) Effect of a reduction of dietary particle size by substituting a mixture of fibrous by-products for lucerne hay on performance and digestion of growing rabbits and lactating does. *Livestock Science* 100, 242–250.

Osborn, R.G., Osweiler, G.D. and Foley, C.W. (1988) Effects of zearalenone on various components of rabbit uterine tubal fluid. *American Journal of Veterinary Research* 49, 1382–1386.

Padilha, M.T.S., Licois, D., Gidenne, T., Carré, B. and Fonty, G. (1995) Relationships between microflora and caecal fermentation in rabbits before and after weaning. *Reproduction Nutrition Development* 35, 375–386.

Perez, J.M. and Leuillet, M. (1986) Composition et valeur nutritive des céréales. *Perspectives Agricoles* 105, 56–61.

Perez, J.M., Gidenne, T., Lebas, F., Caudron, I., Arveux, P., Bourdillon, A., Duperray, J. and Messager, B. (1994) Apports de lignines et alimentation du lapin en croissance. II. Conséquences sur les performances de croissance et la mortalité. *Annales de Zootechnie* 43, 323–332.

Perez, J.M., Gidenne, T., Bouvarel, I., Caudron, I., Arveux, P., Bourdillon, A., Briens, C., Le Naour, J., Messager, B. and Mirabito, L. (1996) Apports de cellulose dans l'alimentation du lapin en croissance. II. Conséquences sur les performances et la mortalité. *Annales de Zootechnie* 45, 299–309.

Perez, J.M., Gidenne, T., Bouvarel, I., Arveux, P., Bourdillon, A., Briens, C., Le Naour, J., Messager, B. and Mirabito, L. (2000) Replacement of digestible fibre by starch in the diet of the growing rabbit. II. Effects on performances and mortality by diarrhoea. *Annales de Zootechnie* 49, 369–377.

Pompa, G., Montesissa, C., Di Lauro, F.M. and Fadini, L. (1986) The metabolism of zearalenone in subcellular fractions from rabbit and hen hepatocytes and its estrogenic activity in rabbits. *Toxicology* 42, 69–75.

Porter, L.P., Borgman, R.F. and Lightsey, S.F. (1988) Effect of water hardness upon lipid and mineral metabolism in rabbits. *Nutrition Research* 8, 31–45.

Prohaszka, L. (1980) Antibacterial effect of volatile fatty acids in enteric *E. coli* infections of rabbits. *Zentralblatt Veterinar Medecine B* 27, 631–639.

Rémois, G. and Rouillère, H. (1998) Effet du sulfate d'aluminium sur les performances des lapins d'engraissement. *7e Journées de la Recherche Cunicole en France, Lyon*. ITAVI, Paris, France, pp. 195–197.

Scheele, C.W. and Bolder, N.M. (1987) Health problems and mortality of young suckling rabbits in relation to dietary composition. In: *Rabbit Production Systems Including Welfare*. Commision of the European Communities, Brussels, Belgium, pp. 115–125.

Skrivanova, V. and Marounek, M. (2006) A note on the effect of triacylglycerols of caprylic and capric fatty acid on performance, mortality, and digestibility of nutrients in young rabbits. *Animal Feed Science and Technology* 127, 161–168.

Skrivanova, E., Molatova, Z. and Marounek, M. (2008) Effects of caprylic acid and triacylglycerols of both caprylic and capric acid in rabbits experimentally infected with enteropathogenic *Escherichia coli* O103. *Veterinary Microbiology* 126, 372–376.

Vanyi, A., Glavits, R., Bata, A., Fekete, S. and Tamas, J. (1989) The pathological effects, metabolism and excretion of T-2 toxin in rabbits. *Journal of Applied Rabbit Research* 12, 194–200.

Verdelhan, S., Bourdillon, A., Morel-Saives, A. and Audoin, E. (2004) Effect of a limited access to water on mortality of fattening rabbits. Effects of source of protein and enzyme supplementation on performance of fattening rabbits. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 1015–1021.

Virag, Gy., Eiben, Cs., Tóth, T. and Schmidt, J. (2008) Colour and pH of rabbit meat and fat deposits as affected by the source and dose of dietary vitamin E supplementation. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 1467–1471.

11 Feed Manufacturing

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11.1 Introduction

Raw materials need to be processed by a combination of different treatments of individual processes to produce an acceptable final feed. This must satisfy all of the nutrient and physical presentation requirements of the animal, except for water.

Before feed manufacturing starts, logistics and nutrition play an important role. Choosing from all the raw materials available in the market suitable for rabbit feeding will be the first step. Feed formulation considering the nutrient requirements of rabbits and the nutritional value of raw materials will produce specific formulae that need to be properly manufactured. Quality control of raw materials is required for achieving good knowledge of their nutritive value. It will also allow checking their stability prior to arriving at the mill, as well as evaluating their organoleptic characteristics. Quality control during the process and on the manufactured feed must be undertaken and, in order to achieve that, hazard analysis and critical control points (HACCP) must be followed, including tracing and tracking.

Once the raw materials arrive at the feed mill and are accepted by the quality control system they will be discharged and placed into bins for further processing. Modern feed mills manufacture the feed in a sequence of processes that run on a batch-type system (Figs 11.1 and 11.2). Following the reception and storage of raw materials, the manufacturing process starts to produce a specific feed associated with a particular type of formulation, previously designed by the nutritionist.

The manufacturing of each feed will always be conditioned by raw material composition and the nutritionist should take in account both individual and collective effects in the feed process itself. The initial process is weighing out according to the formula, followed by grinding for particle size reduction. Following the addition of premixes, homogenization of all the raw materials included in the formula is undertaken. Following an adequate mixing time, manufactured feed is obtained as a mash. However, as will be discussed later, rabbits are not fed mash diets and the feed therefore needs to be further processed by pelleting. Obtaining good quality pellets is one of the main targets of rabbit feed manufacturing and quality control of this step is essential.

There are two main systems used for feed manufacturing. Both use the same individual processes, and the only difference is the sequence in which they are arranged. The pre-milling system is more commonly used in modern feed mills because of economic reasons (e.g. energy cost, investment). In this system, weighing out is done prior to

Fig. 11.1. A modern feed mill.

grinding. The pre-grinding system was used more in the past when fewer raw materials were available and mash was the main means of feed presentation. The pre-milling system sequence will be considered in this chapter as it is more commonly used and better for rabbit feed manufacturing.

11.2 Raw Material Addition

Raw materials are extracted from silos according to their concentration in the formula and transferred to a weigh scale, which records individual data on each of them. Such extractions are done either with screws or slides. Good accuracy can be reached with both systems. Scales are constructed under a metallic bin placed over load cells linked by cable to the automation control of the scale. The design of the scale is important: it must permit appropriate conditions of filling and discharge of the raw materials once weighing is finished.

The standard error of weighing for each raw material is constantly corrected by the automation of the weighing system to minimize deviations between the formulation and the actual weight obtained on the scale. Different ranges of weigh scales are usually found in a modern feed mill in order to manage the weighing of raw materials according to the level of inclusion in the formulation. Weighing should be carried out with precise scales with an error <0.5% to prevent wide variations in the characteristics of the final product. For raw materials that are at high levels in the formulation, such as lucerne and wheat bran, the weighing mechanism and the corresponding scale should allow the weighing in a short period of time, with a final period for weight adjustment. On the other hand, the weighing of raw materials that are present at lower rates, such as limestone or dicalcium phosphate when they are stored as bulk in silos, the weighing system and scale should be more precise in order to avoid oversupply.

Equipment must include safety elements to stop the process whenever a deficiency or an excess during weighing occurs. A printed register on every formula of all individual weights should be kept for assuring control

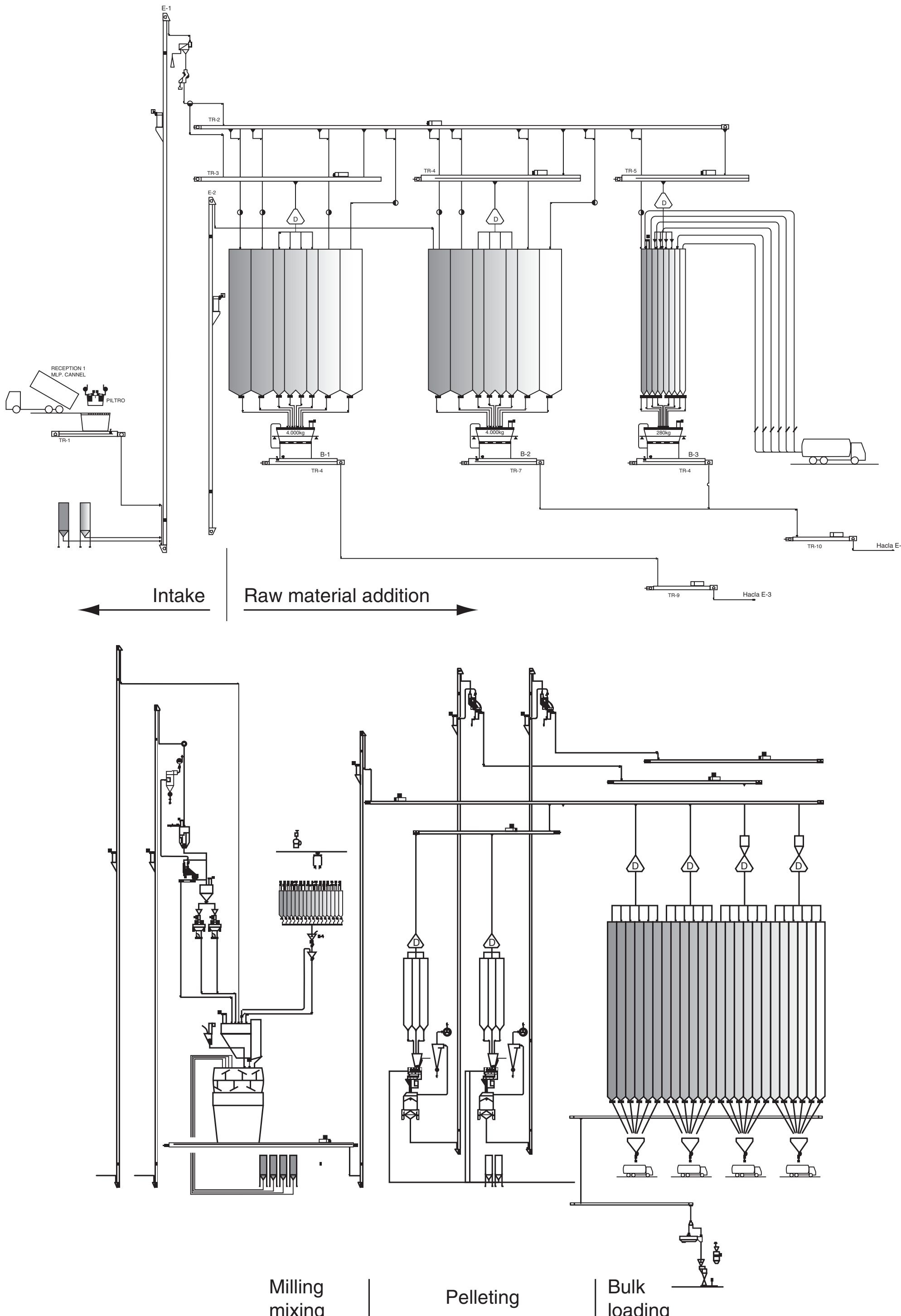

Fig. 11.2. Feed processing in a modern feed mill.

of the process and final product quality, as well as allowing traceability within the whole operation. Adequate software needs to be used for formulating feeds and limitations in terms of minimum amounts included, and rounding of each raw material should be adjusted according to the weighing system limitations of each feed mill.

11.2.1 Premix addition

Vitamins, minerals, amino acids, coccidiostats and other micronutrients should be weighed on specific scales with greater accuracy than the general scales used for the major raw materials included in the feed. It is advisable to prepare a previous mixture with these products before adding them to the main mixer. Regulations on feed manufacturing in the European Union state that any raw material at a rate >2 kg t^{-1} must be added to the main mixer, whereas those at a rate <2 kg t^{-1} need to be premixed in advance to reach this inclusion rate.

All premix materials should be weighed on specific scales, with records of this operation kept, before being added to the main mixer.

11.3 Grinding

Grinding is a critical part of the feed manufacturing process because particle size reduction is a requirement for all types of domestic species and undoubtedly for the rabbit. Raw materials available for feed production vary greatly according to their original texture and particle size: for example, cereals and legumes arrive at the feed mill as whole grains, without any previous treatment, while lucerne, wheat bran, grain co-products and straw arrive as pellets after being ground at the processing plants from which they originate.

Grinding is needed to: (i) reduce particle size in order to increase digestibility in the rabbit; and (ii) obtain an optimum particle size that allows successful mixing of the raw materials and subsequent steam, thus assuring a good pelleting process to obtain pellets of acceptable quality.

As discussed earlier, there are two means of grinding the raw materials, dependent on the feed manufacturing system design: premilling, also called post-grinding (Fig. 11.3), and pre-grinding (Fig. 11.4). With pre-milling, the raw materials come to the grinder together, whereas in pre-grinding each ingredient is ground individually. Both systems have advantages and disadvantages.

11.3.1 Pre-grinding system

Each raw material is ground individually and weighing is later in the sequence, in which mash is used as the weighing component of the feed.

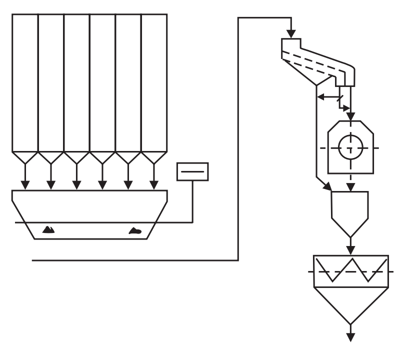

Fig. 11.3. Pre-milling system.

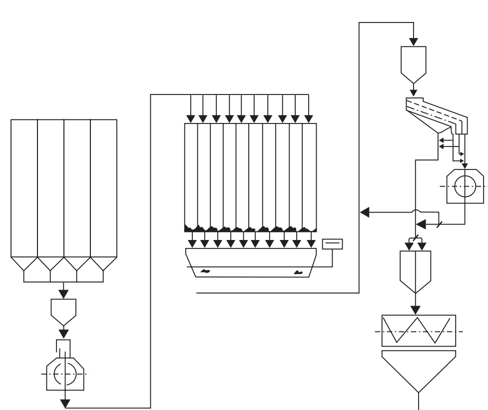

Fig. 11. 4. Pre-grinding system.

The advantages of this system are as follows:

- Particle size distribution for each ingredient can be modified on changing the grinder sieves.
- Advantage can be taken of the maximum grinding capacity because a homogeneous product is ground, so grinding yield is optimized.
- The mixing plant does not depend upon the grinding plant so grinding and mixing can be undertaken separately, while this cannot occur with the pre-milling system.

The disadvantages are as follows:

- There is a risk of final separation problems because of the different particle size distribution of the individual raw materials.
- Raw materials with a high oil content, such as oilseeds, are difficult to grind and cannot be ground individually.
- A larger number of silo-bins is required because the same raw material requires at least two bins, one for reception and another for the ground raw material.
- Materials with a high proportion of hulls, such as oats or barley, are ground more efficiently together with other ingredients such as maize or oilseed meals.
- The storage life of ground products is shorter than that of whole products.

11.3.2 Pre-milling system

When grinding is scheduled after weighing, the whole mix of raw materials travels through the grinding system. As stated before, this system has been more frequently used over the last decade in feed mill design, mainly because of lower production costs and investment for a similar feed manufacturing capacity to the pre-grinding system.

Hammer mill grinders are commonly used with both systems. Two different designs are available according to the shaft layout: vertical or horizontal. The main advantages of the vertical hammer mill are that it requires less space, consumes less energy per tonne ground, gives a lower dispersion of the particle size distribution and has lower maintenance costs (Acedo-Rico, 2006). Control of the hammer mill grinder by an automated system that controls the feeding of the grinder according to energy consumption is necessary. The feeding device should also include one separator of heavy particles and metals.

The use of a sieve before the grinder facilitates the grinding process and saves energy (because particles that are already small bypass the grinder), enlarges the productive life of the grinder (because materials such as minerals, which are very abrasive, can also bypass the grinder) and can increase the efficiency of grinding by decreasing plugging problems at the decompression hoses. A proper air intake will improve the grinding yield and reduce losses due to decreased moisture content.

11.3.3 Particle size

Particle size reduction enables the later pelleting process and consequently favours pellet quality. The finer the grind, the greater the particle size reduction, but the more energy will be expended on the process.

From a physiological standpoint, excessive grinding can increase the retention time of the feed in the intestine (Lang, 1981) and, as a result, nutrient digestibility may increase. In this way it has been found that neutral detergent fibre digestibility is positively correlated with the proportion of particles with a size <0.315 mm. However, an increase in the retention time of feed in the gut is apparently associated with digestive disturbances, which can predispose to diarrhoea (Lebas and Laplace, 1975; Laplace and Lebas, 1977). This increase in the retention time occurs mainly in the caecum, which enlarges, and undesirable fermentation patterns take place. In accordance with this, irregularities of ileo-caecal valve motility with very fine grinding (1 mm sieve) in comparison with coarser grinding (4 mm sieve) have been reported (Pairet *et al.*, 1986).

Table 11.1. Effect of hammer action and sieve size on grinding throughout and particle size.

Variable		Throughput	Particle size
Hammer tip speed	Higher	Decrease	Finer
	Lower	Increase	Coarser
Number of hammers	Smaller	Increase	Coarser
	Greater	Decrease	Finer
Sieve hole diameter	Smaller	Decrease	Finer
	Larger	Increase	Coarser

In practice, fine grinding should be achieved with sieves with a diameter between 2.5 and 3.5 mm, because they permit a good balance between pellet quality and intestinal motility. It is important to take periodic assessments of the particle size distribution to ensure its suitability, because this is influenced by wearing of the parts of the hammer mill (sieves and hammers). Wear due to friction of raw material particles produces coarser mash feeds.

It has been suggested, without any strong experimental evidence, that using two kinds of grinding might be useful: a fine grind for low-fibre ingredients (e.g. cereals, soybean meal) and a coarse grind for fibrous ingredients (e.g. lucerne, straw). It is thought that the former ingredients have greater digestibility when finely ground, while the latter have a mechanical function as ballast when coarsely ground, thus influencing intestinal motility (Mateos and Rial, 1989).

Grinding throughput and particle size vary according to several parameters, as presented in Table 11.1.

In addition, the raw material itself will have a considerable influence on both factors. Identical conditions in a hammer mill will give different particle size distributions depending on the raw material being ground (Table 11.2).

Table 11.2. General guidelines for particle size distribution for rabbit feeds.

Particle size (mm)	Proportion
>1.5	0.15
1.0–1.5	0.20
0.5–1.0	0.40
<0.5	0.25

11.4 Mixing

Mixing is the central process of feed manufacturing. The main objective of the mixing process is to homogenize the different raw materials that have been weighed and ground. Mixing is performed in the main mixer, and the aim is to mix as uniformly as possible particles of different size and density over a short period of time, called the mixing time. The main mixer is the machine in which feed is initially produced, and therefore it is very important to be sure about the mixing capability of the equipment and the uniformity of the complete batch.

It is also important to realize that the process will be mixing raw materials, which represent up to 400–500 kg t^{-1} of the feed, with micro-ingredients that, in extreme cases such as biotin, vitamin B_{12} or selenium, are included at levels of 10–100 mg t^{-1}.

Different types of mixing equipment are found in feed mills, but developments over the last decade have led to the use of horizontal batch-type mixers equipped with one or two axes and paddles (Fig. 11.5). These have the following characteristics:

- Adequate mixing capability (1:100,000).
- Low rotating cycle (33 rpm).
- Low mixing time (<180 s).
- Full discharge for minimal cross-contamination.
- Free inside access to enable cleaning and maintenance.
- Possibility of liquid addition (fats and oils, amino acids, organic acids).
- Stainless steel inside coating (desirable).

In order to check good operating function of the mixer, a homogeneity test must be conducted. Such a test consists of taking

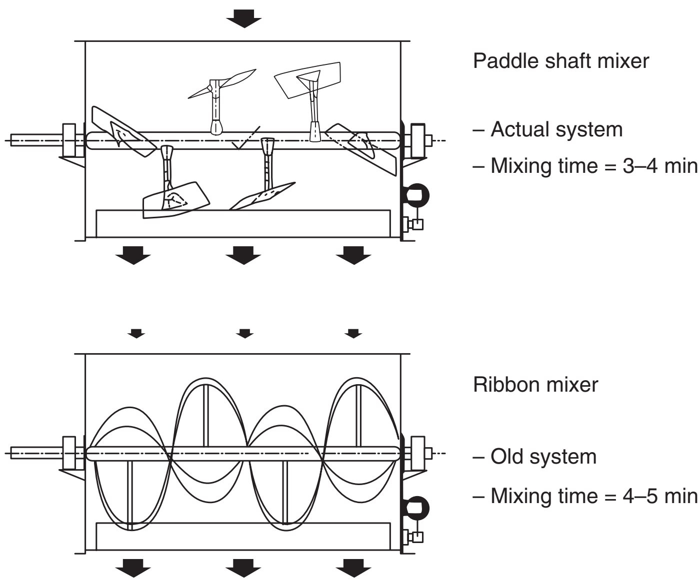

Fig. 11.5. Main mixers.

10–20 samples from the mixer itself or at the outlet at regular time intervals. These samples are ground and divided in the laboratory into 10–20 g samples that are assessed for either a chemical component of the feed (e.g. salt, manganese) or a component that is specially added for this purpose. The latter components are special indicators (micro-tracers) added to the feed at a rate of around 10 g t^{-1}. Another alternative is to use a cobalt mix that can also replace the usual means of adding this mineral to a premix. Feed manufacturing standards consider a mixture to be of good quality when the coefficient of variation is <5% at a defined mixing time. The mixing time should be established for each item of equipment (Fig. 11.6).

Cross-contamination can occur on the main mixer if attention is not paid to details such as the sequence of feed going through the process, the cleaning programme of the mixer and the adequate full discharge of the whole batch. Cross-contamination is of particular concern in the case of some feed additives and pharmacological products, not only because of possible toxicity for the rabbit, but in terms of human health due to the residues that can accumulate in rabbit meat.

In most feed mills, the only way to minimize dangerous cross-contaminations is to have well-designed manufacturing sequence programmes. After mixing medicated feed in the central mixer, only feeds for animals that are not susceptible to the medicines or for animals whose products are not to be consumed by humans should be manufactured.

Rabbits are especially sensitive to some pharmacological medicines such as ampicillin or lincomycin, so special measures must be taken when planning manufacturing sequences with these antibiotics. Currently, it is possible to prevent these situations more easily through the use of software that includes all of the incompatibilities to be considered before manufacturing the feed. The best means of avoiding these situations is the use of an independent manufacturing line for medicated feeds. This strategy has been designed in modern feed mills with double processing lines, in which just one line is used for medicated feeds, while the other never allows drugs or additives with contamination potential to be added to the feeds being manufactured.

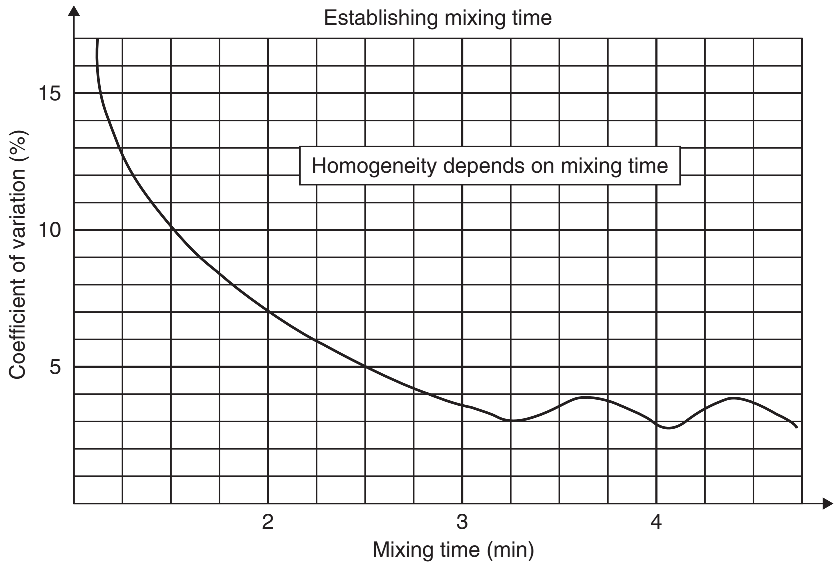

Fig. 11.6. Establishing mixing time.

11.5 Liquid Addition

Some raw materials are available in liquid form and there are different places on the feed manufacturing process in which these liquids are included (Fig. 11.7). With rabbit feed production, the main raw materials used in liquid form are fats, oils and glycerol, molasses, amino acids, liquid flavours and enzymes.

11.5.1 Fats, oils and glycerol

Fats, oils and glycerol are added by spraying in the main mixer. It is important to wait for at least 30 seconds from the beginning of the process before starting fat injection into the mixer in order to ensure that the liquid is added to a more homogeneous environment. To facilitate distribution, liquids must

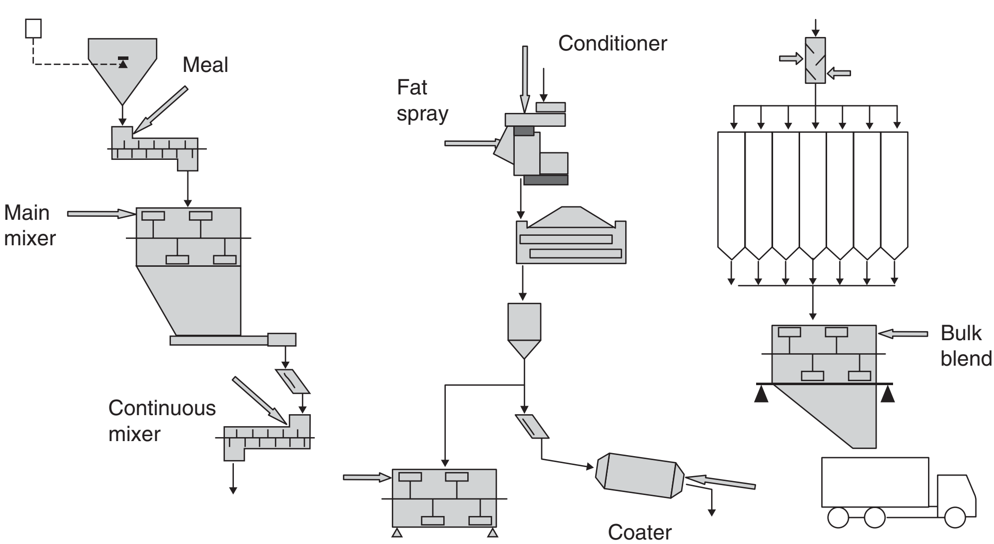

Fig. 11.7. Liquid addition in feed processing.

be injected from at least three different places in the mixer.

When the fat level in the diet is >20–30 kg t^{-1}, it is advisable to use mechanical devices to add the extra fat after pelleting because high fat addition in the mixer impairs pellet quality. Because of its lubricating effect, it is convenient to distribute the fat supply at several places. Generally, fat is first added in the mixer. A second addition point can be at the pellet mill outlet to take advantage of the fact that the pellet is still hot and its absorption capacity is high. This addition system is known as ‘fat spray’, and 20 kg fat t^{-1} fat can be added at this level. Another possible fat addition stage is once the pellet is cool, but here a special mechanism based on a coater where the fat is sprayed onto the pellet is necessary. At this stage, the fat absorption capacity of the pellet is low because it is already cool; in order to increase this capacity, it is convenient to warm the equipment slightly. Pellet thickness is directly related to absorption capacity. Therefore, a 3 mm pellet shows a specific surface that is double that of a 6 mm pellet, and its absorption capacity is 50–100% higher (Walter, 1990).

11.5.2 Molasses

Molasses is added after the main mixer. Small amounts of molasses (20–30 kg t^{-1}) can be added on a continuous mixer placed just after the main mixer, while another 20–30 kg t^{-1} can be added on the pellet conditioner just before pelleting. The inclusion of molasses must be automatically controlled because this is a continuous process and the mash flow through the machine determines the amount of molasses to be added. The longer the mixture remains in the machine and the more regular the advance of the product, the better the quality of the mixture. Because of a high level of sugars, molasses can be caramelized to a solid state. This process occurs when temperatures are >50–60°C; therefore, these temperatures must not be exceeded when adding molasses.

11.5.3 Amino acids

Thermoresistant liquids that are added in small amounts, such as amino acids or choline, must be added in the mixer. Choline should be given special attention due to its aggressive action against other vitamins.

11.5.4 Liquid flavours

Liquid flavours are ideally added after pelleting, in order to keep their aromatic profile.

11.5.5 Enzymes

An important benefit for the poultry industry has been the introduction of enzymes designed to improve the digestibility of diets including barley or wheat. These benefits are not so clear in rabbits, but it is possible that new enzyme activities will be developed for this species. Enzymes can be added as a powder or liquid. As a powder, there is uncertainty about the stability of the product after thermal treatment (pelleting, expansion). To avoid this problem, apart from the technological improvements on the thermal stability of these additives, enzymes can be added as liquids at the cooler outlet, when commercial equipment is available.

11.5.6 Other considerations

Liquid addition is normally through volumetric devices, so some measures must be considered:

- To determine exactly and periodically the product density, because when formulating only weights are used, never volumes.
- To control the amounts added by weighing.
- To have the proper measuring equipment available for assessing flow.

11.6 Pelleting

Pelleting is not a single process; it is the combination of three independent processes that always operate in the following sequence: mash conditioning – pelleting – cooling.

The general objective of the process known as pelleting is to turn mash feed into compact pellets. Crumbling is a final process that is occasionally applied to pellets when the intention is to revert back to a mash-type feed presentation. Rabbit feed is always produced as pellets, with good-quality pellets (a low proportion of fines) and a 3–4 mm diameter.

11.6.1 Conditioning

Mash conditioning is achieved by the addition of steam to the conditioner placed on top of the pellet press. Conditioners are cylindrical containers placed horizontally on top of the press (Fig. 11.8).

There are different types of conditioners with varying parameters, including: (i) volume of the conditioner; (ii) number of conditioners; (iii) inside configuration; (iv) outside coating and heating devices; (v) long-term devices; and (vi) friction-type conditioners.

Conditioning of the mash feed is a consequence of steam addition at levels between 2% and 5%, depending on the type of raw materials used. Mash temperatures rise depending on the amount added. Retention time is the second factor influencing conditioning because it is the time in which mash feed remains heated by steam addition. Optimum conditioning of mash varies depending on the type of raw materials in the formulation: high-starch formulas are able to absorb higher amounts of steam than high-fibre formulas. Retention time is independent of temperature achieved and has more to do with the length of the conditioning period, which has a large influence on the mash.

The consequences of both parameters on mash feed are seen on the following:

- Physical properties: conditioned mash increases its plasticity, is less abrasive and tends to be stickier than non-conditioned mash. It should not get wet because this can cause problems later during pelleting. Fibre tends to become stickier than starch.
- Chemical properties: starch gelatinization can be partially caused by temperatures >60°C. Protein stability may be affected if high temperatures are achieved. Enzyme and vitamin stability can also be affected if over-processing occurs.
- Microbiological conditions: natural microbiological contamination of the raw materials will be lower after conditioning. Specific conditioning processes are used to substantially sterilize mash feed for other types of feed production.

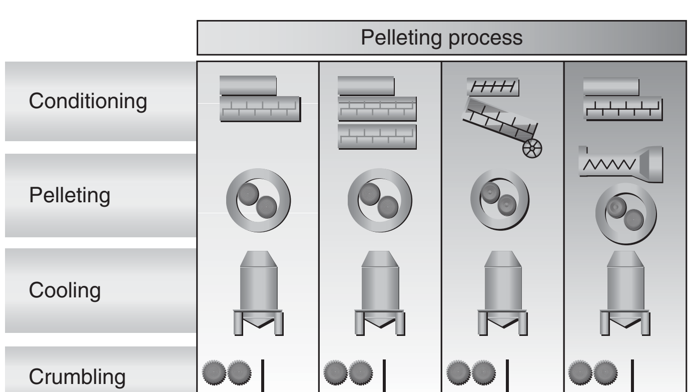

Fig. 11.8. Alternative conditioning systems before pelleting.

As a rule of thumb, the higher the temperature and the longer the retention time, the more the mash feed will be conditioned and the subsequent pelleting process will be improved. Currently, a wide variety of conditioners are available to feed manufacturers and equipment should be installed in the feed mill depending on the specific targets and logistic situation of each manufacturer. Retention time can vary from 20 s on a single-type conventional conditioner to 240 s on a three-step-type long-term conditioner with an external heat coating.

Mash temperature increase will depend on the raw materials in the formulation. The amount of steam added will depend on its water absorption capability, which varies not only between different types of raw materials but also within raw materials of the same group. Grains absorb higher amounts of steam than fibre raw materials. Within grains, wheat can absorb a higher amount of steam than barley or maize.

Conditioners should always be constructed with stainless steel to maximize the hygiene of the feed, allow good cleaning conditions and increase the length of service of the equipment itself. Good inside access must be allowed for cleaning and maintenance, which should be undertaken on a regular basis. Besides steam addition, specific conditioners such as expanders or extruders input energy to the feed through friction. This allows not only increases in temperature but also increases in internal pressure. This will modify the physical structure of the feed due to an increase in specific weight because of the partial gelatinization of starch.

11.6.2 Pelleting

The aim of pelleting is to transform meal into compact pellets of cylindrical shape. As discussed earlier, after conditioning of meal with steam the conditioned product is pressed by rolls to pass through the holes of the pelleting die, which gives the meal the pellet shape.

When pelleting, different parameters associated with the mash, the pellet mill or the process itself will end up affecting final pellet quality. Researchers from Kansas State University, Feed Technology Department, have shown and quantified the main influencing factors (Fig. 11.9) (Behnke, 1996). Formulation is the main factor, but this includes not only the raw materials in the mash feed but also how these have been ground. The particle size of the mash arriving at the pellet mill has a definite influence and should be closely watched. Large particle size (>1.5 mm) hinders pelleting. On the other hand, very small particle size can promote digestive problems in the rabbit. As a consequence, a compromise between both extremes must be sought.

The physicochemical characteristics of the raw materials included in formulations influence the pelleting capacity of

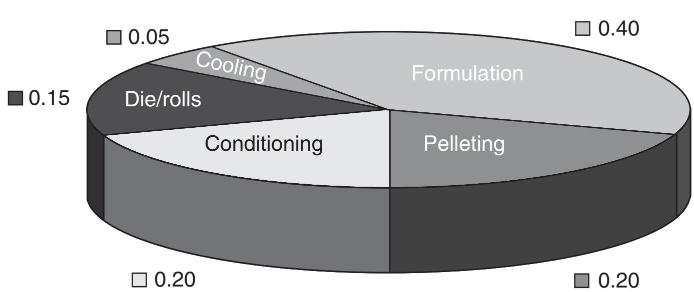

Fig. 11.9. Main influences on pellet quality (Behnke, 1996).

the feed. Ingredients with a high fat level have a bad influence on pellet quality, because the lubricant effect increases the rate of mash flow through the die. The fibre content of ingredients also has to be watched, because the influence is greater according to the type of fibre rather than the rate of inclusion. Lignified fibre tends to impair pellet quality. Ingredients with high cellulose levels are more flexible on processing and the trend is towards a better pellet quality.

As far as starch is concerned, gelatinization starts at around 60°C, and this process favours pelleting. However, starch sources vary as far as their pelleting ability is concerned. Among grains, wheat gives the best pellet quality while maize gives the worst, with barley intermediate. Soybean meal is normally low in oil content; its variation can affect the pellet quality. Oil seeds such as soybean and sunflower have a high oil content, which impairs pellet quality. Among fibrous sources, straw promotes bad-quality pellets, while lucerne and beet pulp favour good-quality pellets. At low levels of inclusion and high temperatures, molasses improves pellet quality because of sugar caramelization.

Beside pellet quality, raw material inclusion on formulation has a big influence not only on pellet quality (Table 11.3) but also on throughput flow on the die (capacity) and abrasiveness effects on dies and rolls (wear). Minerals are abrasive, especially limestone, and therefore small particle-size limestone must be used. Because of their high fibre content, oats are also abrasive for the die. Manioc can contain significant amounts of silica, which is again very abrasive for the die and shortens its operating life.

Considering the influence of raw materials on pellet quality, modern feed mills use a larger number of raw materials than older mills. A wider range of ingredients creates a better opportunity to produce good-quality pellets. The mash density of raw materials has a considerable influence on compacting, as low-density materials tend to create more difficulties in pellet production.

Table 11.3. Pelleting properties of different raw materials (1 = low, 10 = high) (Bühler, 1996).

Raw material	Quality	Capacity	Abrasiveness
Wheat	7	6	4
Maize	5	7	4
Barley	6	7	5
Oats	3	4	7
Full-fat soybean	3	8	4
Soybean meal	6	6	5
Sunflower meal	6	5	5
Rapeseed meal	6	6	6
Palm kernel meal	6	4	6
Coconut meal	6	6	6
Groundnut meal	8	6	5
Cottonseed meal	6	6	5
Carob meal	5	2	9
Dried grains and solubles	3	4	5
Brewers' by-products	7	6	5
Meat and bone meal	4	5	7
Citrus pulp	7	4	6
Beet pulp	8	3	6
Lucerne meal	7	3	7
Wheat bran	6	4	3
Minerals	2	4	10

As stated earlier, conditioning is very important and in general treatment with the highest temperature and retention time will give better pellet quality, not only with low fines but also with good flexibility. Pellet press equipment and spare parts (dies and rolls) obviously have the greatest influence on the quality of rabbit pellets. The die holes most frequently used for rabbit feed are between 3 and 4 mm.

In addition to diameter, the length of die holes (known as compression) should be well defined because both factors must be balanced in order to obtain good-quality pellets and adequate throughput in the press. For a given die hole diameter, more compacting will be achieved with longer lengths and a lower press throughput will be obtained. Energy consumption at the pellet mill is directly related to the compacting capacity and therefore it is related to the die length and hole diameter.

Rolls are used to press mash through the die, and also have a big influence on pellet quality. It is advisable to use a set of rolls with the same die because they will fit better. Die roll distance must be between very narrow limits, around 0.2 mm, in order to reach a maximum pellet mill yield. If the gap is large, pressure on the mash decreases and the product remains inside the hole for longer. This results in lower pellet yields and a risk of excessively toasting the pellet. Dies and rolls will wear and good maintenance procedures should be used, changing both at a time in order to maximize pelleting efficiency. Figure 11.9 shows the influence of dies and rolls on final pellet quality.

11.6.3 Cooling

Since pellets obtained from the pellet mill are hot (50 – 65°C) and moist (140–160 g moisture kg^{-1}), they need to be dried and cooled down before they are stored in a silo. The target is to reduce the moisture content of the pellets to the same level as the mash before conditioning. Temperature should be reduced to ambient levels. Warm pellets are very fragile and deteriorate easily. It is therefore important to avoid deep falls and violent impacts in the pipes or in the subsequent management equipment, otherwise fines will increase.

Coolers are set usually just below the pellet press to avoid the shaking of pellets through pipes or mechanical transports. Counterflow-type coolers are mostly used because of their efficiency and good treatment of pellets. Other types such as belt coolers or the vertical type are still in operation, but are no longer recommended. External air is usually taken from inside the mill and passed through pellets placed inside the cooler. Warm air is extracted through large-diameter galvanized or stainless steel constructed pipes connected to a ventilator and taken outside the mill, usually via the roof. After cooling, good-quality pellets must be passed through a sieve to remove fines that will be directed to reprocessing at the conditioner. Cooling also has an influence on pellet quality (Fig. 11.9).

11.6.4 Pellet quality

Good pellet quality is a main target for feed mills producing this type of feed due to the large influence it has on rabbit production yields. Fines have been shown to have a negative effect on sanitary conditions and are frequently blamed for digestive or respiratory disturbances when present in the final feed. As discussed earlier, different factors affect pellet quality, but most must be considered before processing starts. Problems are usually seen once pelleting has started and there are few possibilities available to change pellet quality:

- Conditioning: the amount of steam can be increased and mash temperature will rise, but excess moisture can make rolls slip on the die and pellet yield will decrease.
- Pelleting: distances between rolls and the die can be adjusted, which can increase or reduce production at the press.

The use of binders such as lignosulonates, vegetable gums, bentonites and sepiolites is sometimes used to improve pellet quality. When poor-quality pellets are obtained, the different process levels should all be analysed in order to improve quality. Consideration should be given to grinding, conditioning, pelleting, cooling and final feed delivery to the farms.

11.7 Other Processing Methods

11.7.1 Expansion

The expander is a thick-walled mixer pipe with an axle endowed with elements for mixing and kneading. The pipe has internal bolts and steam injector valves. The pressure is maintained thanks to the final screw, which modifies the annular gap at the end of the pipe. The expander must be installed before the pellet mill with a bypass circuit to allow the double option of pelleting the product after the expander or transporting the product directly to the cooler.

Energy consumption is lower than for the extrusion process (see next section), but higher than for the pelleting process alone, although this extra energy consumption is partially compensated for by a higher throughput of the pellet mill after expansion. During expansion, mash supports a high temperature and pressure (Fig. 11.10), which greatly facilitates the following pelleting, even when including raw materials with low pelleting ability. The expansion process has an intermediate effect between extrusion and pelleting with regard to vitamin stability.

Expansion is not as severe a treatment as extrusion. Protein denaturation is very limited, so there is not a clear effect on the thermolabile antinutritional factors (ANFs) of legumes. In the same way, starch is only partially gelatinized.

11.7.2 Extrusion

Extrusion has been widely used in the food industry and pet food manufacturing for some considerable time. Application to the feed industry is more recent, and started with oilseed processing basically for denaturing ANFs. Subsequently, extrusion developed for the manufacture of starter-type diets for non-ruminant species. The mechanical process of the extruder combines energy input to mash feed by steam and shearing forces through friction of the mash feed against the inside walls and barrels of the extruder. At the end of the extruder barrel a die is located to allow pellet shape formation of the final extruded product.

Extrusion has three main effects on the nutritional value of the treated feed:

- Proteins are denatured, without affecting the amino acids. Proteins partially lose their tertiary and quaternary structure, but amino acid availability is not altered if the process is run properly.

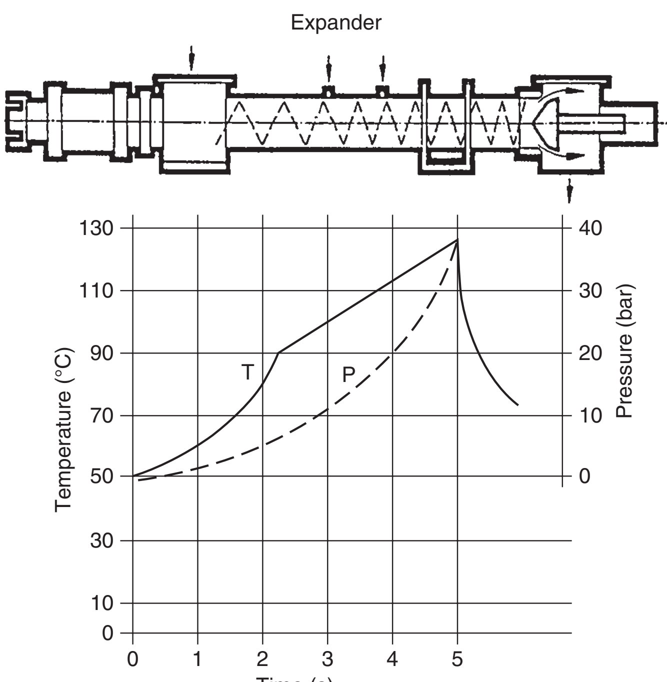

Fig. 11.10. Pressure and temperature curves during the expanding process (Pipa and Frank, 1989).

The immediate consequence is a deactivation of thermally labile ANFs.

- Starch is gelatinized. This makes it more available, especially to young non-ruminants with immature endogenous enzyme production.
- Hygiene is improved. The temperature and pressure result in a low bacterial and mould count of the feed and can partially sterilize it.

However, rabbit feeds have a low starch content and usual protein sources such as soybean meal have been previously heat treated, so extrusion is inappropriate.

The effects on structural changes occurring during the extrusion process on the fermentation pattern in the rabbit hindgut have not been widely studied. Extrusion treatment implies high temperature and the thermal stability of vitamins and other components added to the feed must be known.

Vitamin K destruction will depend on the source used, so either the most stable form is used (menadione bisulfate) or the levels are increased to reach the desired levels in the final feed. Vitamin stability is also affected by other factors, such as the presence of choline chloride in the vitamin-mineral premix. Vitamin K is especially susceptible to choline chloride. Coelho (1996) determined that vitamin K content decreased by 47% of the original activity after 2 months of storage when choline chloride was included in the premix, whereas the activity remained at 98% of the original value when choline chloride was excluded from the premix. This effect will vary according to the choline chloride level in the premix, as well as the proportion carried on the premix. The addition of choline chloride directly to the main mixer in a liquid form can be a useful means of avoiding these problems.

11.8 Feed Presentation

Good-quality pellets are needed because of the aversion of rabbits to fines (Lebas, 1975): if a high proportion of fines is present in the final feed, rabbits almost stop eating for 2–3 days. This is why there is currently virtually no discussion on feed presentation to the rabbit; it is universally accepted that presentation must be as pellets and this is the only physical presentation form used commercially in industrial rabbit production. When considering small-scale rabbit farms without any feed-purchasing possibility, it is more profitable either to use locally grown raw materials or to supply the raw materials as such without any grinding. Whenever essential particle size reduction to facilitate feed manipulation is considered, grinding must be very coarse to prevent as much as possible the presence of fine particles.

An important practical point to consider is the ideal pellet size for the rabbit. A pellet diameter >5 mm promotes feed losses because the animal discards such pellets (Lebas, 1975). Several authors (Maertens, 1994; Maertens and Vermeulen, 1995) have attempted to clarify the situation for smaller diameters by comparing 2.5, 3.2 and 4.8 mm. At weaning, there were no statistically significant differences between the different diameters as far as growth and feed conversion rates are concerned. However, there was a negative trend as the diameter fell, which contradicts the general opinion that a small pellet favours feed intake of rabbit pups. After weaning, the best technical response was obtained with 4.8 mm pellets. Furthermore, if pups consumed a 4.8 mm pellet before weaning and they were then moved to a 2.5 mm pellet, the growth rate decreased. Therefore, the growing/finishing feed for rabbits must never have a diameter lower than the doe–pup feed.

As a general rule, it is advisable to use a pellet diameter between 3 and 5 mm. Pellet length will be between 2- to 2.5-times pellet thickness, that is to say from 6 to 12.5 mm. If the length is greater, the animal can waste feed because of poor cutter adjustment; when trying to eat the pellet, after biting it the rest of the pellet falls down. Another practical rule is to use the same pellet diameter for all rabbit ages and physiological stages. Rabbit feed should be sieved just before bagging or loaded onto bulk trucks to remove fines that could still be present after the cooling and transport of pellets to the loading area of the mill.

11.9 Quality Control

The main objective of a feed mill is to supply properly manufactured compound feeds to customers whenever they need them, and consistently including the necessary nutrients to satisfy animal requirements according to the kind of production (Jones, 1996). Consequently, quality control must cover all of the processes and services that facilitate the fulfilment of this objective, not only based on chemical analysis of raw materials and final feeds. This concept would represent more a quality assurance than a quality control.

Quality assurance is expanding in all economic sectors, including feed manufacturing. Currently, there are several systems that try to guarantee this quality to the consumer. In this way, the ISO 9001 and 22000 rules and others give a guarantee to the consumer that a processing methodology exists and that this methodology is externally audited. There are also other systems, including the Dutch rules on good manufacturing practice (GMP), which have the advantage that they are more orientated to the feed mill industry. It is a company decision to ask for this kind of certification, but, in any case, it is of fundamental importance to carry out these working philosophies in feed milling. GMP is a very wide area but, in general, the following documents should be available:

- Specifications for the raw materials received, previous mixes, additives and medicated premixes.
- Specifications for the feeds to be supplied.
- A descriptive diagram of the feed manufacturing process, with critical points identified.
- A precise description of all the controls and inspections at the critical points of the production process from raw material until the final product.
- A description of the measurement methods to be used in controls, indicating, if necessary, the regulations or bibliography on which they are based.

Development of these approaches, as well as the regulations on residues, diffuse pollution and other aspects of feed milling, are very important for the quality assurance of rabbit feed.

11.10 Raw Material and Feed Control

As previously discussed, the feed manufacturing process consists of particle size reduction (grinding), blending of raw materials (mixing) and feed shaping (pelleting). There is no process that significantly transforms the raw materials; there is only a partial starch gelatinization and/or protein denaturing. Therefore, the nutritional quality of feeds depends on the nutritional quality of the raw materials and, consequently, their control is decisive in feed manufacturing.

Raw material quality must be evaluated through two different methods: (i) analytical values; and (ii) organoleptic parameters. All raw material loads that come to the feed mill, either by truck, railway or ship, must be sampled. The first evaluation of the raw material before unloading is the organoleptic evaluation. This includes the following:

- A check if load identification is correct.
- Detecting the presence of foreign materials such as other raw materials, soil, metals and a great diversity of substances that can contaminate the raw material during the harvest, transport or production process.
- Detecting the presence of insects.
- Recognizing colours that do not correspond to the raw material. These can be due to defects of the process.
- Identifying off or strange odours, which may be due to previous fermentation.

The reception operator of raw materials should thoroughly understand this subject, because it a basic tool for the quality assurance of the feed.

Chemical analysis of raw materials will help to assess their nutritional quality, either directly or indirectly, to complete the quality control. The information obtained can be classified according to origin, supplier and so on. Factors that should be routinely analysed in raw materials as well as in final feeds are moisture, crude protein, fibre, ether extract and ash, as well as urease activity in soybean meal. Other analysis can be sporadically undertaken at the feed mill or external laboratories for amino acids, minerals, vitamins, fatty acid profiles, other type of carbohydrates and so on. Periodic controls of different ANFs of the raw materials used should also be undertaken, including tannins, antitrypsin factors, alkaloids and glucosinolates, depending on the feedstuff used.

11.10.1 Moisture

Water contents >140 g kg^{-1} stimulate microorganism growth, particularly of fungi, which can produce mycotoxins. Another reason to control moisture is that the dilution of the nutritive value of the feedstuff, and therefore a moisture level over that specified in the contract, will result in economic losses.

11.10.2 Crude protein

Protein variation in a raw material, as well as in the feed, implies amino acid compositional changes that can be important in terms of the performance of the animals. Rabbits are sensitive not only to protein deficiency, but also to protein excess because of the risk of digestive disturbances.

11.10.3 Crude fibre

An increase in fibre reduces digestibility and therefore the nutritive value. Because rabbits have specific fibre requirements for the regulation of intestinal motility, this is an important parameter to control not only in feedstuffs, but also in the finished feed. As discussed in other chapters, other kinds of fibre analysis (e.g. acid detergent fibre, neutral detergent fibre) or analysis of fractions (e.g. lignin) may be useful, especially for raw materials with an extreme content of any kind of these fractions (e.g. sugarbeet pulp is high in hemicelluloses, grape marc is rich in lignin), where abnormal intestinal behaviour can be expected.

11.10.4 Ether extract

This analysis is an indicator of the fat content. Because of the high energetic value of fats, it is important to know precisely not only the amount but also the quality of this fat. Raw materials that are very rich in fat, such as oilseeds, must be frequently limited because of their variation according to their origin. At the feed mill, when volumetric systems for fat addition are used, it is necessary to increase controls to prevent deviations from expected formulations.

11.10.5 Ash

This analysis is an indication of the mineral content. Higher than normal figures can indicate some degree of contamination, and a complementary analysis on insoluble material for clorhidric acid would suggest the presence of silica (soil). In feeds, figures under or over those expected indicate incorrect additions of limestone, phosphate or salt. It is remarkable how important it is to have an accurate system for salt addition, because this can be the main reason for feed rejection problems by rabbits. An excess as well as a deficiency can cause problems.

11.10.6 Analysis

Information obtained from chemical analysis must be available as fast as possible to be effective. Action must be taken soon after a deviation from the expected values has been identified because the figure is not so useful after an excessive time. In any case, it is good to have historical values, even if not so recent, in order to identify trends according to the supplier or the year of harvest and hence act to prevent problems that may arise.

As far as fast analysis is concerned, the near infrared reflectance technique allows online information if connected to the production line and, if not, supplies information in a few minutes. Another fast and low-cost technique is microscopy, which allows a qualitative and sometimes semi-quantitative evaluation of feed ingredients. This technique is very useful to detect contamination or adulteration in raw materials (Bates, 1994).

Microbiological analysis (of moulds, yeasts, coliforms, enterobacteria, *Salmonella* and so on) is essential, because rabbits are very sensitive to the bacteriological quality of their feed. With all livestock feeds, it is necessary to ensure the absence of microorganisms that can cause pathological problems, especially those that can transmit disease to the consumer.

Due to the low persistence of *Salmonella* in feed, analytical control is difficult, so it is more interesting to assess enterobacteria, which gives an insight into the effectiveness of treatments. After pelleting, the target value is <100 c.f.u. g^{-1}, with action necessary if values are between 100 and 1000 c.f.u. g^{-1}.

Mycotoxins are metabolites produced by different fungi. They produce various illnesses in animals that consume them and can be harmful at very low concentrations (parts per billion). There are many fungi, but those that can really cause problems are *Aspergillus*, *Fusarium* and *Penicillium* species (Meronuck and Concibido, 1996). The most frequent mycotoxins produced by *Aspergillus* are aflatoxins, specifically B1 and B2. From the genus *Fusarium*, the most common mycotoxins are the trichothecene family, especially T-2, as well as zearalenone and deoxynivalenol.

There is little information available on the effects of mycotoxins in rabbits. Most data come from other species, but, generally speaking, the mycotoxins included in the international regulations are the aflatoxins, where a maximum limit has been established in order to allow a raw material to be marketed. Different countries have limits for other mycotoxins, but currently it is still difficult to establish safe limits for rabbit health and residues. In 2006, the European Union gave guidance values for deoxynivalenol, zearalenone, ochratoxin A, T-2, HT-2 and fumonisins (*Official Journal of the European Union*, August 23, 2006). Mycotoxin detection is an indicator of mould growth in the raw material or in the feed analysed, but many other mycotoxins than those detected can be present.

Mycotoxin control can be undertaken by chromatography and different commercial kits are available to quantitatively determine the presence of the most frequent and dangerous mycotoxins.

11.11 Pellet Quality

Because of their particular way of consuming feed, rabbits are extremely sensitive to the presence of fines in the feeder; when fines are present, they can enter the respiratory system and cause respiratory problems. To solve this situation, feeders with small holes at the bottom that allow the fall of feed fines are used. Unfortunately, this implies a feed loss because the feed falls into the faeces pit. This loss will increase the feed conversion ratio proportionally to the fines content of the feed. This is the reason why a high-durability pellet must be produced at the feed mill. However, pellet hardness must not be too high because this can cause rabbits, especially rabbit pups, to refuse the feed.

Different methods can be used to measure pellet durability and hardness. Hardness is defined as the pellet's resistance to pressure breakage. This parameter is measured by means of a spring device (hard meter), where pressure is gradually increased on the pellet with a screw, up to when the breakage occurs. The pressure on the pellet is recorded on a scale. This parameter assesses pellet hardness, but not durability. A very hard pellet with a low elasticity can be very fragile and can produce a high amount of fines.

The determination of pellet durability is done with a simple mechanism that was originally developed by Professor H.B. Pfost at Kansas State University, and is currently used at many feed mills with minor changes

(Schultz, 1990). This equipment consists of a normalized box, which turns at a rate of 50 rpm for 10 min, with a specific amount of feed inside. Subsequently, the pellets and fines obtained are weighed. As a general rule, pellet durability for rabbit feed must not be <97%. Devices such as the Holmen pellet tester also exist. This can undertake online measures when the pellets are produced and, with this information, corrections on one or several parameters can be made. Both systems are reliable, and each feed compounder should generate its own figures and draw up the optimum standards for every batch of pellet feeds produced (Acedo-Rico, 2006).

11.12 Feed Labelling

Substantial legislation exists on feeds and their labelling rules. The objective is to inform the purchaser on feed composition, the inclusion of additives, recommended periods of use and any withdrawal period, if necessary. Another basic objective of the legislation is to guarantee that feeds do not contain undesirable substances or microorganisms, either for the animals or the consumer. Specific legislation exists for medicated feeds, the main aims of which are the obligatory nature of using licensed products and their inclusion in the feed through veterinary prescription.

11.13 Processing Control

Each feed mill must establish its own processing control, well adapted to its specific requirements and the capacity to follow it (e.g. personnel, laboratory). As a general rule, it is necessary to take into account the following aspects (Jones, 1996):

- Raw materials inventory, which must be taken at least once a day. This measure allows the mill to detect if any raw material has not been properly used.
- Silo-bin cleaning. The frequency will vary according to the raw material stored.
- Checking the equipment at the cleaning stage.
- Grinding.
- Weighing systems.
- Mixing.
- Pelleting and cooling.
- Conditioning temperatures.
- Truck cleaning and control.

The following steps must be followed when a problem arises:

1. Is the analysis correct? First, the laboratory result must be checked or repeated.
2. How was the sample taken? The sample may have been incorrectly taken and it is advisable to repeat the analysis on another sample from the same batch.
3. Is only one nutrient or are several nutrients out of the established range? The latter may indicate the absence of a raw material from the formulated feed.
4. Was the process undertaken by the usual person?
5. Check the inventories to rule out discrepancies that could indicate mistaken identifications.
6. Check the measurement equipment.
7. Check the raw material and finished feed silo bins.
8. Re-evaluate the mixing times.
9. Check the values of the raw materials used to detect possible sporadic lot failures.
10. Check the formulation of the raw material matrix and the formulation itself to verify that the figures are up to date.

It is obvious that if serious problems arise, it is necessary to proceed rapidly to avoid animals consuming a defective feed. Rabbits are probably one of the most sensitive species to feed faults.

11.13.1 Process controls

Documentation is important not only to guide the feed compounder in what is to be done, but also to show to external inspectors how the process works at the feed mill:

- Control of documentation: documents that are required by the feed safety system must be maintained.

- Control of records: records must be established and maintained to provide evidence of compliance with requirements and of the effective operation of the feed safety system.

Management responsibility

Senior management must demonstrate their involvement in the development and implementation of the feed safety system and the continuous improvement of its effectiveness.

Management of resources

Personnel who perform work affecting feed safety must be competent, based on appropriate education, training, skills and experience. The company must have sufficient personnel with the skills and qualifications required for the production of safe feed.

Production must be carried out in an environment in which it is not possible for the presence of potentially hazardous substances to lead to an unacceptable level of those substances in feed.

Production buildings may not stand on or near places that clearly present a danger to feed safety, such as contaminated sites, waste sites and so on. If the environment entails risks for feed safety, the company must show by way of a risk analysis that the risks are sufficiently controlled.

Work environment

- Clearing: dust, dirt and feed remains can form a major breeding ground for bacteria that can contaminate feed materials. The accumulation of such substances must therefore be avoided as much as possible.
- Cleaning: cleaning programmes must be introduced. These should describe responsibilities and methods, frequency and times.
- Pest control: effective programmes must be used for combating harmful organisms. Everything that is reasonably possible and effective must be done to keep birds, pets and vermin away from production areas.
- Waste control: waste and material that is not appropriate as feed must be identified as such and kept separate. If such materials contain hazardous concentrations of feed medicines, contaminants or other hazards, they must be removed in a proper fashion and may not be used as feed. Waste must be collected and stored in clearly designated bins or containers. Places in which waste is collected and stored must be included in the cleaning and disinfection programmes.

Identification and traceability

The factory must take appropriate measures to ensure that the feed produced can be traced effectively. It must maintain a register with the relevant details with respect to purchase, production and sale, which can be used to trace the feed from reception to delivery, including export to the final destination (Product Board Animal Feed, 2008c).

11.14 Carry-over Control

In order to avoid carry-over (or cross-contamination) it is necessary to know the factory risk situation and its limits to avoid it. When measuring the carry-over of additives and veterinary medical products in an installation, there must be an examination using a diagram and the actual layout of the factory to determine which areas may be subject to carry-over. A basic principle in determining carry-over in a factory is that the degree of carry-over as a result of return flows is known and controlled.

The greatest carry-over of additives and veterinary medical products occurs in the weighing process, mixing and transport. The place where premixes are added should be as close to the mixer as possible. In addition, a considerable amount of carry-over can occur in the press line and during loading.

The most popular method of controlling carry-over is based on cobalt. The control procedure includes processing three batches from the same feed mix. The first batch serves to determine the natural cobalt level in the feed in question. A cobalt mix is added to the second batch and the third production batch gives a picture of the carry-over in the installation (Product Board Animal Feed, 2008a). If the undesired carry-over of critical additives and veterinary medical products is expected then a company may take measures by drawing up a mandatory production sequence.

11.15 Hazard Analysis and Critical Control Points

A hazard can be describe as the contamination of animal feed, or a condition leading to the contamination of animal feed, with possible negative implications for human or animal health (Product Board Animal Feed, 2008b).

Three types of hazard can be defined:

- Chemical: residues of pesticides, hormones, additives and veterinary drugs, heavy metals, environmental pollution, mycotoxins, polychlorinated biphenyls, dioxins, cleaning agents, lubricants, mineral oils and so on.
- Microbiological hazards: veterinary risk (animal diseases) and pathogenic organisms such as *Salmonella*, enterobacteria and fungi (the latter group as indicator organisms).
- Physical hazards: glass, plastic, metal parts, stones, bone and pieces of packaging.

From these three types of possible hazard, the HACCP team should determine which are actually a risk – this is a risk assessment. The word 'risk' is defined by two elements: the seriousness and probability of a potential hazard. The hazard must be of such a nature that it realistically could be expected to occur (probability) and that eliminating or reducing it to an acceptable level is essential for manufacturing safe animal feed (seriousness).

References

Acedo-Rico, J. (2006) Problemática de utilización de materias primas fibrosas en fabricas de piensos y plantas de mezclas, influencia sobre la tecnología de fabricación a emplear. *FEDNA Proceedings*. Fundacion Española para el Desarrollo de la Alimentacion Animal. Madrid, Spain, pp. 67–83.

Bates, L.S. (1994) Microscopia de alimentos: secretos para un rápido control de calidad. *Avicultura Profesional* 11, 152–156.

Behnke, K.C. (1996) Feed manufacturing technology: current issues and challenges. *Animal Feed Science and Technology* 62, 49–57.

Bühler, A.G. (1996) *Manual de Tecnología de Fabricación*. Schule für Futtermitteltecnik, Uzwil, Switzerland.

Coelho, M. (1996) Stability of vitamins affected by feed processing. *Feedstuffs* 68, 9–14.

Jones, F. (1996) Quality control in feed manufacturing. *Feedstuffs Reference Issue* 68, 135–138.

Lang, J. (1981) The nutrition of commercial rabbit. Part 1. Physiology, digestibility and nutrition requirements. *Nutrition Abstracts and Reviews, Series B* 51, 192–225.

Laplace, J.P. and Lebas, F. (1977) Digestive transit in the rabbit. 7. Effect of fineness of grind of ingredients before pelleting. *Annales de Zootechnie* 26, 413–420.

Lebas, F. (1975) *Le Lapin de Chair, ses Besoins Nutritionales et son Alimentation Pratique*. ITAVI, Paris, France.

Lebas, F. and Laplace, J.P. (1975) Digestive transit in the rabbit. 5. Variation in fecal excretion according to time of feeding and level of restriction of feed during five consecutive days. *Annales de Zootenhnie* 24, 613–627.

Maertens, L. (1994) Effect of pellet diameter on the growth performance of rabbits before and after weaning. In: *6emes Journées de la Recherche Cunicole en France*. Paris, France, pp. 325–332.

Maertens, L. and Vermeulen, A. (1995) Influence du diamètre du granue sur le croissance des jeunes. *Cuniculture* 237–241.

Mateos, G.G. and Rial, E. (1989) Tecnología de fabricación de piensos compuestos. In: *Alimentación del Conejo*. Mundi-Prensa, Madrid, pp. 101–132.

Meronuck, R. and Concibido, V. (1996) Mycotoxins in feed. *Feedstuffs Reference Issue* 68, 139–145.

Pairet, M., Bouyssou, T., Auvergne, A., Candau, M. and Ruckebusch, Y. (1986) Stimulation physico-chimique d'origine alimentaire et motricité digestive chez le lapin. *Reproduction Nutrition Development* 26, 85–95.

Pipa, F. and Frank, G. (1989) High-pressure conditioning with annular gap expander. A new way of feed processing. *Advances in Feed Technology* 2, 22–31.

Product Board Animal Feed (2008a) *GMP+ Certification Scheme Animal Feed Sector 2006*, Appendix 4: Minimum requirements for Inspections and Audits. © *PDV Including Protocol for the Measurement of Carry-Over*. Available from: http://www.gmpplus.org/en/gmp+_fsa/b-documents.php (accessed 20 January, 2010).

Product Board Animal Feed (2008b) *GMP+ Certification Scheme Animal Feed Sector 2006: HACCP Manual,* Appendix 15. Available from: http://www.pdv.nl/lmbinaries/bijlage_15-uk-.pdf (accessed 18 January, 2010).

Product Board Animal Feed (2008c) *GMP+ Certification Scheme Animal Feed Sector 2006. GMP Standard B1. Production & Processing of Feed for Productive Livestock*. Available from: http://www.pdv.nl/lmbinaries/gmp_b01_-uk-.pdf (accessed 18 January, 2010).

Schultz, R. (1990) The progressive animal feed production and its fundamentals. Pelleting in practice. *Advances in Feed Technology* 3, 6–33.

Walter, M. (1990) The inclusion of liquids in compound feeds. *Advances in Feed Technology* 4, 36–48.

12 Feed Formulation

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12.1 Introduction

This chapter deals with nutritional allowances under practical conditions for intensive meat rabbit production. In recent years, the performance of intensively reared rabbits has regularly increased because of improvements in genetics, management and pathology. Currently, the rate of improvement in productivity is comparable to that obtained in other intensively farmed domestic species. Breeding does are able to wean >60 pups and produce ten times their weight in milk per year, whereas the fast growth rate allows multiplication of their birth weight by 40–50 at the end of the fattening period (60–70 days of age). Rabbits are herbivorous animals and require a high dietary fibre content (about one-third of cell wall constituents on an as-fed basis) to prevent digestive disorders. Furthermore, rabbit diets must be designed to allow a sufficient nutrient intake to meet the high nutritional requirements per unit of body weight. Therefore, factors affecting feed consumption, such as nutrient imbalances, inadequate raw material composition and pellet quality, are a major concern in this species. The average composition of commercial feeds in Spain (Table 12.1) reflects this situation, as feeds typically simultaneously contain a high proportion both of fibrous and highly concentrated ingredients.

Prior to establishing practical feeding standards, the effect of varying dietary energy and nutrient content on rabbit performance will be discussed. This information allows the formulation of diets on a performance–cost basis according to market prices. The effects of diet composition on meat quality and pathology should also be considered, as reviewed in Chapters 9 and 10.

12.2 Level of Fibre

Rabbits are capable of achieving a good growth performance on high-fibre diets as a result of their peculiar digestive physiology (see Chapter 1). As shown in Fig. 12.1, maximal growth rates are reached with diets containing around 180–210 acid detergent fibre (ADF) g kg^{-1}, which corresponds to approximately 9.7–10.3 MJ digestible energy (DE) kg^{-1} when no fat is added.

Above this fibre level, fattening rabbits are not able to maintain DE intake. High-fibre diets (350 g ADF kg^{-1} dry matter (DM)) decrease the average daily gain and feed conversion rate by 30% and 50%, respectively, as compared with diets with the optimal values. This impairment might be higher in young animals (de Blas *et al.*, 1995; Feugier *et al.*, 2006). High-fibrous diets are frequently formulated to limit the incidence of diarrhoea. However, several

Table 12.1. Usual range of ingredient composition of feeds for rabbits in Spain (g kg^{-1}).

Ingredient	Amount
Cereal grains[a]	100–200
Animal and vegetable fats	5–30
Molasses	0–30
Beet, apple and citrus pulp, soy hulls	0–100
Cereal co-products[b]	150–350
Lucerne hay	150–300
Lignified fibrous co-products[c]	50–150
Protein concentrates[d]	120–220

[a]Mainly barley and wheat.
[b]Mainly wheat bran, maize gluten feed and distillers' co-products.
[c]Mainly wheat straw, olive and grape co-products.
[d]Mainly sunflower, soybean, rapeseed and palm kernel meal.

studies have shown that an increase in dietary ADF content from 190–210 to 240–260 g kg^{-1} in fact increases fattening mortality (Gutiérrez *et al.*, 2002; Romero *et al.*, 2009) and sanitary risk (mortality plus morbidity; Feugier *et al.*, 2006) and leads to an impairment in the structure of the mucosa (Álvarez *et al.*, 2007). Conversely, a minimal content of dietary fibre is required to decrease total and caecal mean retention time (see Chapter 5) and to maximize DE intake and weight gain (Fig. 12.1). An adequate fibre level also dilutes dietary and ileal starch and protein content and reduces total microbial growth (García *et al.*, 2000), digestive disorders and fattening mortality (see Chapter 10).

Three long-term studies (>1 year) conducted with rabbit does have compared seven diets containing from 162 to 216 g ADF kg^{-1} and no added fat (Méndez *et al.*, 1986; Barreto and de Blas, 1993; Cervera *et al.*, 1993). The results indicate that rabbit does maintain DE intake by increasing consumption as the dietary fibre content increases. The type of feed had no influence on reproductive performance, but litter weight at weaning decreased (by about 11%) when dietary ADF content was >180 g (equivalent to 10 MJ DE kg^{-1}).

De Blas *et al.* (1995) studied the effect of the substitution of starch for fibre in rabbit does using five iso-energetic diets (10.6 MJ DE kg^{-1}) formulated with increasing levels of ADF (from 167 to 221 g kg^{-1}) and ether extract (from 20 to 51 g kg^{-1}) at the expense of the level of starch, which decreased from 237 to 117 g kg^{-1}. The type of diet had little effect on DM intake. However, regression analyses indicated that dietary levels of neutral detergent fibre (NDF), ADF and starch of around 320, 170 and 180 g kg^{-1}, respectively, were optimal for maximal reproductive performance,

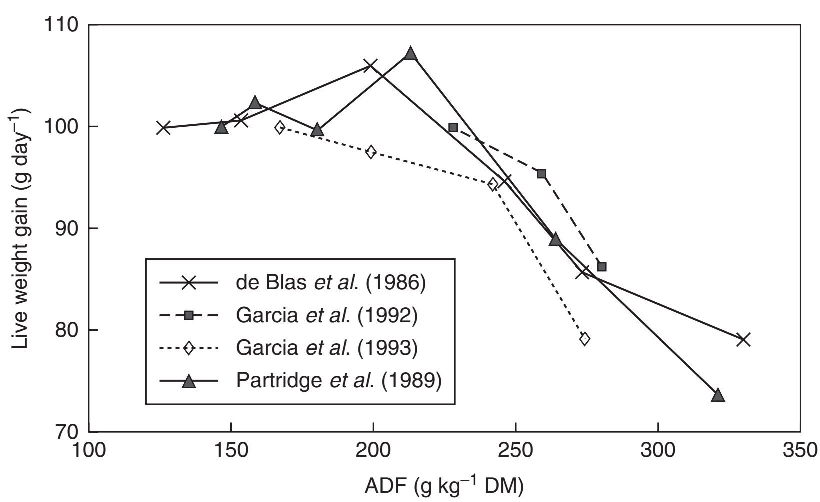

Fig. 12.1. Effect of dietary acid detergent fibre (ADF) content on average daily gain during the fattening period (base 100 = control diet). DM, dry matter.

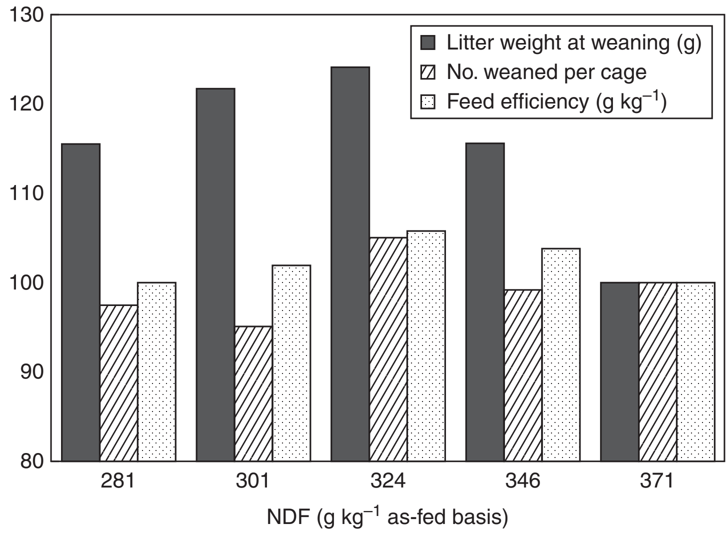

Fig. 12.2. Effect of dietary neutral detergent fibre (NDF) content on the performance of rabbit does and feed conversion rate (base 100 = diet containing 371 g NDF kg^{-1}) (de Blas *et al.*, 1995).

growth of young rabbits and feed efficiency (Fig. 12.2). The impairment observed in rabbits fed the highest levels of fibre might be explained by higher fermentation losses in the caecum, together with an insufficient uptake of glucose from the gut to meet the requirements for pregnancy and milk lactose synthesis. The negative effects of high starch concentrations in the diet were related to an increase in the incidence of diarrhoea.

12.3 Type of Fibre

Several studies have shown that cell wall composition and physical structure influence feed digestion in iso-fibrous diets (see Chapter 5). Lucerne hay is the most widely used fibre source in rabbit diets, accounting for around one-quarter of commercial feeds in Spain (see Table 12.1). Lucerne hay is highly palatable and provides both long and digestible fibre, which allows an adequate transit time of the digesta and a balanced growth of the caecal flora.

Dietary inclusion of fibrous by-products at levels of 100–150 g kg^{-1} has little effect on rabbit performance (Motta *et al.*, 1996; García *et al.*, 2002; Nicodemus *et al.*, 2002). However, an excessive substitution of lucerne hay with highly lignified sources of fibre depresses energy digestibility and caecal fermentative activity (García *et al.*, 1999, 2000) and impairs average daily gain and feed efficiency by 10 and 20%, respectively, in diets with a 50:50 ratio of lucerne hay to grape marc (Parigi-Bini and Chiericato, 1980; Motta *et al.*, 1996).

The inclusion of moderate levels of soluble fibre (120 g soluble NDF kg^{-1}) in post-weaning diets has been shown to improve the immune response and reduce the deterioration of mucosa after weaning, pathogen proliferation in the gut and fattening mortality (Fabre *et al.*, 2006; Gómez-Conde *et al.*, 2007; see Chapters 5 and 10). However, the substitution of high levels of lucerne hay with digestible fibre sources, such as beet and citrus pulps, increases the relative weight of caecal contents and the retention time in the gut (Fraga *et al.*, 1991; García *et al.*, 1993, 1999). As a result, there is a decrease in feed intake and performance in both fattening rabbits and breeding does (Perez *et al.*, 1994; Nicodemus *et al.*, 1999).

Similarly, a minimal proportion of particles >0.315 mm seems to be required to maximize feed intake, growth and lactation performance in rabbits (Nicodemus *et al.*, 2006).

These results indicate a benefit of combining different sources of fibre when trying to substitute a high proportion of lucerne hay in the diet (Nicodemus *et al.*, 2007). However, further research is needed to establish feeding recommendations based upon this issue.

12.4 Fat Supplementation

The effect of the addition of 30 g kg^{-1} of different sources of fat (tallow, lard, deodorized oleins or sunflower oil) in iso-fibrous diets for fattening rabbits has been studied by several authors (Partridge *et al.*, 1986; Santomá *et al.*, 1987; Fernández and Fraga, 1992). In these studies, dietary digestible protein (DP) content was increased with fat addition to keep the DP:DE ratio as constant as possible. Fat inclusion had a positive effect on energy digestibility (5% on average) and feed efficiency (7%), but not on growth rate, as feed intake decreased by 6%. No interaction was found between the type and level of supplemental fat. Therefore, the value of fat addition to fattening feeds should be established on an energy-cost basis also taking into account the effects of fat quality on carcass quality and pellet stability (see Chapters 9 and 11).

Several long-term (9–24 months) studies (Fraga *et al.*, 1987; Maertens and De Groote, 1988a; Barreto and de Blas, 1993; Cervera *et al.*, 1993) have studied the effect of fat addition in iso-fibrous diets (200 g ADF kg^{-1}) on the performance of breeding does. The beneficial effects of fat inclusion were more pronounced for does than for growing rabbits. The inclusion of 35 g fat kg^{-1} in doe diets increased DE intake by 14.5% on average, which promoted an increase in milk yield, and litter weight at weaning by 8.5%. Neither the body weight of breeding does nor fertility or prolificacy were affected by the type of diet, but pup mortality decreased in litters with more than nine pups (Fraga *et al.*, 1987). These results indicate that the use of fat to increase the energy concentration of feeds (>11–11.5 MJ DE kg^{-1}) maximizes milk production and litter growth in highly productive rabbits when the remaining components of the diet (fibre, protein and starch) are kept in balance.

Researchers have also shown that diets enriched in n-3 polyunsaturated fatty acids either from linseed (Maertens *et al.*, 2005) or fish oil (Lleonart, 2005) decrease mortality during lactation and improve the reproductive efficiency of breeding rabbit does. Another study (Castellini *et al.*, 2003) has demonstrated a positive effect of dietary linseed supplementation on the semen quality of bucks.

12.5 Level and Source of Protein

The energy concentration of rabbit diets varies widely. Therefore, it is advisable to express total protein requirements as a ratio between DP and DE. The effect of a variation in this ratio on the performance of fattening rabbits has been studied by de Blas *et al.* (1981) and Fraga *et al.* (1983) using 12 diets containing from 7.9 to 11.7 g DP MJ^{-1} DE. Maximal DE intake and average daily gain were obtained for diets with a DP:DE ratio of 10 g DP MJ^{-1} DE (see Fig. 12.3). Accordingly, the optimal DP content should be increased from 95 to 115 g kg^{-1} when dietary DE increases from 9.5 to 11.5 MJ kg^{-1}. Dietary DP:DE ratios below and above this optimum impair fattening performance and feed efficiency. It has been reported that dietary crude protein contents of around 140 g kg^{-1} do not impair growth performance if the DP:DE ratio is maintained around 9.5–10 g MJ^{-1} and the amino acid supply is adequate (Carabaño *et al.*, 2009). Low DP:DE values (<9.5 g MJ^{-1}) also promote a curvilinear decrease in water and protein and an increase in body fat (see Fig. 12.3). On the other hand, an excess of protein content related to energy increases environmental pollution (Maertens *et al.*, 1997; Xiccato, 2006).

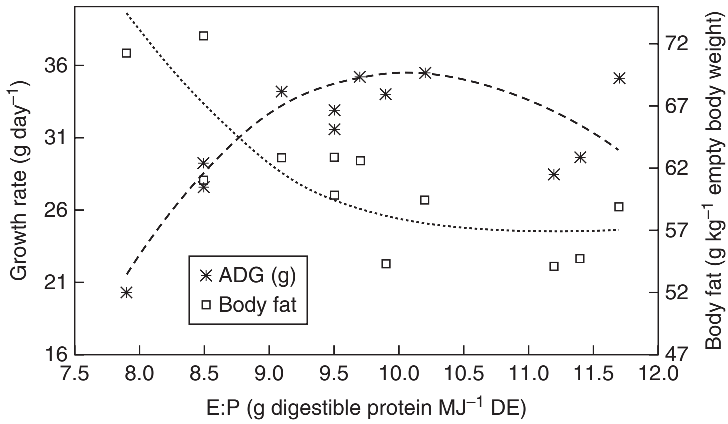

Fig. 12.3. Effect of the dietary digestible protein (DP) to digestible energy (DE) ratio on the average growth rate in the fattening period and content of fat in the empty body of rabbits at 2.25 kg (de Blas *et al.*, 1981; Fraga *et al.*, 1983). ADG, average daily gain; E, energy; P, protein.

Several studies (Gutiérrez *et al.*, 2003; García-Ruiz *et al.*, 2006; Chamorro *et al.*, 2007) have also observed that a reduction in dietary protein content or the use of highly digestible protein sources decreases ileal protein flow and reduces the proliferation of pathogens and mortality during the fattening period (see also Chapter 10).

The effects of the DP:DE ratio in breeding does have been reviewed by Santomá *et al.* (1989) and Xiccato (1996). Optimal recommended values are in the range 11.0–12.5 g DP MJ^{-1} DE; about 20% higher than that for fattening rabbits. The higher values correspond to females under intensive breeding systems. Dietary protein contents below the optimal level decrease milk production, growth of suckling rabbits and fertility and body weight of does.

12.6 Amino Acid Requirements

Until recently, no consideration was given to the quality of protein in rabbit feeds, because all the essential amino acid requirements were believed to be supplied through caecotrophy. However, soft faeces represent only about 0.14 of the total protein intake in intensively reared rabbits (see Chapter 3). Consequently, essential amino acid requirements, along with total protein, must be taken into account in practical feed formulation.

Several authors have studied the amino acid requirements of rabbits on a dose–response basis (Tables 12.2 and 12.3). Dietary amino acid content had a quadratic effect on productivity for some of the traits studied (see Fig. 12.4). This type of response indicates the negative effects of excess amino acids. This problem seems to be of especial interest for threonine. For this amino acid, a level slightly greater than the optimal reduced performance, which indicates the need of establishing a maximal concentration for this nutrient in the diet.

As for other species, there are more data available for growing rabbits than for breeding does, as well as considerable variation between different studies. Part of this variation can be explained by differences in the methods used: purified versus commercial diets, the genetic potential of the animals and the energy concentration of the diets.

Other causes of variability are related to the different availabilities of the sources of amino acids used (see Chapter 3). To take into account this effect, several studies (Taboada *et al.*, 1994, 1996; de Blas *et al.*, 1998) have determined the lysine, sulphur and threonine requirements, expressed in

Table 12.2. Total amino acid requirements of growing-fattening rabbits (g kg^{-1}, as-fed basis).

			Optimal dietary concentrations			
Reference	DE (MJ kg^{-1})	Growth rate (g day^{-1})[a]	Lys	TSAA[b]	Thr	Trp
Adamson and Fisher (1973)	–	25.5	7.0	6.0	5.0	1.5
Colin (1975)	9.41	39.2	5.8	–	–	–
Colin (1978)	11.13	37.6	–	6.3	–	–
Davidson and Spreadbury (1975)	10.46[c]	36.5	9.0	5.5	6.0	2.0
Colin and Allain (1978)	10.88	35.0	6.2	–	–	–
Spreadbury (1978)	–	41.0	9.4	6.2	–	–
Berchiche and Lebas (1994)	11.17	40.2	–	6.2	–	–
Taboada *et al*. (1994)	10.70	40.7	7.6	–	–	–
Taboada *et al*. (1996)	10.75	40.4	–	5.4	–	–
De Blas *et al*. (1998)	10.13	43.2	–	–	6.0	–

DE, digestible energy; Lys, lysine; Thr, threonine; Trp, tryptophan; TSAA, total sulphur amino acids.
[a]At the optimal amino acid concentration.
[b]Methionine must represent at least 0.35 of TSAA (Colin, 1978).
[c]Metabolizable energy.

Table 12.3. Total amino acid requirements of breeding does (g kg^{-1}, as-fed basis).

		Optimal dietary concentrations		
Reference	DE (MJ kg^{-1})	Lys	TSAA	Thr
Maertens and de Groote (1988b)	10.46	8.0	–	–
Taboada *et al*. (1994)	10.70	8.0[a]	–	–
Taboada *et al*. (1996)	10.75	–	6.3	–
De Blas *et al*. (1998)	10.13	–	–	6.4

DE, digestible energy; Lys, lysine; Thr, threonine; TSAA, total sulphur amino acids.
[a]For maximal milk production. Reproductive performance did not improve at >6.8 g kg^{-1}.

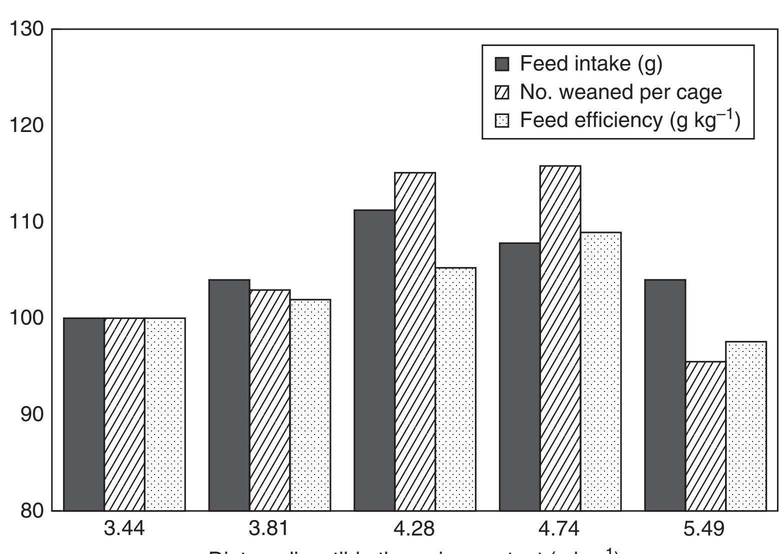

Fig. 12.4. Effect of dietary threonine content on feed intake, reproductive performance and feed efficiency of breeding does (base 100 = diet containing 3.44 g of digestible threonine kg^{-1}) (de Blas *et al*., 1998).

Table 12.4. Digestible (faecal apparent) amino acid requirements of rabbits (g kg^{-1}, as-fed basis).

	Optimal values		
Amino acid	Breeding does	Fattening rabbits	Reference
Lysine	6.4[a]	6.0	Taboada *et al.* (1994)
Methionine + cystine	4.9	4.0	Taboada *et al.* (1996)
Threonine	4.4	4.0	De Blas *et al.* (1998)

[a]For maximal milk production. Reproductive performance was not improved with levels >5.2 g kg^{-1}.

digestible (apparent faecal) instead of crude units. The results are shown in Table 12.4. Optimal values for growth were consistent with those obtained by Moughan *et al.* (1988) based on the amino acid composition of the whole body of 53-day-old rabbits (Table 12.5), although the latter method does not consider the amino acid requirements for maintenance or the amino acid supply by the caecotrophes. Although the use of digestible amino acids in practical feed formulation is still limited, recent information on this subject is provided in Chapter 8.

12.7 Recommended Nutrient Concentration of Diets

The nutrient requirements of intensively reared rabbits are presented in Tables 12.6 and 12.7. Values are given for the three types of diets more commonly used in practice: breeding does, fattening rabbits and a mixed feed for all animals. When rabbits are slaughtered at heavy weights (around 2.5 kg), more than one fattening feed is recommended. In this situation, Carabaño *et al.* (2009) proposed increasing the dietary protein and amino acid content by about 10% for the first 2 weeks after weaning (up to 11 g DP MJ^{-1} DE) to take into account the relatively higher amino acid requirements for tissue accretion, intestinal growth and maintenance of mucosa functionality. The main objective of this feed is to optimize gut health by substituting starch with fat and increasing the concentration of easily fermentable fibre (up to 120 g kg^{-1} soluble NDF or 120 g kg^{-1} hemicellulose, see Chapter 10).

Energy concentrations in Table 12.6 have been determined from estimates based on the optimal proposed levels of carbohydrates and fat. Essential nutrient recommendations have then been referred to those concentrations. However, the DE content of fattening feeds can vary from 9.7 to 11.5 MJ kg^{-1} without detriment to rabbit performance. Changes in the DE concentration with respect to the values given in this table should be accompanied by proportional parallel corrections in the contents of essential nutrients.

Minimal levels of fibre and maximum levels of starch are more critical than maximum levels of fibre and minimum levels of starch, as they affect not only performance but also mortality.

Recommendations for the type of fibre include an optimal concentration for lignin and a minimum level for long fibre particles. Both restrictions should be followed simultaneously, as some highly lignified by-products can have an insufficient content of long fibre.

Only the well-established amino acid requirements are presented in Table 12.6.

Table 12.5. Amino acid composition (mg g^{-1} nitrogen) of the whole body of 53-day-old New Zealand White rabbits (Moughan *et al.*, 1988).

Amino acids	Absolute value	Relative to lysine
Lysine	383	100
Methionine	77.5	20.2
Cystine	158	41.3
Arginine	415	108
Histidine	193	50.4
Threonine	245	64
Leucine	429	112
Iso-leucine	194	50.7
Valine	239	62.4
Phenylalanine	249	65
Tyrosine	192	50.1

Table 12.6. Nutrient requirements of intensively reared rabbits, as concentration kg^{-1} corrected to a dry matter content of 900 g kg^{-1}.

Nutrient	Unit	Breeding does	Fattening rabbits	Mixed feed
Digestible energy	MJ	10.7	10.2	10.2
Metabolizable energy	MJ	10.2	9.8	9.8
NDF[a]	g	320 (310–335)[b]	340 (330–350)	335 (320–340)
ADF	g	175 (165–185)	190 (180–200)	180 (160–180)
Crude fibre	g	145 (140–150)	155 (150–160)	150 (145–155)
ADL	g	*55*[c]	*50*	*55*
Soluble NDF	g	Free	*115*	*80*
Starch	g	170 (160–180)	150 (140–160)	160 (150–170)
Ether extract	g	45	Free	Free
Crude protein	g	175 (165–185)	150 (142–160)	159 (154–162)
Digestible protein[d]	g	128 (115–140)	104 (100–110)	111 (108–113)
Lysine[e]				
Total	g	81	73	78
Digestible	g	64	57	61
Sulphur[f]				
Total	g	63	52	59
Digestible	g	48	40	45
Threonine[g]				
Total	g	67	62	65
Digestible	g	46	43	45
Calcium	g	105	60	100
Phosphorus	g	60	40	57
Sodium	g	23	22	22
Chloride	g	29	28	28

ADF, acid detergent fibre; ADL, acid detergent lignin; NDF, neutral detergent fibre.
[a]The proportion of long fibre particles (>0.3 mm) should be >0.22 for breeding does and >0.205 for fattening rabbits.
[b]Values in parentheses indicate the range of minimal and maximal values recommended.
[c]Values in italics are provisional estimates.
[d]The digestibility of crude protein and essential amino acids is expressed as faecal apparent digestibility.
[e]Total amino acid requirements have been calculated for a contribution of synthetic amino acids of 0.15.
[f]Methionine should provide a minimum of 35% of the total sulphur amino acid requirements.
[g]Maximal levels of 50 and 72 g kg^{-1} of digestible and total threonine, respectively, are recommended for breeding does.

Dietary tryptophan content can be estimated at 0.18–0.20 of the optimal lysine concentration. For other essential amino acids, the ideal protein pattern (Table 12.5) can be of help.

There is a lack of research on mineral and vitamin requirements. the standards proposed in Tables 12.6 and 12.7 are mostly based on the practical levels used by the industry.

Table 12.7. Trace element and vitamin requirements of intensively reared rabbits, as concentration kg^{-1} corrected to a dry matter content of 900 g kg^{-1}.

Nutrient	Unit	Breeding does	Fattening rabbits	Mixed feed
Cobalt	mg	0.3	0.3	0.3
Copper	mg	10	6	10
Iron	mg	50	30	45
Iodine	mg	1.1	0.4	1.0
Manganese	mg	15	8	12
Selenium	mg	0.05	0.05	0.05
Zinc	mg	60	35	60

Continued

Table 12.7. Continued.

Nutrient	Unit	Breeding does	Fattening rabbits	Mixed feed
Vitamin A	mIU	10	6	10
Vitamin D	mIU	0.9	0.9	0.9
Vitamin E	IU	50	15	40
Vitamin K_3	mg	2	1	2
Vitamin B_1	mg	1	0.8	1
Vitamin B_2	mg	5	3	5
Vitamin B_6	mg	1.5	0.5	1.5
Vitamin B_{12}	µg	12	9	12
Folic acid	mg	1.5	0.1	1.5
Niacin	mg	35	35	35
Pantothenic acid	mg	15	8	15
Biotin	µg	100	10	100
Choline	mg	200	100	200

References

Adamson, I. and Fisher, H. (1973) Amino acid requirements of the growing rabbit: an estimate of quantitative needs. *Journal of Nutrition* 103, 1306–1310.

Álvarez, J.L., Margüenda, I., García-Rebollar, P., Carabaño, R., de Blas, C., Corujo, A. and García-Ruiz, A.I. (2007) Effects of type and level of fibre on digestive physiology and performance in reproducing and growing rabbits. *World Rabbit Science* 15, 9–17.

Barreto, G. and de Blas, C. (1993) Effect of dietary fibre and fat content on the reproductive performance of rabbit does bred at two remating times during two seasons. *World Rabbit Science* 1, 77–81.

Berchiche, M. and Lebas, F. (1994) Supplémentation en méthionine d'un aliment à base de feverole: effets sur la croissance, le rendement àl'abattage et la composition de la carcasse chez du lapin. *World Rabbit Science* 2, 135–140.

de Blas, C., Pérez, E., Fraga, M.J., Rodríguez, M. and Gálvez, J. (1981) Effect of diet on feed intake and growth of rabbits from weaning to slaughter at different ages and weights. *Journal of Animal Science* 52, 1225–1232.

de Blas, C., Santomá, G., Carabaño, R. and Fraga, M.J. (1986) Fiber and starch levels in fattening rabbit diets. *Journal of Animal Science* 63, 1897–1904.

de Blas, C., Taboada, E., Mateos, G.G., Nicodemus, N. and Méndez, J. (1995) Effect of substitution of starch for fiber and fat in isoenergetic diets on nutrient digestibility and reproductive performance of rabbits. *Journal of Animal Science* 73, 1131–1137.

de Blas, C., Taboada, E., Nicodemus, N., Campos, R., Piquer, J. and Méndez, J. (1998) Performance response of lactating and growing rabbits to dietary threonine content. *Animal Feed Science and Technology* 70, 151–160.

Carabaño, R., Villamide, M.J., García, J., Nicodemus, N., Llorente, A., Chamorro, S., Menoyo, D., García-Rebollar, P., García-Ruiz, A. and de Blas, C. (2009) New concepts and objectives for protein-amino acid nutrition in rabbits: a review. *World Rabbit Science* 17, 1–14.

Castellini, C., Dal Bosco, A. and Mugnai, C. (2003) Oxidative status and semen characteristics of rabbit bucks as affected by dietary vitamin E, C and n-3 fatty acids. *Reproduction, Nutrition, Development* 43, 41–53.

Cervera, C., Fernández, J., Viudes, P. and Blas, E. (1993) Effect of remating interval and diet on performance of female rabbits and their litters. *Animal Production* 56, 399–405.

Chamorro, S., Gómez-Conde, M.S., Pérez de Rozas, A.M., Badiola, I., Carabaño, R. and de Blas, C. (2007) Effect on digestion and performance of dietary protein content and of increased substitution of lucerne hay with soya-bean protein concentrate in starter diets for young rabbits. *Animal* 1, 651–659.

Colin, M. (1975) Effets sur la croissance du lapin de la supplémentation en L-lysine et en DL-méthionine de régimes végétaux simplifés. *Annales de Zootechnie* 24, 465–474.

Colin, M. (1978) Effets d'une supplementation en méthionine ou en cystine de régimes carencés en acides aminés soufrés sur les performances de croissance du lapin. *Annales de Zootechnie* 27, 9–16.

Colin, M. and Allain, D. (1978) Etude du besoin en lysine du lapin en croissance en relation avec la concentration énérgetique del'aliment. *Annales de Zootechnie* 27, 17–31.
Davidson, J. and Spreadbury, D. (1975) Nutrition of the New Zealand White rabbit. *Proceedings of the Nutrition Society* 34, 75–83.
Fabre, C., Jubero, M.A., Blas, E., Fernández-Carmona, J. and Pascual, J.J. (2006) Utilización de un pienso rico en fibra digestible e indigestible y pobre en almidón en conejos de engorde: ensayo en condiciones de campo. In: *XXXI Symposium de Cunicultura ASESCU*. Murcia, Spain, pp. 67–72.
Fernández, C. and Fraga, M.J. (1992) The effect of source and inclusion level of fat on growth performance. In: Cheeke, P.R. (ed.) *Proceedings of the 5th World Rabbit Congress*. Oregon State University, Corvallis, Oregon, USA, pp. 1071–1078.
Feugier, A., Smit, M.N., Fortun-Lamothe, L. and Gidenne, T. (2006) Fibre and protein requirements of early weaned rabbits and the interaction with weaning age: effects on digestive health and growth performance. *Animal Science* 82, 493–500.
Fraga, M.J., de Blas, C., Pérez, E., Rodríguez, J.M., Pérez, C. and Gálvez, J. (1983) Effets of diet on chemical composition of rabbits slaughtered at fixed body weights. *Journal of Animal Science* 56, 1097–1104.
Fraga, M.J., Lorente, M., Carabaño, R. and de Blas, C. (1987) Effect of diet and of remating interval on milk production and milk composition of the doe rabbit. *Animal Production* 48, 459–466.
Fraga, M.J., Pérez, P., Carabaño, R. and de Blas, J.C. (1991) Effect of type of fiber on the rate of passage and on the contribution of soft feces to nutrient intake of finishing rabbits. *Journal of Animal Science* 69, 1566–1574.
García, G., Gálvez, J.F. and de Blas, C. (1992) Substitution of barley grain by sugar-beet pulp in diets for finishing rabbits. 2. Effect on growth performance. *Journal of Applied Rabbit Research* 15, 1017–1024.
García, G., Gálvez, J.F. and de Blas, C. (1993) Effect of substitution of sugarbeet pulp for barley in diets for finishing rabbits on growth performance and on energy and nitrogen efficiency. *Journal of Animal Science* 71, 1823–1830.
García, J., Carabaño, R. and de Blas, C. (1999) Effect of fiber source on cell wall digestibility and rate of passage in rabbits. *Journal of Animal Science* 77, 898–905.
García, J., Carabaño, R., Perez Alba, L. and de Blas, C. (2000) Effect of fiber source on cecal fermentation and nitrogen recycled through cecotrophy in rabbits. *Journal of Animal Science* 78, 638–646.
García, J., Nicodemus, N., Carabaño, R. and de Blas, C. (2002) Effect of inclusion of defatted grape seed meal in the diet on digestion and performance of growing rabbits. *Journal of Animal Science* 80, 162–170.
García-Ruiz, A.I., García-Palomares, J., García-Rebollar, P., Chamorro, S., Carabaño, R. and de Blas, C. (2006) Effect of protein source and enzyme supplementation on ileal protein digestibility and fattening performance in rabbits. *Spanish Journal of Agricultural Research* 4, 297–303.
Gómez-Conde, M.S., García, J., Chamorro, S., Eiras, P., Rebollar, P.G., Pérez de Rozas, A., Badiola, I., de Blas, C. and Carabaño, R. (2007) Neutral detergent-soluble fiber improves gut barrier function in 25 d old weaned rabbits. *Journal of Animal Science* 85, 3313–3321.
Gutiérrez, I., Espinosa, A., García, J., Carabaño, R. and de Blas, C. (2002) Effect of levels of starch, fiber and lactose on digestion and growth performance of early-weaned rabbits. *Journal Animal Science* 80, 1029–1037.
Gutiérrez, I., Espinosa, A., García, J., Carabaño, R. and de Blas, J.C. (2003) Effect of source of protein on digestion and growth performance of early-weaned rabbits. *Animal Research* 52, 461–472.
Lleonart, F. (2005) Resultados de los ácidos eicosapentanoico y docosahexanoico sobre la fertilidad, prolificidad y producción lechera de conejas. In: *Proceedings of the 30th Symposium de Cunicultura de la ASESCU*. Valladolid, Spain, pp. 91–97.
Maertens, L. and De Groote, G. (1988a) The influence of the dietary energy content on the performance of postpartum breeding does. In: *Proceedings of the 4th World Rabbit Congress, Budapest*. Sandar Holdas, Hercegalom, Hungary, pp. 42–52.
Maertens, L. and De Groote, G. (1988b) The effect of the dietary protein:energy ratio and the lysine content on the breeding results of does. *Archiv für Geflügelkunde* 52, 89–95.
Maertens, L., Luzi, F. and De Groote, G. (1997) Effect of dietary protein and amino acids on the performance, carcass composition and N excretion of growing rabbits. *Annales de Zootechnie* 46, 255–268.
Maertens, L., Aerts, J.M. and De Brabander, D.L. (2005) Effect of a diet rich in n-3 fatty acids on the performances and milk composition of does and the viability of their progeny. In: *Proceedings of the 11èmes Journées de la Recherche Cunicole*. Paris, France, pp. 209–212.
Méndez, J., de Blas, C. and Fraga, M.J. (1986) The effects of diet and remating interval after parturition on the reproductive performance of the commercial doe rabbit. *Journal of Animal Science* 86, 1624–1634.

Motta, W., Fraga, M.J. and Carabaño, R. (1996) Inclusion of grape pomace in substitution for alfalfa hay, in diets for growing rabbits. *Animal Science* 63, 167–174.

Moughan, P.J., Schultze, W.H. and Smith, W.C. (1988) Amino acid requirements of the growing meat rabbit. 1. The amino acid composition of rabbit whole body tissue – a theoretical estimate of ideal amino acid balance. *Animal Production* 47, 297–301.

Nicodemus, N., Carabaño, R., García, J., Méndez, J. and de Blas C. (1999) Performance response of lactating and growing rabbits to dietary lignin content. *Animal Feed Science and Technology* 80, 43–54.

Nicodemus, N., García, J., Carabaño, R. and de Blas, C. (2002) Effect of the inclusion of sunflower hulls in the diet on performance, disaccharidase activity in the small intestine and caecal traits of growing rabbits. *Animal Science* 75, 237–243.

Nicodemus, N., García, J., Carabaño, R. and de Blas, C. (2006) Effect of a reduction of dietary particle size by substituting a mixture of fibrous by-products for lucerne hay on performance and digestion of growing rabbits and lactating does. *Livestock Science* 100, 242–250.

Nicodemus, N., García, J., Carabaño, R. and de Blas, C. (2007) Effect of substitution of a soybean hull and grape seed meal mixture for traditional fiber sources on digestion and performance of growing rabbits and lactating does. *Journal of Animal Science* 85, 181–187.

Parigi-Bini, R. and Chiericato, G.M. (1980) Utlization of various fruit pomaces products by growing rabbits. In: Camps, J. (ed.) *Proceedings of the 2nd World Rabbit Congress*. Asociación Española de Cunicultura, Barcelona, Spain, pp. 204–213.

Partridge, G.G., Findlay, M. and Fordyce, R.A. (1986) Fat supplementation of diets for growing rabbits. *Animal Feed Science and Technology* 16, 109–117.

Partridge, G.G., Garthwaite, P.H. and Findlay, M. (1989) Protein and energy retention by growing rabbits offered diets with increasing proportions of fibre. *Journal of Agricultural Science* 112, 171–178.

Perez, J.M., Gidenne, T., Lebas, F., Caudron, Y., Arveux, P., Boudillon, A., Duperray, J. and Messager, B. (1994) Apports de lignines et alimentation du lapin en croissance. 2. Conséquences sur les performances et la mortalité. *Annales Zootechnie* 43, 323–332.

Romero, C., Nicodemus, N., García-Rebollar, P., García-Ruiz, A.I. and de Blas, C. (2009) Dietary level of fibre and age at weaning affect the proliferation of *Clostridium perfringens* in the caecum, the incidence of epizootic rabbit enteropathy and the performance of fattening rabbits. *Animal Feed Science and Technology* 153, 131–140.

Santomá, G., de Blas, C., Carabaño, R. and Fraga, M.J. (1987) The effects of different fats and their inclusion level in diets for growing rabbits. *Animal Production* 45, 291–300.

Santomá, G., de Blas, C., Carabaño, R. and Fraga, M.J. (1989) Nutrition of rabbits. In: Haresign, W. and Cole, D.J.A. (eds) *Recent Advances in Animal Nutrition*. Butterworths, London, UK, pp. 109–138.

Spreadbury, D. (1978) A study of the protein and amino acid requirements of the growing New Zealand White rabbit with emphasis on lysine and sulphur-containing amino acids. *British Journal of Nutrition* 39, 601–603.

Taboada, E., Méndez, J., Maeos, G.G. and de Blas, C. (1994) The response of highly productive rabbits to dietary lysine content. *Livestock Production Science* 40, 329–337.

Taboada, E., Méndez, J. and Blas, C. (1996) The response of highly productive rabbits to dietary sulphur amino acid content for reproduction and growth. *Reproduction, Nutrition and Development* 36, 191–203.

Xiccato, G. (1996) Nutrition of lactating does. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 175–180.

Xiccato, G. (2006) Nutrition of the young and growing rabbit: a comparative approach with the doe. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Sciences*. Ilvo, Merelbeke, Belgium, pp. 239–246.

13 Feeding Behaviour of Rabbits

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13.1 Introduction

As a non-ruminant herbivore, the rabbit has a unique feeding behaviour compared to other domestic animals. It belongs to the Lagomorpha order (Leporidae family: rabbits and hares; Grassé and Dekeuser, 1955) and, consequently, expresses a main specificity that is caecotrophy. In brief (see details in Chapter 1) caecotrophy is a complete behaviour involving the excretion and immediate consumption of specific faeces, named soft faeces or 'caecotrophes'. Consequently, the daily intake behaviour of the rabbit is comprised of two meals: feeds and caecotrophes. Although the rabbit is not a rodent, one of the main features of its feeding behaviour is to gnaw. Information about feeding behaviour has mainly been obtained with the domestic rabbit, bred for meat or fur production or as a laboratory animal. It has basically involved rabbits receiving *ad libitum* a balanced complete pelleted feed, supplemented or not with dry forages or straw, but generally without a real free choice of feed.

This chapter reviews regulation of the intake behaviour according to several factors: age, type of feed and so on. The last part of the chapter is devoted to the feeding behaviour of wild and domestic rabbits in a situation of free choice.

13.2 The Behaviour of Caecotrophy

Caecotrophy plays an important role in rabbit nutrition, providing proteins and B vitamins from bacterial sources. The physiological mechanisms implicated in caecotrophy are detailed in Chapter 1. It is not fully known when caecotrophy behaviour commences in young rabbits, but it probably starts around 25 days of age, when a significant dry feed intake occurs that leads to both caecal and colon filling (Gidenne *et al.*, 2002a; Orengo and Gidenne, 2007).

Hard pellets are voided, but soft pellets are recovered by the rabbit directly upon being expelled from the anus. To do this the rabbit twists itself around, sucks in the soft faeces as they emerge from the anus and then swallows without chewing them. The rabbit can retrieve the soft pellets easily, even from a mesh floor. By the end of the morning there is a large number of these pellets inside the stomach, where they may comprise three-quarters of the total contents. The intriguing presence of these soft pellets in the stomach was at the origin of the first correct description of caecotrophy by Morot (1882): the production of two types of faeces and the systematic ingestion of one of the two types (the soft ones). This makes caecotrophy different from the coprophagy classically described for rats or pigs, where only one type of faeces is produced.

13.3 Feeding Behaviour in the Domestic Rabbit

13.3.1 Feeding behaviour of the young rabbit: from milk to solid food

Females give birth to naked and blind young in a nest after 31 days of gestation. There is subsequently a period of rapid development for the young, ending in weaning around 1 month later. During this period, kits progress from a diet comprised almost exclusively of milk, available only once a day, to several meals of solid food.

Milk intake

Initial nursing occurs during parturition. Suckling is induced by the mother when she stands motionless over the kits in the nest. She gives no direct assistance to the offspring to suck (Hudson and Distel, 1982, 1983). Therefore, locating the nipples and ingesting milk depends on the individual abilities of each kit to behave efficiently under the female.

It was demonstrated a long time ago that the rabbit suckles her litter for 4–5 min once a day only during the initial 2 weeks after birth (Zarrow *et al.*, 1965). More recently, however, data have suggested that some does (wild or domestic) nurse their young twice a day (Hoy and Selzer, 2002). In any case, suckling represents <0.0035 of the time budget of kits. Under experimental conditions, if two different females are presented to the litter, the young are able to suckle twice a day or more (Gyarmati *et al.*, 2000). However, double suckling on its own offers few if any nutritional benefits: the weight of kits at 21 days increased by 4.6% according to Etchegaray-Torres *et al.* (2004) and was clearly not influenced by suckling frequency according to Tudela et Balmisse (2003). On the other hand, under normal breeding conditions, it may happen relatively frequently that one or two kits from the same litter do not obtain milk at one nursing (0.14 of the litter on day 1 according to Coureaud *et al.*, 2007).

The first suckling bouts occur after parturition and within the first hour after the birth (colostrum), and are essential to the subsequent survival of kits. Starvation is indeed one of the key causes of mortality, usually peaking during the initial days post-partum (Coureaud and Schaal, 2000; Coureaud *et al.*, 2000), in addition to other factors such as maternal inexperience and behaviour (Verga *et al.*, 1978, 1986). During suckling, competition for access to nipples is very high. Indeed, in domestic rabbit breeds there are frequently more kits in the litter than nipples, with seven to ten kits per litter according to breed and selection and generally four pairs of nipples (Drummond *et al.*, 2000; Hudson *et al.*, 2000; Bautista *et al.*, 2005), although does from breeds or lines selected for prolificacy may have up to 12 nipples (Fleischhauer *et al.*, 1985; De Rochambeau *et al.*, 1988; Szendrö *et al.*, 1991; Coisne, 2000). Notwithstanding the actual number of nipples available, newborn rabbits do not appropriate a single nipple but change from one to another approximately every 20 seconds within the same sucking bout. This is contrary to other newborn mammals (e.g. kittens, piglets), where newborns retain the same nipple throughout lactation. Bautista *et al.* (2005) showed that the availability of milk across the eight nipples is equal during the first days post-partum, but that more milk is available from the two middle pairs by the end of the first week.

During the first week post-partum, kits drink about 0.15 of their live weight (LW) in milk each day in one nursing session, and up to 0.25 for some individuals (around 15–25 g; Lebas, 1969). Their nipple-searching behaviour is very stereotyped and controlled by a pheromonal signal (Schaal *et al.*, 2003). During the first week post-partum (between 4 and 6 days of age) kits also consume some hard faeces deposited by the doe in the nest, thus stimulating the caecal microbiota maturation (Kovacs *et al.*, 2004). Thereafter, individual milk intake increases gradually to reach a peak of about 25 g day^{-1} between 17 and 25 days of age (Fig. 13.1). During this period, milk intake is highly variable between kits due to individual ability, competition between littermates and milk availability (Fortun-Lamothe and Gidenne, 2000). After day 20–25, maternal milk production progressively decreases. If food resources are sufficient and the female is not fertilized again, milk production can continue to 5–6 weeks or even longer. If the female is fertilized just after parturition, however, and sustains a concurrent pregnancy and lactation, milk production decrease significantly at the end of pregnancy and ceases 2–3 days before the following parturition (Lebas, 1972; Fortun-Lamothe *et al.*, 1999). This frequently occurs in wild rabbits in the spring, when females mate again on the day of parturition. In this situation, young rabbits may be weaned from 3 weeks of age. In commercial systems, weaning is generally carried out between 28 and 35 days of age, even if milk production has not completely stopped.

Solid food intake and evolution of nutrient and energy supply

Young rabbits begin to eat significant quantities of solid food at around 16–18 days of age, when there are able leave the nest and move easily to access a feeder (with pelleted feed) and drinker. Nevertheless, the first contacts with solids occur during the first week of life, when the young consume some hard faeces deposited by the doe in the nest during suckling (Kovacs *et al.*, 2004; Moncomble *et al.*, 2004).

Initially, the young eat very small quantities of feed (<2 g day^{-1} per rabbit before 20 days of age). The solid food intake increases from 25 days of age to reach 40–50 g day^{-1} by 35 days (Gidenne *et al.*, 2002b), although this is highly variable between litters. Consequently, the feeding behaviour changes considerably in a few days, as the young switch from a single daily meal of milk to 25–30 solid and liquid (water) meals in 24 h. The ingestion of solid food and water exceeds that of milk during the fourth week of life.

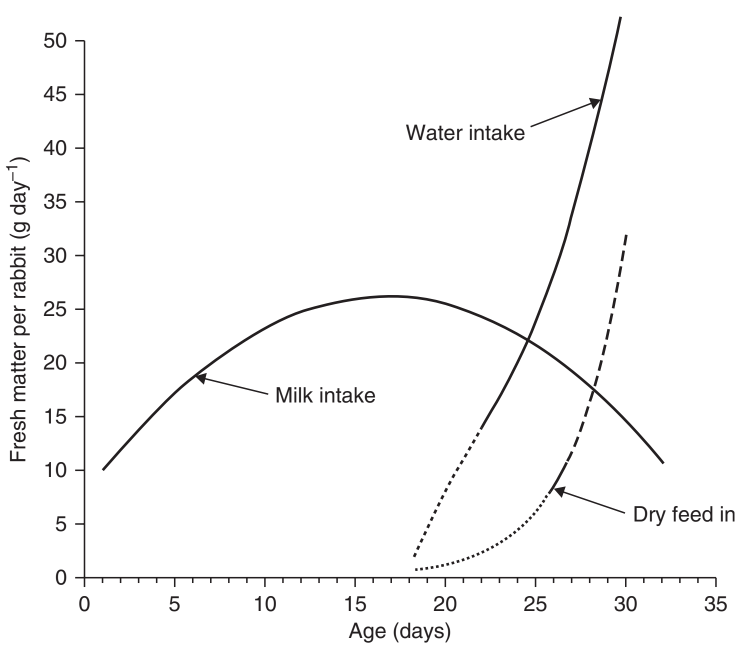

Fig. 13.1. Milk, water and dry feed intake of the young rabbit (adapted from Szendrö *et al.*, 1999; Fortun-Lamothe and Gidenne, 2000). Values are means for litters of seven to nine kits with pelleted dry feed and one lactating doe available, and weaned at 30 days (does remated 11 days after kindling).

It is interesting to note that when suckling rabbits begin to eat solid food, they prefer to eat from the same feeder as their mother rather than from a specific feeder for young animals (Fortun-Lamothe and Gidenne, 2003). This suggests that the start of solid food ingestion is influenced by initiation or imitation of the mother. In addition, for the early-weaned rabbit, the watering system (nipple or 'open air' drinker) does not affect solid feed intake, while a too-small pellet diameter increases hardness and impairs feed intake (Gidenne *et al.*, 2002a, 2003).

In parallel to modifications in feeding behaviour, the nutrients ingested by young rabbits change significantly between birth and weaning (Fig. 13.2). Indeed, rabbit milk is very rich in lipids (13 g 100 g^{-1}) and proteins (12 g 100 g^{-1}), but contains only traces of lactose (Maertens *et al.*, 2006). On the other hand, pelleted feed mainly contains carbohydrates (80 g 100 g^{-1}, with varying digestibility ranging from very high for starch to low for fibre), some protein (15–18 g 100 g^{-1}) and only a small quantity of lipids (2–5 g 100 g^{-1}), all of vegetable origin. Therefore, digestive capacities must evolve rapidly, in parallel with the evolution of feeding patterns (Gidenne and Fortun-Lamothe, 2002). The ingestion of vegetable proteins becomes equal to that from the milk at around 25 days of age, and then exceeds it within a few days. Conversely, lipids come mainly from milk until weaning. While the ingestion of carbohydrates is virtually zero (<0.3 g day^{-1}) until 17 days of age, it becomes significant from day 21 in the form of fibre and starch. However, proteins and fats in the milk constitute the main sources of energy until weaning.

Regulation of feeding behaviour in young rabbits

The individual feeding behaviour of kits before weaning and its regulation are not easy to study due to the interactions of each kit with its littermates and mother. Nevertheless, it is well known that the availability of milk is a key regulating factor of solid food ingestion before weaning. Thus, if the size of the litter is reduced from ten to four kits or if milk production increases, the start of solid food ingestion is

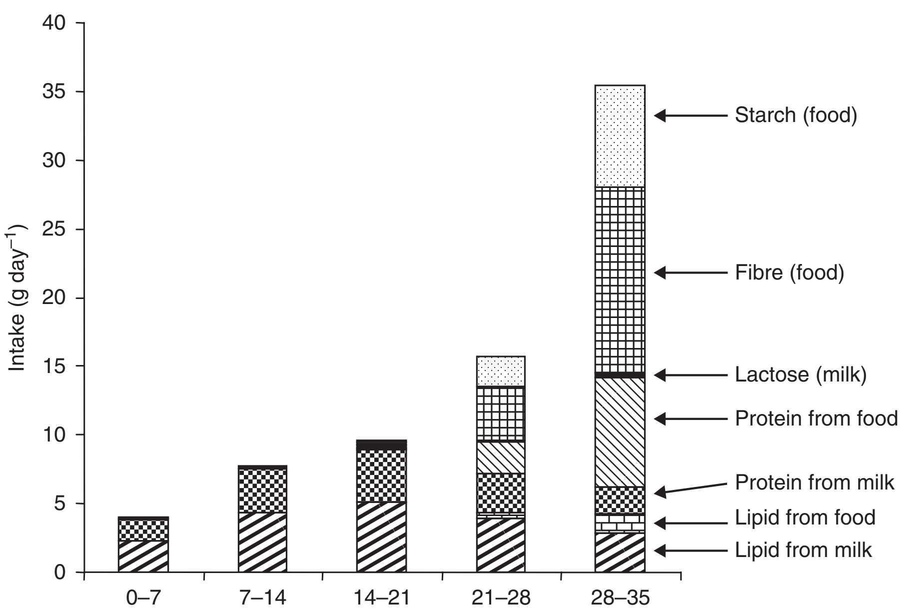

Fig. 13.2. Evolution of the composition of food ingested by young rabbits between birth (day 0) and weaning (day 35) in breeding conditions.

delayed by 2–4 days (Fortun-Lamothe and Gidenne, 2000) and the feed intake of the whole litter is reduced (Pascual *et al.*, 2001). Similarly, offering a second milking to the young (using a second doe) delays dry feed intake (Gyarmati *et al.*, 2000). On the other hand, early weaning (before 25 days of age) stimulates and considerably accelerates dry feed intake (Gallois *et al.*, 2005; Xiccato *et al.*, 2005).

The influence of feed nutritional composition on feeding behaviour is poorly understood, although some authors have used an original model of a cage to measure the intake of the litter without separation from the mother (Fortun-Lamothe *et al.*, 2000). However, results obtained with young rabbits indicate that the variability among littermates is very high (up to 45%) and that the control of intake before weaning through the nutrient and energy supply is not consistent. For instance, Pascual *et al.* (1998, 1999) suggested that suckling rabbits regulate their food consumption according to dietary digestible energy (DE) content, as do weaned rabbits. Conversely, greater feed intake has been found for a high- compared to a moderate-energy diet (Debray *et al.*, 2002; Gidenne *et al.*, 2004). Finally, other factors, such the form of presentation of food and pellet size and quality (e.g. hardness, durability) probably play a key role in the starting of solid feeding behaviour.

Despite this, the *individual* feeding behaviour of kits (e.g. regulation factors, number of meals) is largely unknown, since no method is presently available to assess their intake when reared collectively (until weaning).

13.3.2 Feeding behaviour of the growing and adult rabbit

From weaning (classically between 4 and 5 weeks), the daily feed intake of the domestic rabbit (fed a complete pellet feed) increases in relation to metabolic LW (Fig. 13.3) and stabilizes at about 5 months of age. Taking as a reference an adult animal fed *ad libitum* (140–150 g dry matter DM day^{-1}, for example, for a 4 kg New Zealand White): (i) at 4 weeks a young rabbit eats 0.25 of the amount an adult eats, but its LW is only 0.14 of that of the adult; (ii) at 8 weeks the

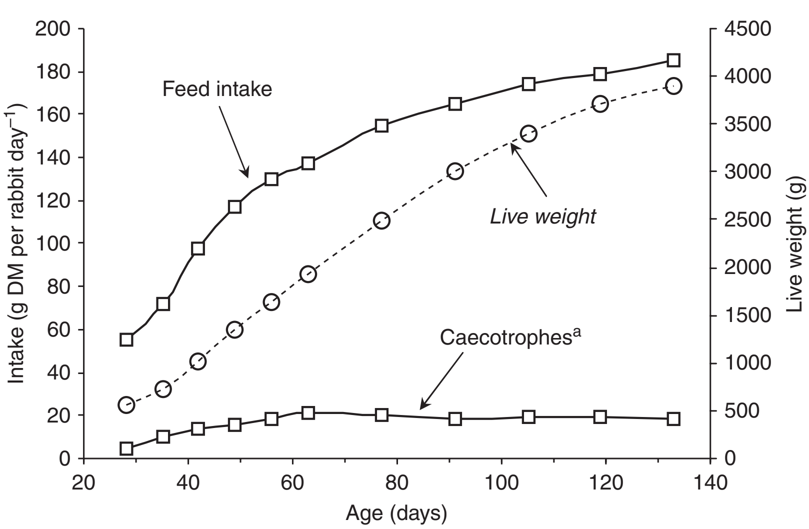

Fig. 13.3. Dry matter (DM) intake from pelleted feed and caecotrophes and live weight from weaning (28 days) until adulthood. Data are from the domestic rabbit, fed a pelleted feed *ad libitum* (Gidenne and Lebas, 1987). [a]Data on caecotrophe excretion were obtained from rabbits wearing a collar.

relative proportions are 0.62 and 0.42; and (iii) at 16 weeks, 1.00–1.10 and 0.87, respectively. Between weaning (4–5 weeks) and 8 weeks of age, weight gain is at its highest (Table 13.1) and feed conversion is optimal. The rate of increase of feed intake and growth rate subsequently decrease, with intake stabilizing at around 12 weeks of age for current hybrid lines of domestic rabbit.

Similarly to other mammals, the rabbit regulates its feed intake according to energy requirements. Chemostatic mechanisms are involved, by means of the nervous system and blood levels of compounds used in energy metabolism. In non-ruminants, however, glycaemia plays a key role in food intake regulation, while in ruminants the plasma levels of volatile fatty acids have a major role. Since the rabbit is a non-ruminant herbivore, the main blood component regulating feed intake is not clear, but it is probably glucose. Voluntary intake, proportional to metabolic LW ($LW^{0.75}$), is about 900–1000 kJ DE day^{-1} kg^{-1} $LW^{0.75}$, and chemostatic regulation appears only with a dietary DE concentration >9–9.5 MJ kg^{-1} (see Chapter 6). Below this level, a physical-type regulation is prevalent and linked to gut fill.

The intake of soft faeces increases only until 2 months of age and then remains steady (Fig. 13.3). Expressed as fresh matter, the intake of soft faeces increases from 10 g day^{-1} (1 month old) to 55 g day^{-1} (2 months), thus representing 0.15–0.35 of the feed intake (Gidenne and Lebas, 1987). However, the classic method of calculating caecotrophy probably underestimates this proportion, since installing a collar around the neck of the rabbit to avoid the intake of soft faeces from the anus is stressful. Recently, Belenguer *et al.* (2008) developed methods based on microbial marker analysis that are less intrusive for the animal.

The rabbit divides its voluntary solid intake into numerous meals: about 40 at 6 weeks of age, and a slightly lower number in adulthood (Table 13.2). This meal fractionation is probably linked to the relatively weak storage capacity of the stomach (as detailed in Chapter 1), particularly when compared to herbivorous animals or even carnivorous or omnivorous ones (such as dogs and pigs).

For 6-week-old rabbits fed a pelleted diet, the time spent feeding every 24 h is slightly >3 h. Subsequently, it drops rapidly to <2 h. If a ground non-pelleted diet is offered, the time spent on eating doubles (Lebas, 1973). The number of liquid meals increases in parallel to that of feed, and less time is spent drinking than eating. Furthermore, at any age, feeds containing >0.70 water, such as green forage, provide rabbits with sufficient water at temperatures <20°C and, in these circumstances, rabbits may not drink at all. In growing rabbits fed with pellets, the normal ratio of water to DM is about 1.6–1.8. In the adult or breeding doe it is increased up to 2.0–2.1.

Table 13.1. Feeding behaviour of the domestic rabbit after weaning. Mean values from rabbits (current commercial lines) fed a pelleted diet (890 g dry matter kg^{-1}) *ad libitum* and with free access to drinkable water.

	Age (weeks)	
	5–7	7–10
Solid feed intake (g day^{-1})	100–120	140–170
Weight gain (g day^{-1})	45–50	35–45
Food conversion	2.2–2.4	3.4–3.8

Table 13.2. Feeding and drinking behaviour of the domestic rabbit from 6 to 18 weeks old. Mean values from nine New Zealand White rabbits fed a pelleted diet (89 g dry matter kg^{-1}) *ad libitum* and with free access to drinkable water (Prud'hon *et al.*, 1975b).

	Age in weeks		
	6	12	18
Solid feed (pellets, 890 g dry matter kg^{-1})			
Solid feed intake (g day^{-1})	98	194	160
No. of meals per day	39	40	34
Average quantity per meal (g)	2.6	4.9	4.9
Drinking water			
Water intake (g day^{-1})	153	320	297
No. of drinks per day	31	28.5	36
Average weight of one drink (g)	5.1	11.5	9.1

Solid intake fluctuates over a 24-h period (Fig. 13.4). Over 0.60 of the solid feed (excluding soft faeces) is consumed in the dark period for a domestic rabbit submitted to a 12-h light, 12-h dark schedule. The circadian changes in liquid meals are strictly parallel to those of solid meals for the domestic rabbit fed pellets (Prud'hon *et al.*, 1975b), but no correlation can be established between the time or intervals of solid and water meals. Peak intake is observed at the end of the light period, about 1 h before the start of the dark period. Prud'hon *et al.* (1975b) reported intense feed consumption in the 6-week-old rabbit. According to Horton *et al.* (1974) and Jolivet *et al.* (1983), intake is usually spread over two periods: (i) one at the end of the dark interval (or early in the day); and (ii) another more important period at the end of the light interval (or early night).

With older rabbits, the nocturnal feeding behaviour becomes more pronounced. The feeding habits of wild rabbits are even more nocturnal than those of domesticated rabbits. In fact, the domestic rabbit no longer has prolonged periods without eating, since it has >20 meals of dry feed a day, and it also consumes caecotrophes (early in the light period). Moreover, Hirakawa (2001) pointed out that leporids (inc luding rabbits) also consume a portion of their own hard faeces, which are masticated (in contrast to soft faeces, which are swallowed). In rabbits, meals of soft faeces (and sometimes hard) increase in proportion when feed availability is insufficient.

Obviously, the feed intake level is modulated by the physiological status of the animal. For instance, the voluntary intake of does varies considerably during the reproductive cycle (Fig. 13.5), with intake falling markedly during the final days of pregnancy. Some does refuse solid food just before kindling. Water intake, however, never stops completely. After kindling, feed intake increases very rapidly and can exceed 100 g DM kg^{-1} LW day^{-1}. Water intake is also increased at that time, from 200 to 250 g day^{-1} kg^{-1} LW. When a doe is both pregnant and lactating, she eats a similar amount to a doe that is only lactating.

13.4 External Factors Modulating the Feeding Behaviour of the Domestic Rabbit

13.4.1 Feed composition and presentation form

One of the main dietary components implicated in feed intake regulation, after weaning, is the DE concentration. The domestic rabbit (fed a pelleted balanced diet) is able to regulate its DE intake (and thus its growth) when the dietary DE concentration is between

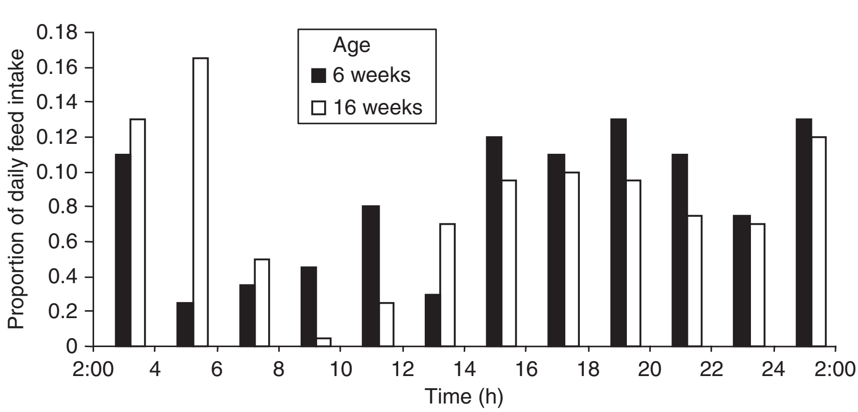

Fig. 13.4. Circadian pattern of feed intake in the growing or adult rabbit. Mean values for domestic rabbits (n = 6) fed a pelleted feed *ad libitum* (daily feed intake of 80 and 189 g day^{-1}, respectively, for 6- and 16-week-old rabbits) and bred under a 7:00–19:00 light schedule (Bellier *et al.*, 1995).

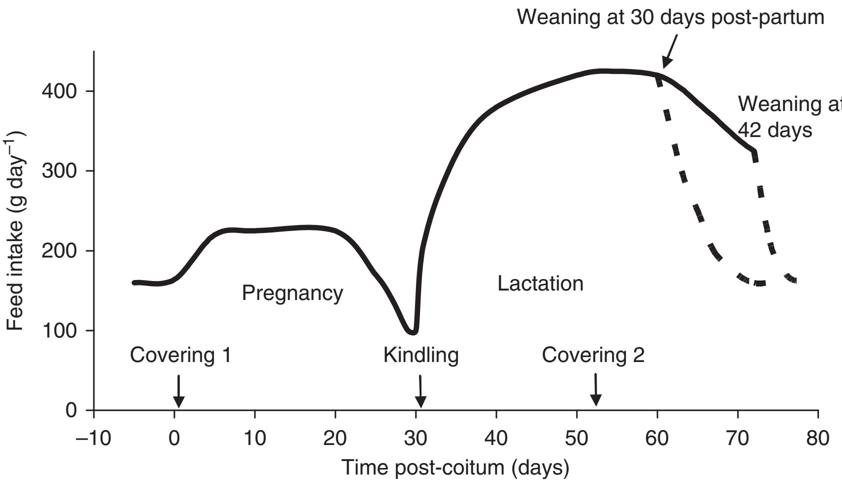

Fig. 13.5. Intake behaviour of a doe during gestation and lactation (data from Lebas, 1975). Data from a domestic rabbit fed a balanced pelleted feed (890 g DM kg^{-1}).

9 and 11.5 MJ kg^{-1}, or when the dietary fibre level is between 10% and 25% acid detergent fibre. The intake level is thus well correlated with the dietary fibre level, compared to the dietary DE content (Fig. 13.6). However, the incorporation of fat in the diet – while maintaining the dietary fibre level – increases the dietary DE level, but leads to a slight reduction in intake. Other nutrients in the diet, such proteins and amino acids, are able to modify the food intake (Tome, 2004). For example, an excess of methionine has been observed to reduce the feed intake of the growing rabbit by at least 10% (Colin *et al.*, 1973; Gidenne *et al.*, 2002b).

The presentation of the diet is an important factor that modulates feeding behaviour in the rabbit. Compared to meals, pelleted feeds are preferred at 97% when offered in free choice (Harris *et al.*, 1983). Furthermore, meals seem to modify the circadian cycle of feed intake (Lebas and Laplace, 1977). Pellet size and quality (hardness, durability) also affect feeding behaviour (see Chapter 14). A reduction in pellet diameter, which also increases the hardness, reduces the feed intake of young and growing rabbits (Maertens, 1994; Gidenne *et al.*, 2003), although the time spent on feeding is increased.

13.4.2 Environmental factors affecting the feeding behaviour of the rabbit

Energy expenditure and hence the requirements and feed intake of the rabbit depend on the ambient temperature. Studies on growing rabbits have shown that the intake of pelleted feed drops from 180 to 120 g day^{-1} and water intake rises from 330 to 390 g day^{-1} at temperatures between 5°C and 30°C (Table 13.3) (Eberhart, 1980). A closer analysis of feeding behaviour shows that the number of solid meals eaten in 24 h drops as temperature increases, from 37 solid feeds at 10°C to only 27 feeds at 30°C (for 6-week-old New Zealand White rabbits; Prud'hon, 1976). The amount eaten at each meal also decreases with higher temperatures (from 5.7 g per meal at 10–20°C to 4.4 g per meal at 30°C). Water intake increases, however, from 11.4 to 16.2 g per meal between 10°C and 30°C (Prud'hon, 1976).

The negative effect of hot ambient temperatures (29–32° C) on daily feed intake may be partly counterbalanced by distribution of cooler drinking water (16–20° C). With 'cold' water distribution, the average feed intake may be increased by 4–6% for fatteners and breeding does, with corresponding improvements in performance (Duperray *et al.*, 1998).

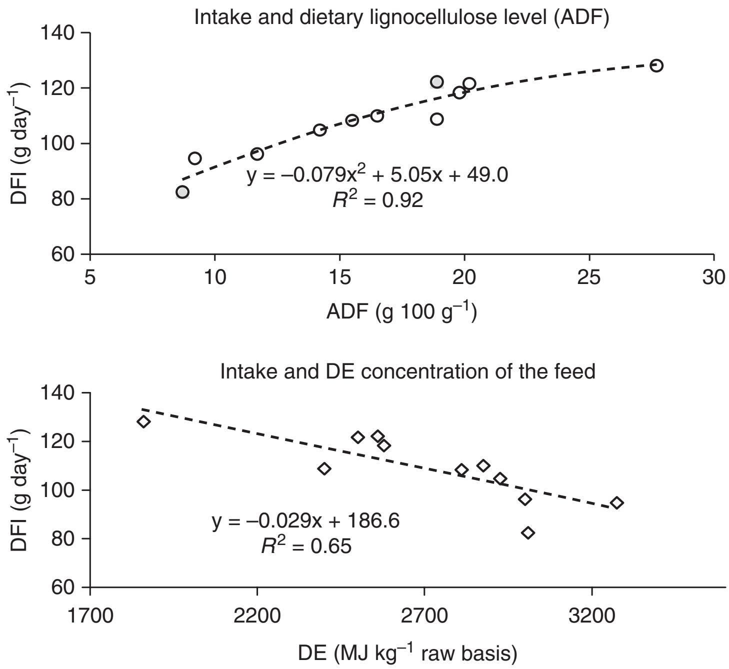

Fig. 13.6. Feed intake prediction in the domestic rabbit after weaning. ADF, acid detergent fibre; DE, digestible energy; DFI, daily feed intake (measured between weaning (4 weeks) and 11 weeks of age) (Gidenne, 2000).

Table 13.3. Feeding behaviour of the growing rabbit according to ambient temperature (data from Eberhart, 1980).

	Ambient temperature		
	5°C	18°C	30°C
Relative humidity (%)	80	70	60
Pelleted feed eaten (g day^{-1})	182	158	123
Water intake (g day^{-1})	328	271	386
Water to feed ratio	1.80	1.71	3.14
Average weight gain (g day^{-1})	35. 1	37.4	25.4

In an experiment by Selim *et al.* (2004), feed intake w as increased by up to 11% for 7-week-old fatteners with 6 h of hot temperatures (29–32°C) during each 24-h cycle. The feeding and drinking behaviour of does and their litters according to climatic conditions is discussed in Chapter 15.

If drinking water is not provided and the only feed available is dry with a moisture content of <140 g kg^{-1}, DM intake drops to zero within 24 h. With no water at all, and depending on temperature and humidity, an adult rabbit can survive from 4 to 8 days without any irreversible damage, although its weight may drop by 20–30% in less than a week (Cizek, 1961). Rabbits with access to drinking water but no solid feed can survive for 3–4 weeks. Within a few days they will drink four to six times as much water as normal. Sodium chloride in the water (0.0045 g kg^{-1}) reduces this high water intake, but potassium chloride has no effect (sodium loss through urination). Although the rabbit is very resistant to hunger and relatively resistant to thirst, any reduction in the water supply, in terms of water requirements, causes a proportional reduction in DM intake, with a consequent drop in most performance criteria. For example, limiting water availability for breeding does to 20 min day^{-1} decreases their feed intake, milk production and growth of kits by about 17–18%, but has no effect on reproduction parameters or kit mortality (Carles and Prud'hon, 1980).

Other environmental factors have also been studied in the domestic rabbit, including the lighting schedule and housing systems. In the absence of light (24-h dark) the feed intake of fattening rabbits is increased, as compared to rabbits submitted to a natural sunlight programme (Lebas, 1977). Under these conditions, rabbits organize their feeding pattern in a regular 23.5- to 23.8-h programme, with about 5–6 h devoted to soft faeces ingestion and the remaining part of the cycle to feed intake. Under continuous lighting, the feeding pattern is organized in an approximate 25-h programme (Jilge, 1982; Reyne and Goussopoulos, 1984). For breeding does, reduction of the lighting duration during a 24-h cycle by the introduction of two 4-h periods of dark during the normal 12 h of lighting in a 12-h light, 12-h dark programme (intermittent lighting) did not modify the average daily feed intake, despite an increase in milk output leading to a better feed efficiency for milk production (Virag *et al.*, 2000).

As previously mentioned, the type of caging also influences the daily feed intake and feeding pattern of rabbits. For instance, feed intake is affected by the stocking density of rabbits in the cage. An increase in stocking density seems to lead to greater competition for feeders among the animals and reduced feed intake (Aubret and Duperray, 1993). However, this is not necessarily a result of a competition for feeders since it is also observed with rabbits in individual cages (Xiccato *et al.*, 1999).

In comparisons of cage and pen housing, enlarging the cage size for a group (with or without variations in stocking density) allows rabbits to move more and reduces daily feed intake (Maertens and Van Herck, 2000). At the same density, rabbits caged in groups of two or six had the same daily feed intake, but those in cages of two spent a lower proportion of their time budget on feed consumption: 0.058 versus 0.099 during the 10-h light period during which they were observed (Mirabito *et al.*, 1999). Finally, according to the feeding pattern, the number of places at a feeder (one to six) for a group of ten rabbits did not influence daily feed intake (Lebas, 1971).

13.5 Feeding Behaviour in Situations of Choice

All of the studies described above were conducted with domestic rabbits, generally fed with complete and more-or-less balanced diets. In the wild or in situations of free choice for caged rabbits, another dimension must be added to the feeding behaviour: how rabbits select feeds.

13.5.1 Feeding behaviour of the wild rabbit or the rabbit in an open situation (grazing)

The feed resources available to wild rabbits invariably include a wide range of plant material. Rabbits clearly prefer graminaceous plants (*Festuca*, *Brachypodium* or *Digitaria* species) and graze only a few dicotyledons if insufficient grasses are available (Williams *et al.*, 1974; Leslie *et al.*, 2004). Within the dicotyledonous plants, rabbits graze some leguminous plants and some *Compositae*. However, it should be underlined that consumption of carrots (*Daucus carota*) is minimal, and this plant is not preferred by rabbits (CTGREF, 1978).

The proportion of grazing of dicotyledonous species may increase during some seasons, depending on the availability of plants (Bhadresa, 1977). In winter and early spring, grazing of cultivated cereals by rabbits may completely compromise the crop, especially up to a distance of 30–100 m from the warren (Biadi and Guenezan, 1992). When rabbits can choose between winter cereals cultivated with or without mineral fertilization (phosphorus and/or nitrogen), they clearly prefer the latter (Spence and Smith, 1965).

Grazing rabbits may be very selective and, for example, choose one type or part of the plant with the highest nitrogen concentration (Lebas, 2002). Similarly, in a test performed in Ireland, wild rabbits grazed one variety of spring barley more intensively than four others, probably in relation to the plant's composition. However, differences in the sugar content of the varieties did not fully explain this varietal selection (Bell and Watson, 1993).

The considerable winter appetence of rabbits for buds and young stems of some woody plants is important. Grazing of very young trees or shoots may completely compromise the regeneration of forests (CTGREF, 1980) or, more specifically, the regeneration of shrubs such as juniper (Lebas, 2002) or common broom (M. Sabourdy, personal communication, 1971). In winter rabbits like to eat the bark of some cultivated trees (not only young stems), especially that of apple trees and, to some extent, cherry and peach trees. The bark of pear, plum or apricot trees is generally not so frequently consumed (CTGREF, 1980). In forests rabbits clearly prefer broad leaved trees, but may also consume the bark of conifers (mainly spruce and some types of pines); however, when very young trees are available, rabbits prefer to eat apical or lateral sprouts of spruces or firs instead of oaks (CTGREF, 1978).

The basic reasons for these choices remain unclear, even if they are constant. They are regulated by the hypothalamus, since hypothalamic lesions clearly modify the choice pattern of rabbits (Balinska, 1966).

Many experiments have been conducted, especially in Australia and New Zealand, to study the behaviour of wild rabbits when offered different manufactured baits (the ultimate objective being the eradication of imported wild rabbits). Many variations have been observed, depending on both the type of bait and the season. For example pollard plus bran pellets (5:1 in weight) is consumed throughout the year; in contrast, the acceptability of carrots or oats varies seasonally. The addition of salt (10 or 50 g NaCl kg^{-1}) or lucerne meal (150 g kg^{-1}) to the pollard plus bran pellets significantly reduces bait consumption (Ross and Bell, 1979).

13.5.2 Free choice for the domestic caged rabbit

When a choice is proposed between a control diet and the same diet plus an appetiser, rabbits generally prefer the latter. However, when the same two diets are offered alone to rabbits, both the daily feed intake and growth performance are exactly the same (Fekete and Lebas, 1983). This means that the pleasant smell of the proposed food is not essential for feed intake regulation. This has also been shown with a repellent diet (the addition of formalin), which was clearly rejected in a free-choice test but consumed in the same quantity in a long-term single food test (Lebas, unpublished data).

Similarly Cheeke *et al.* (1977) have demonstrated that rabbits prefer lucerne with a saponin (a bitter component) content of up to 3 mg g^{-1} diet, whereas rats always prefer the control diet without saponin in the range of 0.4–5 mg g^{-1} (Fig. 13.7). However,

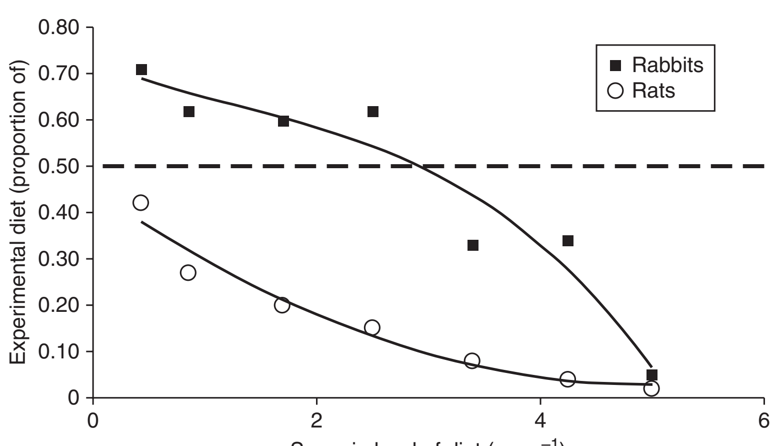

Fig. 13.7. Relative feed intake of a lucerne-based diet with various levels of saponin in rats and rabbits in a situation of free choice between this diet and a control diet without saponin (Cheeke *et al.*, 1977).

when single feeds with different levels of saponin are offered to rabbits (saponin from 1.8 to 6.4 mg g^{-1} complete diet), feed intakes and growth rates are independent of the saponin level (Auxilia *et al.*, 1983).

Conversely, when a toxin is present (e.g. aflatoxins) rabbits completely refuse to consume the diet or consume it in very low quantities (Fehr *et al.*, 1968; Morisse *et al.*, 1981; Saubois and Nepote, 1994). This regulation may be relevant in protecting the animal against food-borne pathologies.

When a concentrate (low-fibre compound diet) and a fibrous material are offered as free choice to rabbits, they prefer the former. The fibrous material is consumed in only small quantities and the growth rate may be reduced (Lebas *et al.*, 1997). A further consequence is an immediate increase in the health risk for rabbits with digestive disorders through lack of fibre (Gidenne, 2003). This is the result of the specific search by rabbits for energy sources (scarce in the wild), the dominant regulation system of feed intake in rabbits.

In fact when Gidenne (1985) offered a free choice of two energy concentrates with a complete diet and fresh green bananas, the growth rate was similar among the two groups and the DE daily intake was identical. Nevertheless, it must be underlined that, in this study, the proportion of bananas in the DM intake decreased from 0.40 at weaning (5 weeks) to 0.28 at the end of the experiment 7 weeks later.

Similarly, rabbits receiving a diet deficient in one essential amino acid (lysine or sulphur amino acids) and drinking water with or without the missing amino acid in solution clearly prefer the solution with the missing amino acid (Lebas and Greppi, 1980).

To add a last constituent to this section on free choice, it should be remembered that a simple variation in the humidity of one component may change the balance of choice. For example, when dehydrated lucerne and normally dried maize grains (110 g moisture kg^{-1}) are offered *ad libitum*, the result of the choice is 65% lucerne to 35% maize. If, however, the water content of the maize grains is increased up to 140–150 g kg^{-1} the balance changes to 45% lucerne and 55% maize (Lebas, 2002). In this case the choice seems motivated more by the immediate palatability of the feeds than by their nutritive value.

As described above, regulation of intake in a free-choice situation is difficult to predict. Thus, in most practical situations of rabbit production, the utilization of a complete balanced diet is advisable.

13.6 Feeding Behaviour in a Situation of Feed Restriction

13.6.1 Quantitative limitation

When a limited quantity of pelleted food is distributed to a rabbit, the animal consumes its daily allocation within a few hours. For example, for rabbits caged individually or in pairs, a quantity representing 0.85 of the *ad libitum* intake is ingested in a maximum of 16 h; if the quantity is reduced to 0.70, however, the time taken to ingest this quantity is reduced to 10 h (Bergaoui *et al.*, 2008).

When restricted-fed rabbits are caged in groups, the time spent on feed intake is shorter and depends on the number of rabbits able to eat pellets at the same time. For example, according to Tudela and Lebas (2006), fattening rabbits caged in groups of eight, with feed restriction at 0.85 of *ad libitum*, will consume all of the daily allocation within 8 h if only one rabbit has access to the feeder; but if two rabbits can access the feeder simultaneously, only 0.89 of the daily allocation is consumed in the same 8 h. According to the same authors, if the daily allocation is distributed in two equal halves at 8:00 and 18:00 to groups of eight fattening rabbits with only enough space for one at the feeder, all of the feed is consumed within 2 h of distribution (0.93 during the first hour). If two rabbits can consume feed simultaneously, 3 h are necessary (0.76 during the first hour).

A feed restriction at 0.85 of *ad libitum* is not associated with real competition for feed intake between eight rabbits (as indicated from LW measurements), whether there are one or two places at the feeder or one or two meal distributions per day. Moreover, the within-cage standard deviation of LW is also independent of these factors and identical to that of the *ad libitum* control group. If the feed restriction is more severe (0.60 of *ad libitum*), however, the average LW is not affected by the number of feed distributions, but the standard deviation of LW is significantly increased by 20% when compared to that obtained with groups restricted at 0.85 or fed *ad libitum* – a situation that can be interpreted as the result of real competition between rabbits (Tudela and Lebas, 2006).

13.6.2 Limitation of daily access to the feeder or drinker

Restricted access to the feeder

Feed intake is reduced if access to the feeder is <14–16 h day^{-1}, as demonstrated by the different studies conducted in Hungary (Szendrö *et al*., 1988; Tal El Den *et al*., 1988) and summarized by Lebas (2007) in Fig. 13.8. For example feed restriction to 8 h day^{-1} was associated, on average, with a reduction in feed intake of 0.80 of *ad libitum*. Nevertheless, it must be highlighted that reducing access time to feeders induces a greater reduction in the intake of young rabbits than in older fattening rabbits. A reduction to 0.64, 0.73 and finally 0.81 of the *ad libitum* intake during each of the 3 weeks following weaning at 32 days has been demonstrated with 8 h of access to feeders (Foubert *et al*., 2007); a reduction to 0.73 of the *ad libitum* intake is seen with 4- to 5-week-old rabbits feeding for 9 h day^{-1} (Matics *et al*., 2008); and intake almost identical to that of an *ad libitum* control at 12 weeks is seen with continuous 8 h day^{-1} limitation of access (Szendrö *et al*., 1988). If a breeder hopes to induce a known quantitative restriction for a group of fattening rabbits (e.g. 0.85 of *ad libitum*, or adjustment to a theoretical curve of intake) by reducing the feeding time, it would be necessary to regularly determine the real feed intake in some cages in order to adjust, once or twice per week, the duration of access to feeders for the whole group.

The same Hungarian group has also observed the time taken by rabbits to consume their food in conditions of restricted access to feeders. The total number of meals per day is not affected by time limitations (30–35 day^{-1} on average at 12 weeks), but meals are concentrated in the smaller number of hours ‘available’, without a significant increase in the duration of each meal. Nevertheless, with 9 h day^{-1} available for feed

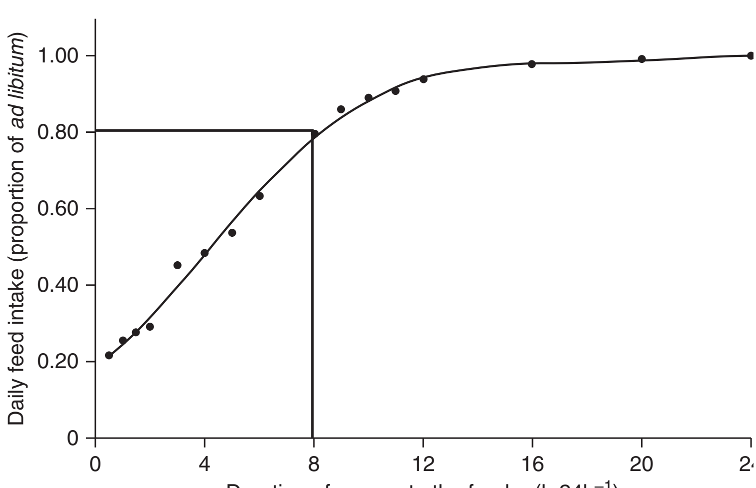

Fig. 13.8. Pelleted feed intake of rabbit with a limited time of access to the feeder (Lebas, 2007; data from Szendrö *et al*., 1988; Tag El Den *et al*., 1988).

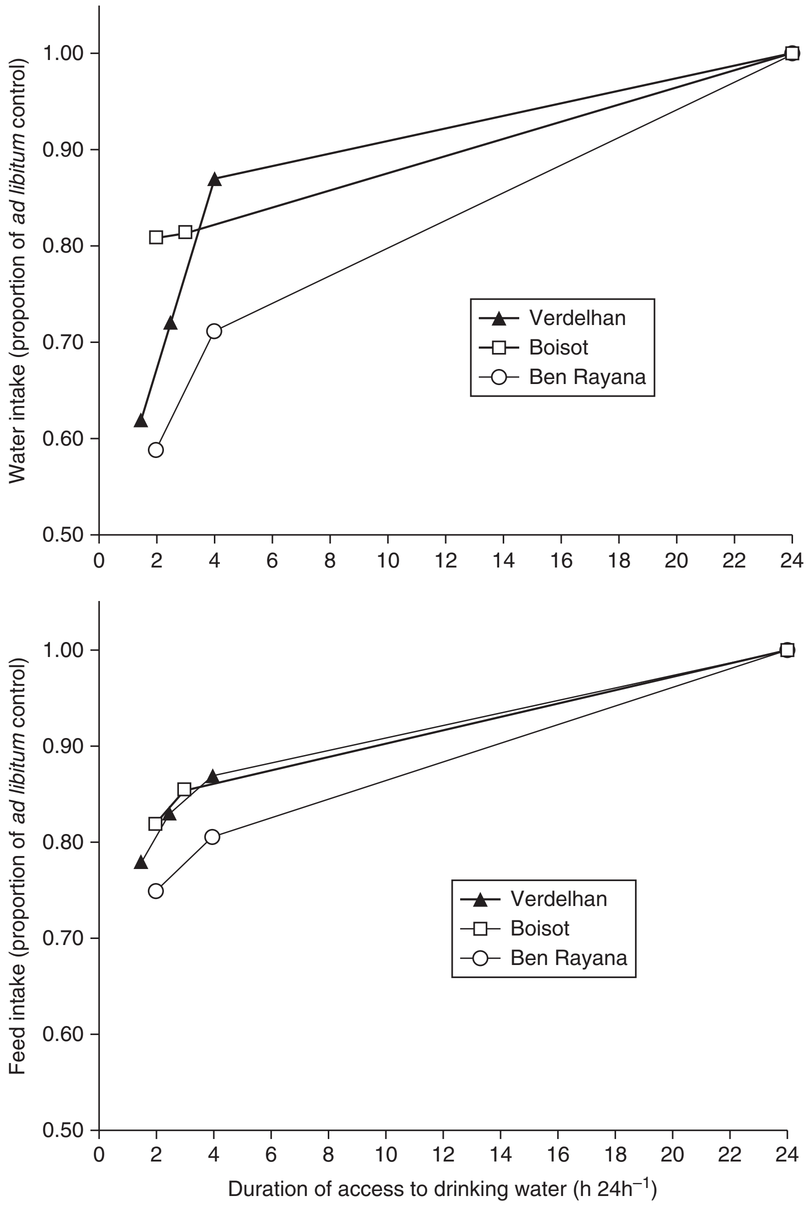

Fig. 13.9. Average water and feed intakes of fattening rabbits able to drink water for a limited duration every day (1.5–4 h 24 h^{-1}) but fed *ad libitum* (Boisot *et al*., 2004; Verdelhan *et al*., 2004; Ben Rayana *et al*., 2008). Results are expressed as percentage of the control rabbits, watered and fed *ad libitum*.

intake, the total duration spent on feed consumption is 1 h 20 min day^{-1}, compared to the 1 h 45 min day^{-1} spent by rabbits of the same age fed *ad libitum* (Szendrö *et al*., 1988).

Restricted access to drinking water

Limitation of the access time to drinkers is another method by which to reduce feed intake. Some time ago, Prud'hon *et al.*

(1975a) demonstrated that after 1 week of adaptation, rabbits receiving free access to drinking water for only 10 min day^{-1} reduced their feed intake to 0.76–0.86 of that of rabbits drinking *ad libitum*, depending on the age: 0.86 for 6- to 9-week-old rabbits, 0.84 for 11- to 14-week-old rabbits and 0.76 for adults. The adaptation period was introduced because of the drastic reductions in water and feed intake (–63% and –53%, respectively) in the 1–2 days following the institution of the restriction, followed by 6–8 days of adaptation to the new situation (Lebas and Delaveau, 1975).

In practical conditions with fattening rabbits, limiting access to drinking water to 1.5–4 h induces a reduction in water intake that is proportionally greater than the concomitant reduction in pelleted food intake, mainly for short durations of watering (Fig. 13.9). As a consequence, the water to feed ratio is reduced from 1:74 for rabbits fed *ad libitum* to 1:54 for those receiving water during only 1.5–4 h day^{-1}.

It must be pointed out that with restricted access to drinkers, the water to feed ratio is always reduced as consequence of the drastic reduction in water intake compared to the *ad libitum* control. However, when feed intake is reduced even more than after water access restriction (Boisot *et al.*, 2005), the water intake is clearly enhanced above the *ad libitum* intake (Table 13.4) and the water to feed ratio is increased above that of the control.

13.7 Conclusion

The feeding behaviour of rabbits is very particular compared to that of other mammals, with special features such caecotrophy associated with a particular digestive physiology, intermediate between the non-ruminant and the herbivore. As a herbivore, the feeding strategy of the rabbit is almost opposite to that of ruminants. The feeding strategy of ruminants consists or retaining food particles in the rumen until they reach a sufficiently small size. The rabbit has adopted the reverse strategy, characterized by a preferential retention of fine digesta particles in the fermentative segment (caecum and proximal colon), with rapid removal of the coarse particles (such as poorly digested fibre) in hard faeces. This is associated with numerous meals, thus favouring a quick digesta rate of passage and digestion of the most digestible fibre fractions.

Therefore, the rabbit is adapted to various feeding environments, from desert to temperate and even cold climates, and is able to consume a very wide variety of feeds, from seeds to herbaceous plants.

Table 13.4. Effect of limiting daily drinking duration or reducing the quantity of pellets distributed on relative water and feed intakes (Boisot *et al.*, 2005). Observation during the 3 weeks following weaning at 31 days.

Feeding and watering conditions	*Ad libitum* control	Water available for 1 h day^{-1}	Quantitative feed restriction (theoretical 0.65)
Feed intake	136 g day^{-1} = 1.00	0.78	0.66
Water intake	228 g day^{-1} = 1.00	0.56	1.36
Water to feed ratio	1.7	1.2	3.5

References

Aubret, J.M. and Dupperay, J. (1993) Effets d'une trop forte densité dans les cages d'engraissement. *Cuniculture* 109, 3–6.

Auxilia, M.T., Bergoglio, G., Masoero, G., Mazzocco, P., Ponsetto, P.D. and Terramoccia, S. (1983) Feeding meat rabbits. Use of lucerne with different saponin content. *Coniglicoltura* 20, 51–58.

Balinska, H. (1966) Food preference in rabbits with hypothalamic lesions. *Revue Roumaine de Biologie* 11, 243–247.

Bautista, A., Mendoza-Degante, M., Coureaud, G., Martina-Gomez, M. and Hudson, R. (2005) Scramble competition in newborn domestic rabbits for an unusually limited milk supply. *Animal Behaviour* 70, 997–1002.

Belenguer, A., Balcells, J., Fondevila, A., Abecia, L. and Solanas, E. (2008) Alternative methodologies to estimate ingestion of caecotrophes in growing rabbits. *Livestock Science* 115, 13–19.

Bell, A.C. and Watson, S. (1993) Preferential grazing of five varieties of spring barley by wild rabbits (*Oryctolagus cuniculus*). *Annals of Applied Biology* 122, 637–641.

Bellier, R., Gidenne, T., Vernay, M. and Colin, M. (1995) *In vivo* study of circadian variations of the cecal fermentation pattern in postweaned and adult rabbits. *Journal of Animal Science* 73, 128–135.

Ben Rayana, A., Ben Hamouda, M. and Bergaoui, R. (2008) Effect of water restriction times of 2 and 4 hours per day on performances of growing rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche E Zootechniche, Brescia, Italy, pp. 541–545.

Bergaoui, R., Kammoun, M. and Ouerdiane, K. (2008) Effects of feed restriction on the performance and carcass of growing rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche E Zootechniche, Brescia, Italy, pp. 547–550.

Bhadresa, R. (1977) Food preferences of rabbits *Oryctolagus cuniculus* (L.) at Holkham Sand Dunes, Norfolk. *Journal of Applied Ecology* 14, 287–291.

Biadi, F. and Guenezan, M. (1992) Le lapin de garenne. *Bulletin Technique d'Information* 3, 89–95.

Boisot, P., Duperray, J., Dugenetais, X. and Guyonvarch, A. (2004) Interest of hydric restriction times of 2 and 3 hours per day to induce feed restriction in growing rabbits. In: Becerril, C. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 759–764.

Boisot, P., Duperray, J. and Guyonvarch, A. (2005) Intérêt d'une restriction hydrique en comparaison au rationnement alimentaire en bonnes conditions sanitaires et lors d'une reproduction expérimentale del'Entéropathie Epizootique du lapin (EEL). *11ème Journées Recherche Cunicole*. Paris, France, pp. 133–136.

Carles, Y. and Prud'hon, M. (1980) Influence d'une restriction de la durée quotidienne d'abreuvement sur les performances de la lapine reproductrice. In: Camps, J. (ed.) *Proceedings of the 2nd World Rabbit Congress*, Vol. 1. Asociación Española de Cunicultura, Barcelona, Spain, pp. 424–430.

Cheeke, P.R., Kinzell, J.H. and Pedersen, M.W. (1977) Influence of saponins on alfalfa utilisation by rats, rabbits and swine. *Journal of Animal Science* 46, 476–481.

Cizek, L.J. (1961) Relationship between food and water ingestion in the rabbit. *American Journal of Physiology* 201, 557–566.

Coisne, F. (2000) Sélection des lapines sur leur nombre de mamelles. *Cuniculture* 27, 115–117.

Colin, M., Arkhurst, G. and Lebas, F. (1973) Effet del'adition de methionine au régime alimentaire sur les performances de croissance chez le lapin. *Annales de Zootechnie* 22, 485–491.

Coureaud, G. and Schaal, B. (2000) Attraction of newborn rabbits to abdominal odors of adult conspecifics differing in sex and physiological state. *Developmental Psychobiology* 36, 271–281.

Coureaud, G., Schaal, B., Coudert, P., Rideaud, P., Fortun-Lamothe, L., Hudson, R. and Orgeur, P. (2000) Immediate postnatal suckling in the rabbit: its influence on pup survival and growth. *Reproduction Nutrition Development* 40, 19–32.

Coureaud, G., Fortun-Lamothe, L., Langlois, D. and Schaal, B. (2007) The reactivity of neonatal rabbits to the mammary pheromone as a probe for viability. *Animal* 1, 1026–1032.

CTGREF (1978) Observations sur les préférences alimentaires du lapin de garenne et les dégâts causés aux plantations forestières. *Le Saint-Hubert* Fév, 74–76.

CTGREF (1980) Protection des cultures agricoles et des régénérations forestières contre le lapin de garenne. *CTGREF Informations Techniques,* cahier 39 note 4, 5.

Debray, L., Fortun-Lamothe, L. and Gidenne, T. (2002) Influence of low dietary starch/fibre ratio around weaning on intake behaviour, performance and health status of young and rabbit does. *Animal Research* 51, 63–75.

De Rochambeau, H., Tudela, F. and Chabert, J. (1988) Some results about number of teats on three strains of rabbits. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 2. Sandor Holdas, Hercegalom, Hungary, pp. 261–268.

Drummond, H., Vázquez, E., Sánchez-Colón, S., Martínez-Gómez, M. and Hudson, R. (2000) Competition for milk in the domestic rabbit: survivors benefit from littermate deaths. *Ethology* 106, 511–526.

Duperray, J., Eckenfelder, B. and Le Scouarnec, J. (1998) Effet de la température del'eau de boisson sur les performances zootechniques du lapin de chair. In: Perez, J.M. (ed.) *7èmes Journées de la Recherche Cunicole, Lyon*. ITAVI, Paris, France, pp. 199–202.

Eberhart, S. (1980) The influence of environmental temperatures on meat rabbits of different breeds. In: *Proceedings of the 2nd World Rabbit Congress*, Vol. 1. Asociación Española de Cunicultura, Barcelona, Spain, pp. 399–409.

Echegaray-Torres, J.L., Rebolledo, Ch.O., Rodríguez, H.J.C. and Salcedo-Baca, R. (2004) Effect of nursing frequency on rabbits productive performance. In: Becerril, C. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 1122–1127.

Fehr, P.M., Delage, J. and Richir, C. (1968) Répercutions del'ingestion d'aflatoxine sur le lapin en croissance. *Cahiers de Nutrition et Diététique* 5, 62–64.

Fekete, S. and Lebas, F. (1983) Effect of a natural flavour (thyme extract) on the spontaneous feed ingestion, digestion coefficients and fattening parameters. *Magyar Allatow Lapja* 38, 121–125.

Fleischhauer, H., Schlolaut, W. and Lange, K. (1985) Influence of number of teats on rearing performance of rabbits. *Journal of Applied Rabbit Research* 8, 174–176.

Fortun-Lamothe, L. and Gidenne, T. (2000) The effect of size of suckled litter on intake behaviour, performance and health status of young and reproducing rabbits. *Annales de Zootechnie* 49, 517–529.

Fortun-Lamothe, L. and Gidenne, T. (2003) Les lapereaux préfèrent manger dans la même mangeoire que leur mère. In: Bolet, G. (ed.) *Proceedings of the 10ème ées de la Recherche Cunicole, Paris*. ITAVI, Paris, France, pp. 111–114.

Fortun-Lamothe, L., Prunier, A., Bolet, G. and Lebas, F. (1999) Physiological mechanisms involved in the effects of concurrent pregnacy and lactation on foetal growth and motality in the rabbit. *Livestock Production Science* 60, 229–241.

Fortun-Lamothe, L., Gidenne, T., Lapanouse, A. and De Dapper, J. (2000) Technical note: an original system to separately control litter and female feed intake without modification of the mother–young relations. *World Rabbit Science* 8, 177–180.

Foubert, C., Boisot, P., Duperray, J. and Guyonvarch, A. (2007) Intérêt d'un accès limité à la mangeoire de 6h, 8h et 10h par jour pour engendrer un rationnement alimentaire chez le lapin en engraissement. In: *12èmes Journées de la Recherche Cunicole, Le Mans*. ITAVI, Paris, France, pp.123–126.

Gallois, M., Gidenne, T., Fortun-Lamothe, L., Le Huerou-Luron, I. and Lallès, J.P. (2005) An early stimulation of solid feed intake stimulation slightly influences the morphological gut maturation in the rabbit. *Reproduction Nutrition Development* 45, 109–122.

Gidenne, T. (1985) Effet d'un apport de banane en complément d'un aliment concentré sur la digestion des lapereaux àl'engraissement. *Cuni-Sciences* 3, 1–6.

Gidenne, T. (2003) Fibres in rabbit feeding for digestive troubles prevention: respective role of low-digested and digestible fibre. *Livestock Production Science* 81, 105–117.

Gidenne, T. and Fortun-Lamothe, L. (2002) Feeding strategy for young rabbit around weaning: a review of digestive capacity and nutritional needs. *Animal Science* 75, 169–184.

Gidenne, T. and Lebas, F. (1987) Estimation quantitative de la caecotrophie chez le lapin en croissance: variations en fonctions del'âge. *Annales de Zootechnie* 36, 225–236.

Gidenne, T., Fortun-Lamothe, L., Lapanouse, A., Aymard, P. and De Dapper, J. (2002a) Feeding behaviour in the early weaned rabbit: effect of drinking system. In: Boiti, C., Gidenne, T. and Sabbiani, E. (eds) *European Meeting COST848, Joint Workshop on Reproduction and Nutrition, Varese*. JRC Ispra, Ispra, Italy, p. 32.

Gidenne, T., Jehl, N., Segura, M. and Michalet-Doreau, B. (2002b) Microbial activity in the caecum of the rabbit around weaning: impact of a dietary fibre deficiency and of intake level. *Animal Feed Science and Technology* 99, 107–118.

Gidenne, T., Lapanouse, A. and Fortun-Lamothe, L. (2003) Comportement alimentaire du lapereau sevré précocement: effet du diamètre du granulé. In: Bolet, G. (ed.) *10th Journées de la Recherche Cunicole, Paris*. ITAVI, Paris, France, pp. 17–19.

Gidenne, T., Lapanouse, A. and Fortun-Lamothe, L. (2004) Feeding strategy for the early weaned rabbit: interest of a high energy and protein starter diet on growth and health status. In: Becerril, C. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 853–860.

Grassé, P.P. and Dekeuser, P.L. (1955) Ordre des lagomorphes. In: Grassé P.P. (ed.) *Traité de Zoologie. Anatomie, Systématique, Biologie. Tome XVII Mammifères Les Ordres: Anatomie, Ethologie, Systématique*. Masson et Cie Editeur, Paris, France, pp. 1285–1320.

Gyarmati, T., Szendrö, Z., Maertens, L., Biró-Németh, E., Radnai, I., Milisits, G. and Matics, Z. (2000) Effect of suckling twice a day on the performance of suckling and growing rabbits. In Blasco, A. (ed.) *Proceedings of the 7th World Rabbit Congress, Valencia*, Vol. C. Valencia University Publications, Valencia, Spain, pp. 283–290.

Harris, D.J., Cheeke, P.R. and Patton, N.M. (1983) Feed preference and growth performance of rabbits fed pelleted versus unpelleted diets. *Journal of Applied Rabbit Research* 6, 15–17.

Hirakawa, H. (2001) Coprophagy in leporids and other mammalian herbivores. *Mammal Review* 31, 61–80.

Horton, B.J., Turley, S.D. and West, C.E. (1974) Diurnal variation in the feeding pattern of rabbits. *Life Science* 15, 1895–1907.

Hoy, S. and Selzer, D. (2002) Frequency and time of nursing in wild and domestic rabbits housed outdoors in free range. *World Rabbit Science* 10, 77–84.

Hudson, R. and Distel, H. (1982) The pattern of behaviour of rabbit pups in the nest. *Behaviour* 79, 255–271.

Hudson, R. and Distel, H. (1983) Nipple location by newborn rabbits: evidence for pheromonal guidance. *Behaviour* 82, 260–275.

Hudson, R., Schaal, B., Martinez-Gomez, M. and Distel, H. (2000) Mother–young relations in the European rabbit: physiological and behavioral locks and keys. *World Rabbit Science* 8, 85–90.

Jilge, B. (1982) Monophasic and diphasic patterns of the circadian caecotrophy rythm of rabbits. *Laboratory Animals* 16, 1–6.

Jolivet, E., Reyne, Y. and Teyssier, J. (1983) A methodological approach to the study of the circadian pattern of feed intake in the growing domestic rabbit. *Reproduction Nutrition Development* 23, 13–24.

Kovacs, M., Szendrö, Z., Csutoras, I., Bota, B., Bencsne, K.Z., Orova, Z., Radnai, I., Birone, N.E. and Horn, P. (2004) Development of the caecal microflora of newborn rabbits during the first ten days after birth. In: Becerril, C. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 1091–1096.

Lebas, F. (1969) Alimentation lactée et croissance pondérale du lapin avant sevrage. *Annales de Zootechnie* 18, 197–208.

Lebas, F. (1971) Nombre de postes de consommation pour des groupes de lapins en croissance. *Bulletin Technique d'Information* 260, 561–564.

Lebas, F. (1972) Effet de la simultanéité de la gestation et de la lactation sur les performances laitières chez la lapine. *Annales de Zootechnie* 18, 197–208.

Lebas, F. (1973) Possibilités d'alimentation du lapin en croissance avec des régimes présentés sous forme de farine. *Annales de Zootechnie* 22, 249–251.

Lebas, F. (1975) Etude chez les lapines del'influence du niveau d'alimentation durant la gestation. I. Sur les performances de reproduction. *Annales de Zootechnie* 24, 267–279.

Lebas, F. (1977) Faut-il éclairer les lapins durant l'engraissement? *Cuniculture* 5, 233–234.

Lebas, F. (2002) Biologie du lapin. 4.4 Comportement alimentaire. Available from: http://www.cuniculture.info/Docs/Biologie/biologie-04-4.htm (accessed 25 January 2010).

Lebas, F. (2007) L'utilisation de la restriction alimentaire dans la filière cunicole et les différents modes de contrôle utilisés sur le terrain. *12èmes Journées de la Recherche Cunicole, INRA-ITAVI, Le Mans*. Présentation orale lors de la table ronde. Available from: http://www.asfc-lapin.com/Docs/Activite/T-ronde-2007/T-ronde2007-1.htm (accessed 25 January 2010).

Lebas, F. and Delaveau, A. (1975) Influence de la restriction du temps d'accès à la boisson sur la consommation alimentaire et la croissance du lapin. *Annales de Zootechnie* 24, 311–313.

Lebas, F. and Greppi, G. (1980) Ingestion d'eau et d'aliment chez le jeune lapin disposant d'un aliment carencé en méthionine ou en lysine et pour boisson, en libre choix, d'une solution de cet acide aminé ou d'eau pure. *Reproduction Nutrition Development* 20, 1661–1665.

Lebas, F. and Laplace, J.P. (1977) Le transit digestif chez le lapin. VI. Influence de la granulation des aliments. *Annales de Zootechnie* 26, 83–91.

Lebas, F., Coudert, P., De Rochambeau, H., and Thébault, R.G. (1997) *The Rabbit – Husbandry, Health and Production*, 2nd edn. FAO Publishing, Rome, Italy.

Leslie, T.K., Dalton, L. and Phillips, C.J.C. (2004) Preference of domestic rabbits for grass or coarse mix feeds. *Animal Welfare* 13, 57–62.

Maertens, L. (1994) Infuence du diamètre du granulé sur les performances des lapereaux avant sevrage. In: Coudert, P. (ed.) *Proceedings of the VIèmes ées de la Recherche Cunicole, La Rochelle*, Vol. 2. ITAVI, Paris, France, pp. 325–332.

Maertens, L. and Van Herck, A. (2000) Performances of weaned rabbits raised in pens or in classical cages: first results. In: Blasco, A. (ed.) *Proceedings of the 7th World Rabbit Congress, Valencia*. Valencia University Publications, Valencia, Spain, pp. 435–440.
Maertens, L., Lebas, F. and Szendrö, Zs. (2006) Rabbit milk: a review of quantity, quality and non dietetary affecting factors. *World Rabbit Science* 14, 205–230.
Matics, Zs., Dalle Zotte, A., Radnai, I., Kovács, M., Metzger, Sz. and Szendrö, Zs. (2008) Effect of restricted feeding after weaning on the productive and carcass traits of growing rabbits. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche E Zootechniche, Brescia, Italy, pp. 741–745.
Mirabito, L., Galliot, P., Souchet, C. and Pierre, V. (1999) Logement des lapins en engraissement en cages de 2 ou 6 individus: etude du budget-temps. In: Perez, J.M. (ed.) *8èmes Journées de la Recherche Cunicole, Paris*. ITAVI, Paris, France, pp. 55–58.
Moncomble, A.S., Quennedy, B., Coureaud, G., Langlois, D., Perrier, G. and Schaal B. (2004) Newborn rabbit attraction toward maternal faecal pellets. *Developmental Psychobiology* 45, 277.
Morisse, J.P., Wyers, M. and Drouin, P. (1981) Aflatoxicose chronique chez le lapin. Essai de reproduction expérimentale. *Recueil de Médecine Vétérinaire* 157, 363–368.
Morot, C. (1882) Mémoire relatif aux pelotes stomacales des léporidés. *Recueil de Médecine Vétérinaire* 59, 635–646.
Orengo, J. and Gidenne, T. (2007) Feeding behaviour and caecotrophy in the young rabbit before weaning: an approach by analysing the digestive contents. *Applied Animal Behaviour Science* 102, 106–118.
Pascual, J.J., Cervera, C., Blas, E. and Fernandez-Carmona, J. (1998) Effect of high fat diets on the performance and food intake of primiparous and multiparous rabbit does. *Animal Science* 66, 491–499.
Pascual, J.J., Tolosa, C., Cervera, C., Blas, E. and Fernandez-Carmona, J. (1999) Effect of diets with different digestible energy content on the performance of rabbit does. *Animal Feed Science Technology* 81, 105–117.
Pascual, J.J., Cervera, C. and Fernandez-Carmona, J. (2001) Effect of solid food intake before weaning on the performance of growing rabbits. *Proceedings of the 2nd Meeting of Workgroups 3 and 4*. COST Action, Godollo, Hungary, p. 48.
Prud'hon, M. (1976) Comportement alimentaire du lapin soumis aux températures de 10, 20 et 30°C. In: Lebas, F. (ed.) *Proceedings of the 1st World Rabbit Congress*. WRSA, Dijon, France.
Prud'hon, M., Cherubin, M., Carles, Y. and Goussopoulos, J. (1975a) Effets de différents niveaux de restriction hydrique sur l'ingestion d'aliments solides par le lapin. *Annales de Zootechnie* 24, 299–310.
Prud'hon, M., Cherubin, M., Goussopoulos, J. and Carles, Y. (1975b) Evolution au cours de la croissance des caractéristiques de la consommation d'aliments solides et liquides du lapin domestique nourri ad libitum. *Annales de Zootechnie* 24, 289–298.
Reyne, Y. and Goussopoulos, J. (1984) Caractéristiques du système endogène responsable des rythmes circadiens de la prise de nourriture et d'eau de boisson chez le lapin de garenne: étude en lumière permanente et en obscurité permanente. In: Finzi, A. (ed.) *Proceedings of the 3rd World Rabbit Congress,* Vol. 2. Rome, Italy, pp. 473–480.
Ross, W.D. and Bell, J. (1979) A field study on preference for pollard and bran pellets by wild rabbits. *New Zealand Journal of Experimental Agriculture* 7, 95–97.
Saubois, A. and Nepote, M.C. (1994) Aflatoxins in mixed feeds for rabbits. *Bolletin Micologico* 9, 115–120.
Schaal, B., Coureaud, G., Langlois, D., Ginies, C., Semon, E. and Perrier, G. (2003) Chemical and behavioural characterization of the rabbit mammary pheromone. *Nature* 424, 68–72.
Selim, A.D., Soliman, A.Z. and Abdel-Khalek, A.M. (2004) Effect of drinking water temperature and some dietary feed additives on performance of heat stressed rabbits. In: Becerril, C. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 981–990.
Spence, T.B. and Smith, A.N. (1965) Selective grazing by rabbits. *Agriculture Gazette of N.S. Wales* 76, 614–615.
Szendrö, Z., Szabo, S. and Hullar, I. (1988) Effect of reduction of eating time on production of growing rabbits. In: *Proceedings of the 4th World Rabbit Congress, Budapest*. Sandor Holdas, Hercegalom, Hungary, pp. 104–114.
Szendrö, Z., Mohamed, M.M.A. and Biro-Nemeth, E. (1991) Teat number of new-born rabbits depending on the teat number of their parents. *Journal of Applied Rabbit Research* 14, 133–135.
Szendrö, Z., Papp, Z. and Kustos, K. (1999) Effect of environmental temperature and restricted feeding on production of rabbit does. *CIHEAM, Cahiers Options Méditeranéennes* 41, 11–17.

Tag El Den, T.H., Mervat, T.H. and Ali Molnar, A. (1988) Effect of restricting feeding periods on feed intake and digestibility of dry matter in rabbits. In: *Proceedings of the 4th World Rabbit Congress, Budapest*. Sandor Holdas, Hercegalom, Hungary, pp. 213–222.

Tome, D. (2004) Protein, amino acids and the control of food intake. *British Journal of Nutrition* 92, S27–S30.

Tudela, F. and Balmisse, E. (2003) Influence du nombre journalier de tétées sur la production laitière des lapines. In: Bolet, G. (ed.) *10ème Journées de la Recherche Cunicole*. INRA-ITAVI, Paris, France.

Tudela, F. and Lebas, F. (2006) Modalités du rationnement des lapins en engraissement. Effets du mode de distribution de la ration quotidienne sur la vitesse de croissance, le comportement alimentaire etl'homogénéité des poids. *Cuniculture Magazine* 33, 21–27.

Verdelhan, S., Bourdillon, A. and Morel-Saives, A. (2004) Effect of a limited access to water on water consumption, feed intake and growth of fattening rabbits. In: Becerril, C. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 1015–1021.

Verga, M., Dell'Orto, V. and Carenzi, C. (1978) A general review and survey of maternal behaviour in the rabbit. *Applied Animal Ethology* 4, 235–252.

Verga, M., Canali, E., Pizzi, F. and Crimella, C. (1986) Induced reactions in young rabbits of dams of different parity and reared on two different nursing schedules. *Applied Animal Behavioural Science* 16, 285–293.

Virag, G., Papp, Z., Rafai, P., Jakab, L. and Kennessy, A. (2000) Effects of an intermittent lighting schedule on doe and suckling rabbit's performance. In: Blasco, A. (ed.) *Proceedings of the 7th World Rabbit Congress, Valencia*, Vol. 8. Valencia University Publications, Valencia, Spain, pp. 477–481.

Williams, O.B., Wells, T.C.E. and Wells, D.A. (1974) Grazing management of Woodwalton Fen: seasonal changes in the diet of cattle and rabbits. *Journal of Applied Ecology* 11, 499–516.

Xiccato, G., Verga, M., Trocino, A., Ferrante, V., Queaque, P.I. and Sartori, A. (1999) Influence del'effectif et de la densité par cage sur les performances productives, la qualité bouchère et le comportement chez le lapin. In: Perez, J.M. (ed) *8th Journées de la Recherche Cunicole, Paris*. ITAVI, Paris, France, pp. 59–62.

Xiccato, G., Trocino, A., Boiti, C. and Brecchia, G. (2005) Reproductive rhythm and litter weaning age as they affect rabbit doe performance and body energy balance. *Animal Science* 81, 289–296.

Zarrow, M.X., Denenberg, V.H. and Anderson, C.O. (1965) Rabbit: frequency of suckling in the pup. *Science* 150, 1835–1836.

14 Feeding Systems for Intensive Production

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14.1 Introduction

Rabbits are primarily raised as meat producers and secondarily as wool or fur producers. They are also an important pet and exhibition animal in many countries and widely used in biomedical research. In recent years, interest has been increasing in biotechnology to produce protein-linked substances with transgenic rabbits. The high daily output (50 g kg^{-1} live weight day^{-1}) of protein-rich milk (120 g kg^{-1}) makes female rabbits suitable for the production of human drugs (Maertens *et al.*, 2006).

In intensive production systems, rabbits are almost exclusively fed with a balanced compound diet in order to fulfil their dietary requirements, with a view to optimizing their production records and feeding management. The size of commercial farms has been dramatically increased due to the introduction of artificial insemination and the batch management system. As an example of intensive rabbit production, the average production records obtained from commercial farms in France are presented in Table 14.1.

In rabbit meat production, as with other animal species, feeding costs represent the largest part of the production costs. Depending mainly on investment costs, they amount to 0.60–0.70 of the total costs. In fact, the production costs for meat rabbits are twice as high as those for broilers and 25–35% higher than for pigs. In view of being competitive with these animal productions, a reduction in feeding costs is of primary importance. For this reason, this chapter considers in detail different possibilities for optimizing the feed conversion ratio (FCR) in rabbit production.

14.2 Diet Presentation

In intensive rabbit production, dried and ground raw materials are used to prepare balanced compound diets. These concentrated diets are generally pelleted because rabbits show a strong preference for pellets over the same diet in meal or mash form. The processing costs for pelleting rabbit diets are more than compensated for by a number of benefits. Significantly lower amounts of feed are consumed on meal diets, resulting in lower daily weight gain, inferior FCR and lower slaughter yield (Table 14.2). When offered a choice between meal and pellet form, 0.97 of the total feed intake is of the pelleted diet (Harris *et al.*, 1983).

Other benefits of pelleting are comparable to those for other animals: segregation or selection between the different raw materials is impossible, higher amounts of

Table 14.1. Average production records in 2006 from commercial rabbit farms in France (Lebas, 2007).

Criteria	2006
Number of farms considered	1089
Does per farm	495
Replacement rate (%)	113
Kindling per artificial insemination (%)	79.4
Number of parities per female year^{-1}	6.85
Litter size at birth (alive)	9.5
Litter size at weaning	8.1
Mortality (plus eliminated) before weaning (%)	15.8
Mortality after weaning (%)	8.5
Fatteners produced per doe year^{-1}	50.7
Market weight of growers (kg)	2.45
Feed per rabbits produced (kg)[a]	3.58

[a]Total feed consumption including young parent stock, does and fatteners.

co-products can be fed and feed wastage is minimal. Pellets further reduce dust problems in houses and automatic or semi-automatic rabbit feeders work much more easily with pellets than with meal or mash.

Several efforts have been undertaken with other presentation forms. When a limited number of raw materials are mixed and supplied, rabbits select those raw materials according to their palatability. As a result of this unbalanced intake, performances deteriorate (Schlolaut, 1995). When cereals and protein sources are covered with molasses, however, rabbits are less able to distinguish between the different dietary components.

With low cereal prices, efforts have been made to feed high amounts of whole grains together with a concentrated pellet. Rommers *et al.* (1996) compared pellets with a mixture of 0.85 pellets and 0.15 whole wheat or barley for fatteners. The biological performance was not significantly different. However, the cereals accumulated in the feeders because the rabbits showed preference for the pellets. Because feed wastage was not observed, mainly due to the construction of the feeders, it was concluded that a mixture of pellets and whole grains could reduce feeding costs. However, attention must be drawn to the need to avoid feed wastage and to feed a homogenous mixture.

Instead of pelleting, rabbit diets can be extruded. Rabbits accept such a presentation if the durability and hardness are acceptable, but performances tended to decrease, especially in young rabbits (Maertens and Luzi, 1995). The decreased daily weight gain could be related to degradations in protein quality due to the high temperature during the extrusion. Moreover, the intended higher starch digestibility was not obtained and consequently extrusion failed to reduce the mortality rate of high-starch diets (Maertens and Luzi, 1995). Similarly, Fernández-Carmona *et al.* (1983) obtained a lower intake and growth rate and considerable variability in FCR when rabbits from 18 to 42 days of age were fed an extruded diet as opposed to the pelleted diet. The authors

Table 14.2. Effect of diet presentation on the performances of growing rabbits (as a proportion of pellet).

Reference	Presentation form	DWG	DFI	FCR[a]
Lebas (1973)	Pellet	=1.00	=1.00	=1.00
	Meal	0.87	0.83	1.06
King (1974)	Pellet	=1.00	=1.00	=1.00
	Meal	0.93	0.90	1.03
Machin *et al.* (1980)	Pellet	=1.00	=1.00	=1.00
	Meal	0.98	0.80	1.23
	Mash (0.40 water)	0.75	0.84	0.89
Candau *et al.* (1986)	Pellet	=1.00	=1.00	=1.00
	Meal	0.60	0.75	1.23
Sánchez *et al.* (1984)	Pellet	=1.00	=1.00	=1.00
	Meal	0.64	0.52	2.79

DWG, daily weight gain; DFI, daily feed intake; FCR, feed conversion ratio.
[a]A higher figure indicates worse FCR.

explained these inferior results by the lower quality of the pellets in the extruded diet (lower durability and hardness).

In intensive rabbit meat production, a pelleted balanced diet is the basis for meeting nutrient and energy requirements for maximizing biological performance. All 'alternative' methods (e.g. meal, mash, roughages, mixture of raw materials) decrease the daily dry matter intake. Most of these methods are labour-intensive because they have to be fed daily and are difficult to distribute automatically; they are therefore not suitable for large-scale production.

14.2.1 Pellet size and quality

The length of pellets is preferentially between 0.8 and 1 cm. If longer, there is a higher risk of breaking during handling. Moreover, losses of single pellets or parts of pellets by the rabbits are more frequent at sizes >1 cm. The preferential pellet diameter is in the range of 3–4 mm, which is also suitable for use in rabbit feeders and minimizes production costs. At diameters >5 mm, the risk of pellet wastage increases (Lebas, 1975a).

Small pellet size (diameter <2.5 mm) tends to decrease feed intake, probably due to the increased feeding time (Maertens, 1994). This effect was partly confirmed in the choice feeding trial of Gidenne *et al.* (2003a) with early-weaned rabbits. Between 18 and 31 days of age, rabbits consumed 40% fewer 2.5 mm pellets than control pellets of 3.5 mm. However, the hardness of the small pellets was 18% higher and it is not clear if a small pellet size or higher durability is responsible for the decreased feed intake at a smaller size.

Changing from a large pellet diameter (4.8 mm) to a small diameter (2.5 mm) at weaning leads to a 20% reduction in feed intake and weight gain, while the opposite change induces a 10% increase in feed intake and weight gain shortly after weaning (Maertens, 1994).

Pellet durability and hardness are the major quality characteristics of rabbit pellets because rabbits do not eat the fines between the pellets. Several types of device for measuring pellet quality have been used by the industry. Generally, these devices can be classified into those testing the resistance of pellets to crushing (hardness) or those testing fragmentation when rubbed or shaken (durability). The pneumatic-powered hardness testers determine the power (in kg) for crushing pellets (Payne *et al.*, 1994). Although this method is quick, sufficient pellets (>ten) have to be tested in order to have good repeatability. Equipment using a motor drive instead of manual handling is preferable because it excludes effects due to the operator. A minimum hardness of 8 kg is necessary to avoid excessive fines being produced during handling or transport, especially when using automatic feeders.

To test the durability of pellets, a standard method using a square tumbling can has been developed (Phost, 1963). The can is rotated at a speed of 50 rpm using a perpendicular axis centred on both sides. A quantity of 500 g of pellets, after sieving out the fines, is used for the test. The sample is placed in the tumbling can and rotated for 10 min; after sieving again (standard mesh size just smaller than the nominal pellet diameter), the remaining pellets are weighed and the pellet durability index (g pellets after/g pellets before tumbling × 10) is obtained. Under correct processing and handling conditions, <0.02 of 'fines' may be produced in quality pellets during transport, in silos or bags and in tubes and rabbit feeders.

When the resistance to crushing is between 7 and 13 kg, the biological performance of rabbits is not influenced by the hardness of the pellets (Morisse *et al.*, 1985).

14.3 Feed Storage

With the increasing size of rabbit breeding units, feeds are mainly delivered in bulk. Packaging in bags is still used for small units or for special feeds (e.g. weaning diets). Storage time should be limited to 3–4 weeks, employing outdoor silos. Due to temperature variations, feed may sweat and become mouldy. Taking into account the fact that about 4 t feed week^{-1} is consumed in a unit of 500 does and corresponding fatteners, a storage capacity of about 15 t is necessary for bulk feed. When only two different diets are supplied (for lactation and growth), the minimal silo sizes are 5 and 10 t for does and fatteners, respectively.

If stored in a dry location, rabbit feed with a dry matter content of at least 890 g kg^{-1} can be stored for several months, which implies fewer diet changes for a growing cycle. Cages equipped with manual-filled feeders must contain at least the quantity consumed daily. With automatic feeding systems, tubes supplying a number of cages (rather than individual feeders) are used. In such systems, feed is distributed several times per day. Although it is claimed that rabbits eat more when fresh feed is served, no experiments have demonstrated this.

14.4 Number of Diets

With increasing knowledge of the specific requirements of the different categories of rabbits, a series of diets can be proposed. However, based on practical considerations and because of the relatively small differences in nutrient and energy concentrations between the different diets, the number of diets should be limited.

In practice, two or three silos (diets) are economically optimum for a middle-sized rabbitry, otherwise the quantities involved are too small to operate with bulk feed. Furthermore, automatic or semi-automatic feeding systems are increasingly being used in large units. These do not allow the distribution of a different diet to each category. However, rabbit management is changed from individual handling to a batch system. In this way, animals in the same reproductive phase or of the same age are grouped together in one building or battery. Such a management system allows the use of phase feeding programmes. The same silo is progressively filled with an adapted diet according to the age of the fatteners.

In a rabbitry, depending on the weaning date and slaughter weight, about 0.5–0.6 of the feed is consumed in the fattening unit and 0.4–0.5 in the reproduction unit (Fig. 14.1a). Young parent stock, males (if present) and does in pre-gestation cages have similar requirements. They can be fed a fattening diet, sometimes on a restricted basis.

After weaning, about 0.65 of the feed is consumed in the second half of the fattening period (Fig. 14.1b). A specific finishing diet (high energy level, reduced protein content) favours feed efficiency and reduces the nitrogen output to the environment.

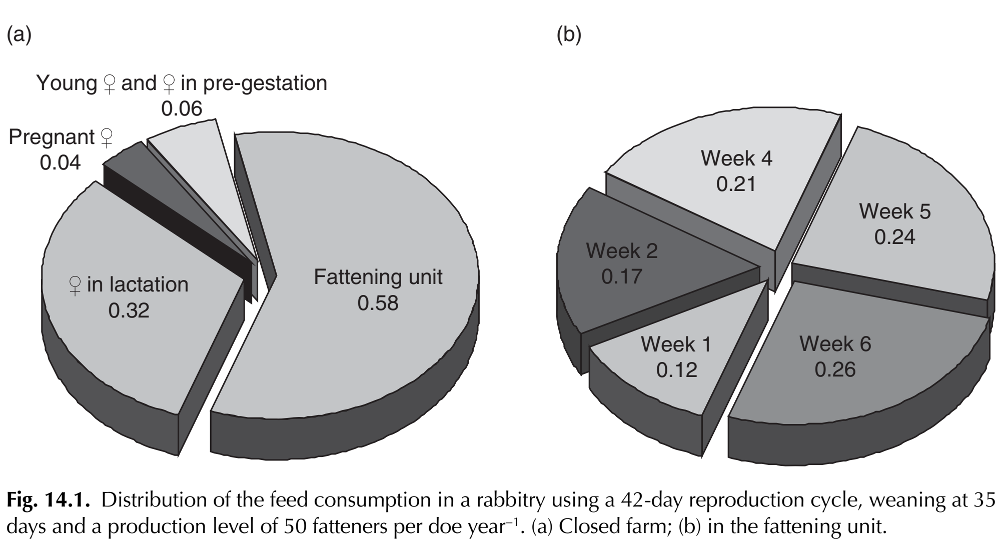

Fig. 14.1. Distribution of the feed consumption in a rabbitry using a 42-day reproduction cycle, weaning at 35 days and a production level of 50 fatteners per doe year^{-1}. (a) Closed farm; (b) in the fattening unit.

14.5 Feed and Water Intake

The average feed intakes and feed efficiencies of weaned rabbits until slaughter weight are given in Table 14.3. Data reflect results obtained with a quickly growing strain and weaning at 30 days. Growth, and consequently feed intake curves, show an irregular development during the fattening period; therefore, the data presented in Table 14.3 are mean values of several batches of hybrid rabbits. Feed intake increases with age, but not when expressed as kg^{-1} live body weight. The highest feed intake per unit of weight is reached before the maximal growth rate occurs.

The feed intake of does varies considerably during the reproductive cycle, from 150 to 450 g day^{-1}. Feed intake patterns during the lactation period correspond largely to the milk yield of the doe and consequently drop dramatically after weaning.

Rabbits fed a pelleted diet have a water requirement that exceeds the dry matter intake. The ratio of feed to water consumption increases during the fattening period, from 1.55 to 1.65 (Laffolay, 1985). This ratio is about 1.9 for non-lactating does or adults at maintenance. Lactating does have a water intake that is about twice the feed intake.

If water is not available, feed intake drops quickly and will stop within 24 h. Limited drinking water leads to reduced feed intake and is sometimes used as an indirect way of inducing restricted feeding (Boisot *et al.*, 2004). However, this management system cannot be defended from a welfare viewpoint.

14.6 Practical Feeding of the Different Categories of Rabbits

In commercial rabbitries, as a rule, rabbits are fed on an *ad libitum* basis (Table 14.4). This is not only for practical considerations, but also because of the rapid reproductive rate and the adjustment of the voluntary feed intake in response to changes in dietary energy concentration. However, some categories of rabbits are fed on a restricted basis to prevent excessive fattening or overcome digestive disturbances. In Table 14.4, a feeding scheme for commercial rabbit meat production is presented.

When restricted feeding is applied with a pelleted diet, no advantage has been found for feeding the total quantity once a day instead of spreading it over two meals (Tudela and Lebas, 2006). Moreover, in fatteners the number of feeding places does not need to be increased when feed is restricted (Tudela and Lebas, 2006).

14.6.1 Young parent stock

Feeding young parent stock properly is very important because it affects the lifetime reproductive capacity of the animals. The optimal feeding regime for young does

Table 14.3. Average values of weight gain, feed consumption and technical feed conversion ratio during the fattening period.[a]

Age (days)	Weight (g)	Weight gain (g day^{-1})	Feed intake		Feed conversion ratio	
			g day^{-1}	g kg^{-1} LW	$Week^{-1}$	Cumulative
21–30	400–740	38	35 + milk	–	–	–
30–37	740–1050	44	84	94	1.91	1.91
37–44	1050–1395	49	114	93	2.33	2.13
44–51	1395–1750	51	136	86	2.67	2.32
51–58	1750–2085	48	148	77	3.08	2.51
58–65	2085–2395	44	160	71	3.64	2.72
65–72	2395–2680	41	171	67	4.17	2.94

LW, live weight.

[a]Diet: 10 MJ digestible energy kg^{-1}. Moderate temperature conditions (15–23°C), no assumed mortality.

Table 14.4. Feeding scheme for commercial rabbit meat production.

Category	Quantity	Diet
Young does		
Early mating (15–16 weeks)	*Ad libitum*	Fatteners
Late mating (17–20 weeks)	Restricted (40 g kg^{-1} live weight, followed by a 4-day flushing before insemination)	Fatteners or specific rearing diet
Does		
Late gestation	*Ad libitum*	Lactation
Lactating	*Ad libitum*:	
	Kits <3 weeks	Lactation
	Kits >3 weeks	Weaning
In pre-gestation cages	Restricted (40 g kg^{-1} live weight), but *ad libitum* 4 days prior to insemination	Fatteners
Males		
Young (until 18 weeks)	*Ad libitum*	Fatteners
Adult	Restricted (40 g kg^{-1} live weight)	Fatteners
Weaned rabbits		
4–6/7 weeks	Restricted, 0.75 of *ad libitum*	Fatteners
6/7–10/11 weeks	*Ad libitum*	Fatteners/finishing

depends to a large extent on the age of the first desired mating. Although evidence is found in the literature that *ad libitum* feeding together with early mating (0.75–0.80 of the adult weight) leads to favourable results in obtaining a first litter, in practice it is recommended to restrict feeding in young does and postpone the first mating until the age of at least 17 weeks, with a target of 0.85–0.90 of the adult weight (Rommers *et al.*, 2006). This improves litter size. Restricted feeding during rearing also results in a higher milk yield and increased weaning weight of the kits at the end of the first lactation. Flushing 4–5 days prior to mating or insemination leads to oestrous synchronization, high pregnancy rates and a greater number of follicles (Rommers *et al.*, 2006).

Another method to restrict feeding in young parent stock is to use a low-energy, high-fibre diet (<8 MJ digestible energy (DE) kg^{-1}). Such a diet, even fed *ad libitum*, induces growth retardation, but the rabbit develops a higher intake capacity in the first lactation (Cervera *et al.*, 2008).

When restricted-fed, a daily quantity of 40 g kg^{-1} live weight is sufficient to cover the nutrient and energy requirements when a moderately concentrated diet (9.5 MJ DE kg^{-1}) is fed. Requirements increase rapidly during the second half of the gestation period, but the intake capacity decreases because of the development of fetuses in the abdomen. Subsequently, feed is provided *ad libitum*.

After weaning, non-pregnant does or does in early pregnancy have to be restricted-fed to prevent excessive fattening, which would lead to both high perinatal mortality and suppression of voluntary feed intake in early lactation (Partridge *et al.*, 1986; Pascual *et al.*, 2003). The same quantity as mentioned for young does is recommended. In late gestation, feed restriction is no longer necessary.

14.6.2 Males

Males increase their voluntary feed intake until the age of 5 months. Subsequently, feed intake drops by about 0.30 or a natural feed restriction takes place. In comparison with restricted-fed littermates (0.75 of *ad libitum*), libido or semen characteristics are not negatively influenced by *ad libitum* feeding (Luzi *et al.*, 1996). Excessive feed restriction in males is therefore not recommended. However, males from heavy lines frequently show sore hocks in wire mesh cages; feed restriction reduces their adult weight by about 0.5 kg and consequently favourable effects on longevity may be expected.

14.6.3 Lactating does and their young

Lactating females have a high nutrient and energy demand due to their concentrated milk production (Maertens *et al.*, 2006). A concentrated high-energy lactation diet stimulates daily nutrient and energy intake, and reduces the energy deficit at the end of the lactation (see Chapter 6). Feed intake gradually increases as milk production increases (Fig. 14.2). Feeding the lactating doe *ad libitum* is a common habit to fulfil the high nutrient and energy demands.

Lactating does and their young eat out of the same feeder. Specific starter diets (creep feeding) as for piglets are not commonly used with rabbits. Although a system has been developed to feed the female and her litter separately (Fortun-Lamothe *et al.*, 2000), it is not actually in use in practice. Before the age of 3 weeks, only small amounts of feed are consumed by the young. Therefore, a lactation diet adapted to the requirements of the doe is fed in early lactation.

Once the young start to eat significant amounts of solid feed, from the age of 3 weeks, preference can be given to the young. A diet more adapted to their requirements has been shown to not only promote higher weaning weights, but also favour their intestinal health (Xiccato *et al.*, 2006; Gidenne *et al.*, 2007).

14.6.4 Weaned young

If a specific weaning diet is fed from the age of 3 weeks, this may be continued after weaning until the age of 7–8 weeks. Once the critical period is passed (7–8 weeks of age), rabbits are fed a more concentrated fattening diet. The increased energy concentration favours the conversion ratio.

A phase-feeding programme during the fattening period is designed to reduce mortality, increase biological performance and minimize mineral excretion in order to protect the environment. However, such a programme requires scheduled production in large groups.

Besides dietary qualitative aspects, quantitative aspects of feeding have proved to be helpful in overcoming losses due to diarrhoea (see Chapter 10). Based on studies and success under practical conditions, many farms no longer feed weanlings *ad libitum*. A reduction of feed intake by at least 25% has proven to be very helpful in overcoming enteritis problems between the ages of 5 and 8 weeks. Gidenne *et al.* (2003b) found the mortality rate to be halved in restricted-fed rabbits compared to those fed *ad libitum*. On intensive farms, increasing use is therefore being made of automatic feeding systems, which allow the distribution of a restricted quantity of

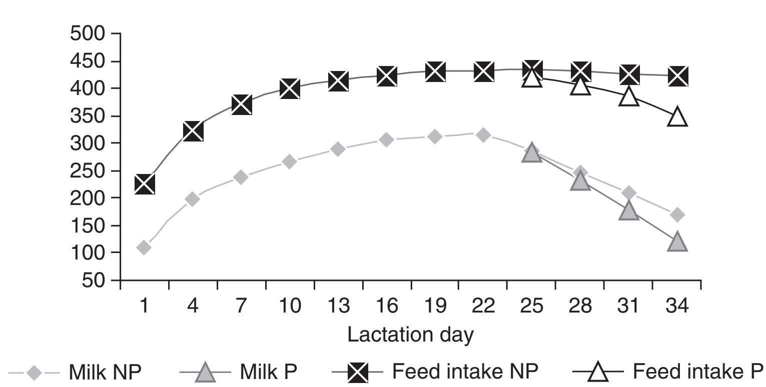

Fig. 14.2. Feed intake (g day^{-1})and milk yield (g day^{-1}) of does if pregnant (P) or non-pregnant (NP) 11 days post-parturition (data from Maertens and De Groote, 1991; Maertens *et al.*, 2006).

feed in relation to the age of the growing rabbit.

An indirect method of restricting feed intake is to restrict water intake. Rabbits have a relatively small stomach, which limits a high water and feed intake during a short period of time. Therefore, when the water distribution is limited to 2–3 h (continuous) day^{-1}, feed intake is only 0.70 of the *ad libitum* intake. Under such conditions, both in experimental studies as on commercial farms, positive results have been obtained in reducing enteritis and losses due to diarrhoea (Boisot *et al.*, 2003, 2004). As already mentioned, however, restricting water cannot be defended from a welfare viewpoint and direct feed restriction should be applied to prevent enteritis in young rabbits (see also Chapter 11).

14.7 Feed Conversion Ratio

Numerous experimental FCR data are available in fatteners, but only very few data are available for the reproduction unit. Nevertheless, to improve FCR, possibilities have to be considered for both females and fatteners. The most important factors are the use of efficient stock, the quality of the feed, limitation of losses (mortality) and farm management (e.g. reproduction efficiency, slaughter age). The impact of some of the factors of primary importance in reducing FCR will be discussed.

14.7.1 Definition of feed conversion ratio

When speaking about FCR, in practice the most extensively used parameter for estimatimating feed efficiency in intensive systems is the overall *global* (farm) *feed conversion ratio*. This global FCR is defined for a closed unit (maternity and fattening) as the ratio between the kg of feed consumed (bought) per kg of rabbits produced (sold). Consequently, it is very valuable from a practical and economic viewpoint.

Recent overviews of farm data have shown average FCRs of 3.60, 3.82 and 3.63 in France, Italy and Spain, respectively (Lebas, 2007; Rosell and González, 2007; Xiccato *et al.*, 2007). However, all of these studies stressed the big differences between farms (from <3.0 to >4.5) (Fig. 14.3). In this index, reproduction efficiency and

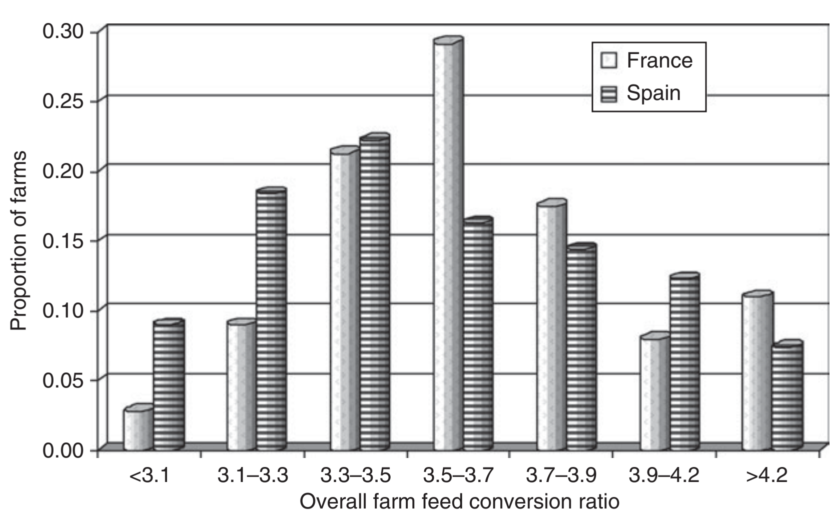

Fig. 14.3. Variations in farm feed conversion ratios in France and Spain (Lebas, 2007; Rosell and González, 2007).

slaughter weight are the main factors that influence the FCR. When the same mortality is considered (10% after weaning), the cummulative effect of both variables results in an increase from 3.07 to 4.03, or 31.3% (Table 14.5).

When the FCR is calculated in fatteners then the FCR is defined as the ratio of kg of feed consumed per kg weight gain of rabbits (finishing weight minus weaning weight). In this FCR, the feed consumption of rabbits lost (mortality and removed) is included, while no weight gain for them is considered. This is correct from an economic viewpoint, and is therefore defined as the *economic FCR*. If we calculate this FCR in the reproduction unit then the FCR is the ratio between kg of feed consumed and kg of rabbits weaned plus sold old females.

However, if mortality is not one of the target variables in nutrition experiments, the effect of mortality is eliminated and the result is the *technical FCR*. In this method, only the feed consumed by rabbits reaching the end of the experimental period is taken into account. As a consequence, the technical FCR is lower than the economic FCR. For this correction, it is assumed that no feed was consumed during the 2 days preceding death (Maertens *et al.*, 2005b).

In addition to FCR, efficacy of the feed utilization is sometimes presented as *feed efficiency* (de Blas *et al.*, 1998). From a scientific point of view this inverse ratio, namely kg of weight gain per kg feed consumed, shows a figure that better expresses the efficiency and is therefore suggested for experimental purposes.

14.7.2 Feed conversion ratio as affected by age

Young and fast-growing animals have a far more favourable FCR in the early fattening stage than when near slaughter weight. The different content of tissue accretion (fat versus protein and water) and increased maintenance requirements are responsible for the very fast increase in FCR above a weight of 2.0 kg (FCR >3.25). In Table 14.3, recent data obtained at our experimental unit are presented for the technical FCR with a fast-growing strain.

14.7.3 Diet concentration

Feed effciency is negatively correlated with dietary DE content, as was originally shown 30 years ago by Lebas (1975b) and confirmed in many later experiments. A rabbit regulates its feed intake according to energy requirements, as for other mammals. In non-ruminants glycaemia plays a key role in food intake regulation, while in ruminants the levels of plasma volatile fatty acids have a major role. Since rabbit is a non-ruminant herbivore, the main blood component regulating feed intake is unclear, but it is likely to be the blood glucose level (Gidenne and Lebas, 2005). However, because of the close relationship between dietary fibre and DE content, daily feed intake (and by consequence FCR) is even more correlated with lower digestible fibre (acid detergent fibre) than with the higher DE content (Gidenne and Lebas, 2005).

Based on the relationship between dietary DE content and intake, an improved

Table 14.5. Global feed conversion ratio for different slaughter weights and the number of rabbits produced per doe year^{-1} (adapted from Maertens *et al.*, 2005a).

	Number of rabbits produced per doe year^{-1}			
Slaughter weight (kg)	40	45	50	55
2.00	3.64	3.39	3.21	3.07
2.25	3.79	3.53	3.34	3.19
2.50	4.03	3.75	3.55	3.39

FCR can be obtained with diets of high energy concentration. However, due to the dietary fibre requirements of rabbits and the low digestibility of different fibre classes (Gidenne, 2003), rabbit diets have a low energy content (DE or metabolizable energy) compared to poultry and pig diets.

While respecting fibre requirements, diets of high energy concentration can be obtained by adding fat (and to a lesser content digestible fibre). The DE content of fats (or oils) is nearly three times as high as that of cereals (Maertens *et al.*, 2002). However, because of the necessity for rabbit diets to be pelleted, the addition is limited to 20–30 g kg^{-1} because of its negative impact on the pellet quality (Maertens, 1998). If it is taken into account that a replacement of 20 g cereal with 20 g fat (oil) kg^{-1} results in an increase in the dietary DE content of 0.44 MJ kg^{-1}, a decrease in the FCR by about 0.15, or 5–7%, can be expected. This effect has again been demonstrated by Corrent *et al.* (2007); rabbits did not reduce their feed intake, and consequently the higher daily energy intake resulted in a more favourable FCR (Table 14.6). Because amino acids were adjusted to the dietary DE content, daily weight gain also tended to be higher with the higher energy concentration diets.

The use of diets of higher energy concentration to improve the FCR is especially interesting during the finishing stage. Shortly after weaning, feed consumption is low and optimizing digestive health is of primary importance. However, in the second fattening stage rabbits are less sensitive to digestive disorders and about 0.66 of the feed is consumed during this period. A phase feeding programme, including higher energy concentration diets in the finishing stage, improves FCR. Based on several studies, an improvement in FCR of 0.15–0.20 for 0.5 MJ DE kg^{-1} can be expected (Maertens, 2009). However, more trials are necessary to verify if this relationship is linear (especially with fat addition) and between which margins of dietary energy content.

14.7.4 Mortality

It is evident that mortality has a very large impact on FCR (Table 14.7). For this calculation, the weight gain and feed intake data for a 5-week fattening period (between 30 and 65 days of age), as presented in Table 14.3, were used. The effect of both increasing mortality (from 0% to 20%) and the timing of mortality (week 1, week 2–3 or during the last week) are presented.

If mortality occurs in the early fattening stage, the economic FCR deteriorates only slightly. However, if the losses (mortality and culled rabbits) are concentrated at the end of the fattening period, the FCR is 11.2% and 26.1% worse for mortality rates of 10% and 20%, respectively (Table 14.7) (Maertens, 2009).

Losses in the fattening unit also have consequences on the FCR in the reproduction unit. Before weaning these rabbits have consumed feed and, moreover, the feed consumption of the mother has to be divided between fewer weaned rabbits. This will be discussed further in the management paragraph.

Table 14.6. Effect of dietary digestible energy (DE) content on growth and feed conversion ratio during the finishing period (Corrent *et al.*, 2007).

	Energy content of diet (MJ DE kg^{-1})[a]			
	10.25 (24.5)	10.67 (34.4)	11.08 (39.5)	*P* value
Weight gain (g day^{-1}) between 48 and 70 days	47.2	48.2	50.3	0.06
Feed intake (g day^{-1}) between 48 and 70 days	168.8	163.5	168.4	>0.10
Feed conversion ratio	3.60	3.40[b]	3.36[c]	<0.01

[a] Values in parentheses are ether extract (g kg^{-1}).
[b,c] Data with different letter are statistically different (P <0.05).

Table 14.7. Economic feed conversion ratio in the fattening unit as affected by mortality and age of losses.

Age when mortality occurs	Mortality (%) 0	5	10	15	20
Week 1	2.72	2.74	2.76	2.78	2.81
Week 2–3	2.72	2.78	2.85	2.92	3.00
Week 4–5	2.72	2.86	3.02	3.20	3.43

14.7.5 Management

In practice, a 42-day reproduction cycle is generally used. However, fertility rate, litter size and pre-weaning mortality have a very large impact on the number of rabbits weaned per doe and, as a consequence, on the FCR of the reproduction unit. Data concerning this FCR are very scarce in the literature. Therefore, a calculation is presented for a rabbit unit with weaning at 35 days, based on recent feed intake data obtained at our institute (Table 14.8) (Maertens, 2009). During the entire lactation period, productive does and their young consume on average 18.5 kg of feed. Furthermore, their feed consumption outside of the lactation period has to be considered (110 days year^{-1}), in addition to the feed consumption of the young females and females in pre-gestation cages (together 45 days year^{-1}). For the calculation of FCR in a productive maternity, we have assumed an average of 7.3 litters per doe year^{-1} and 8.5 weaned kits per litter.

The FCR obtained in such a productive maternity unit is only 2.79, but this does not take into account weanlings losses in the fattening unit. The feed consumption before weaning of these rabbits is lost and the FCR worsens in the maternity unit. In Table 14.9, the effect of post-weaning losses is presented for different production levels.

When 10% losses are considered, the FCR worsens to 3.45 at a production level of 57 young. On the other hand, an increase of five young per doe year^{-1} leads to an improved FCR of 3.09, a decrease of 11%. The simultaneous impact of an increase of five weaned young and a decrease of 5% of post-weaning mortality results in an improved FCR (e.g. from 3.45 to 2.93) (Table 14.9).

14.7.6 Other factors involved in the FCR

Restricted feeding in the fattening unit has proved to be helpful in overcoming digestive disorders, especially shortly after weaning, but it has also a favourable effect on FCR. According to Gidenne *et al.* (2003b), the following relationship is found during the 5-week fattening period:

$$\text{FCR} = 2.88 - 0.021 \times \text{feed restriction (proportion)}$$

This means that an improvement of 0.21 in FCR can be obtained when rabbits are 0.10 restricted-fed (i.e. 0.9 of *ad libitum*). However, this gain has to be considered

Table 14.8. Calculation of the feed conversion ratio in a productive reproduction unit (for 100 does).

Feed consumed: Stage	Per 100 does (kg)	Product: Rabbits produced	Weight (kg)
Lactation: 18.5 kg per litter × 7.3 litters per doe year^{-1}	13.505	8.5 weaned per litter × 7.3 litters or 62 weaned per doe year^{-1} with a weight of 1.0 kg	6.200
Only pregnant: 110 days × 160 g day^{-1}	1.760	Sold females: 50 with an economic weight of 3 kg	150
Young females and females in rearing cages: 45 × 365 days × 150 g day^{-1}	2.464		
Total	17.729	Total	6.350
FCR		2.79	

Table 14.9. Feed conversion ratio in the maternity unit, as affected by post-weaning mortality and production level.

Losses in the fattening unit (%)	No. of weaned young per doe year^{-1}		
	62	57	52
0	2.79	3.03	3.31
5	2.93	3.27	3.59
10	3.09	3.45	3.79
15	3.27	3.66	4.01

under the restriction plan that was applied in their trials.

Females that do not immediately become pregnant have to be restricted-fed because over-fattening impairs their subsequent reproductive performance and leads to a reduced output in the subsequent lactation (Pascual *et al.*, 2003). Based on the data in Table 14.8, an over-consumption of 10 g day^{-1} leads to a deterioration of 2–3% of the FCR in the maternity unit.

Fattening rabbits are mainly caged in a group size of six to eight. However, several comparative trials have shown that individually caged rabbits have a higher daily weight gain and better FCR. In a Spanish study, the difference in favour of individual caging was 11.8% (Garcia-Palomares *et al.*, 2006). Housing in large groups (pens) or on an alternative floor (e.g. straw) always leads to a deterioration in the FCR (Dal Bosco *et al.*, 2002).

In addition, environmental conditions affect the FCR because of their effect on the requirements for thermoregulation. During the summer, a better FCR is obtained than during the winter despite the lower growth rate. On the other hand, higher growth rates but worse FCRs are observed at low temperatures (winter) compared to fattening under heat stress (Ramon *et al.*, 1996).

Finally, feed wastage due to the feeder design or meal losses can have a significant impact on the FCR. Pregnant females can waste large amounts of feed by scratching it out of un-modified feeders. Another important wastage results from rabbits not eating fines. Any mash present in the pellets or formed in the feeding system worsens FCR. Farm data indicate that this loss can approach 1.5–2% of the total amount of feed.

References

Boisot, P., Licois, D. and Gidenne, T. (2003) Feed restriction reduces the sanitary impact of an experimental reproduction of epizootic rabbit enteropathy syndrome (ERE), in the growing rabbit. In: Bolet, G. (ed.) *Proceedings 10ème Journées Recherche Cunicole, Le Mans*. ITAVI, Paris, France, pp. 267–270.

Boisot, P., Duperray, J., Dugenetais, X. and Guyonvarch, A. (2004) Interest of hydric restriction times of 2 and 3 hours per day to induce feed restriction in growing rabbits. In: Becerril, C.M. and Pro, A. (eds) *Proceedings of the 8th World Rabbit Congress, Puebla*. Colegio de Postgraduados, Montecillo, Spain, pp. 759–764.

Candau, M., Auvergne, A., Comes, F. and Bouillier-Oudot, M. (1986) Influence de la forme de présentation et de la finesse de mouture del'aliment sur les performances zootechniques et de la fonction caecale chez le lapin en croissance. *Annales de Zootechnie* 35, 373–386.

Cervera, C., Juncos, A., Martínez, E., Ródenas, L., Blas, E. and Pascual, J.J. (2008) Effect of different feeding systems for young rabbit does on their development and performance until first weaning: preliminary results. In: Xiccato, G., Trocino, A. and Lukefahr, S.D. (eds) *Proceedings of the 9th World Rabbit Congress, Verona*. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, Italy, pp. 579–582.

Corrent, E., Launay, C., Troislouches, G., Viard, F., Davoust, C. and Leroux, C. (2007) Impact d'une substitution d'amidon par des lipides surl'indice de consommation du lapin en fin d'engraissement. In: *Proceedings 12èmes Journées Recherche Cunicole, Le Mans*. ITAVI, Paris, France, pp. 97–100.

Dal Bosco, A., Castellini, C. and Mungai, C. (2002) Rearing rabbits on a wire net floor or straw litter: behaviour, growth and meat qualitative traits. *Livestock Production Science* 75, 149–156.

de Blas, J.C., Taboada, E., Nicodemus, N., Campos, R., Piquer, J. and Méndez, J. (1998) Performance response of lactating and growing rabbits to dietary threonine content. *Animal Feed Science and Technology* 70, 151–160.

Fernández-Carmona, J., Cervera, C. and Blas, E. (1983) Utilización de piensos extrusionados en el destete de gazapos. In: ASESCU (ed.) *Proceedings IX Symposium de Cunicultura, Granollers*. ASESCU, pp. 55–57.

Fortun-Lamothe, L., Gidenne, T., Lapanouse, A. and De Dapper, J. (2000) Technical note: an original system to separately control litter and female feed intake without modification of the mother–young relations. *World Rabbit Science* 8, 177–180.

Garcia-Palomares, J., Carabaño, R., Garcia-Rebollar, P., de Blas, J.C., Corujo, A. and Garcia-Ruiz, A.I. (2006) Effects of dietary protein reduction and enzyme supplementation on growth performance in the fattening period. *World Rabbit Science* 14, 231–236.

Gidenne, T. (2003) Fibres in rabbit feeding for digestive troubles prevention: respective role of low-digested and digestible fibre. *Livestock Production Science* 81, 105–117.

Gidenne, T. and Lebas, F. (2005) Le comportement alimentaire du lapin. In: *Proceedings 11èmes Journées Recherche Cunicole, Paris*. ITAVI, Paris, France, pp. 183–196.

Gidenne, T., Fortun-Lamothe, L. and Lapanouse, A. (2003a) Comportement alimentaire du lapereau sevré précocement: effet du diamètre du granulé. In: Bolet, G. (ed.) *Proceedings 10ème Journées Recherche Cunicole, Le Mans*. ITAVI, Paris, France, pp. 17–19.

Gidenne, T., Feugier, A., Jehl, N., Arveux, P., Boisot, P., Briens, C., Corrent, E., Fortune, H., Montessuy, S. and Verdelhan, S. (2003b) A post-weaning quantitative feed restriction reduces the incidence of diarrhoea, without major impairment of growth performances: results of multi-site study. In: Bolet, G. (ed.) *Proceedings 10ème Journées Recherche Cunicole, Le Mans*. ITAVI, Paris, France, pp. 29–32.

Gidenne, T., De Dapper, J., Lapanouse, A. and Aymard, P. (2007) Adaption du lapereau à un aliment fibreux distribué avant sevrage: comportement d'ingestion, croissance et santé digestive. In: *Proceedings 12èmes Journées Recherche Cunicole, Le Mans*. ITAVI, Paris, France, pp. 109–112.

Harris, D.J., Cheeke, P.R. and Patton, N.M. (1983) Feed preference and growth performance of rabbits fed pelleted versus unpelleted diets. *Journal of Applied Rabbit Research* 6, 15–17.

King, J.O.L. (1974) The effects of pelleting rations with and without an antibiotic on the growth rate of rabbits. *Veterinary Record* 94, 586–588.

Laffolay, B. (1985) Croissance journalière du lapin. *Cuniculture* 12, 331–336.

Lebas, F. (1973) Possibilités d'alimentation du lapin en croissance avec des régimes présentés sous forme de farine. *Annales de Zootechnie* 23, 249–251.

Lebas, F. (1975a) *Le Lapin de Chair, ces Besoins Nutritionnels et son Alimentation Pratique*. ITAVI, Paris, France.

Lebas, F. (1975b) Influence de la teneur en énergie del'aliment sur les performances de croissance chez le lapin. *Annales de Zootechnie* 24, 281–288.

Lebas, F. (2007) Productivité des élevages cunicoles professionnels en 2006. Résultats de RENELAP et RENACEB. *Cuniculture Magazine* 34, 31–39.

Luzi, F., Maertens, L., Mijten, P. and Pizzi, F. (1996) Effect of feeding level and dietary protein content on libido and semen characteristics of bucks. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 2. Association Française de Cuniculture, Lempdes, France, pp. 87–92.

Machin, D.H., Butcher, C., Owen, E., Gryant, M. and Owen, J.E. (1980) The effects of dietary metabolizable energy concentration and physical form of the diet on the performances of growing rabbits. In: Camps, J. (ed.) *Proceedings of the 2nd World Rabbit Congress*, Vol. 2. Asociación Española de Cunicultura, Barcelona, Spain, pp. 65–75.

Maertens, L. (1994) Influence du diamètre du granulé sur les performances des lapereaux avant et après sevrage. In: *Proceedings 6èmes Journées de la Recherche Cunicole, La Rochelle*, Vol. 2. ITAVI, Paris, France, pp. 325–332.

Maertens, L. (1998) Fats in rabbit nutrition: a review. *World Rabbit Science* 6, 341–348.

Maertens, L. (2009) Possibilities to reduce the feed conversion in rabbit production. In: *Proceedings Giornate di Coniglicoltura*. ASIC, Forli, Italy, pp. 1–10.

Maertens, L. and De Groote, G. (1991) The nutrition of highly productive rabbit does and kits before weaning. *Revue del'Agriculture* 44, 725–737.

Maertens, L. and Luzi, F. (1995) The effect of extrusion in diets with different starch levels on the performance and digestibility of young rabbits. In: 9. *Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere. Celle*. Deutsche Vet. Medizinische Gesellschaft e.V., Giessen, pp. 131–138.

Maertens, L., Perez, J.M., Villamide, M., Cervera, C., Gidenne, T. and Xiccato, G. (2002) Nutritive value of raw materials for rabbits: EGRAN tables 2002. *World Rabbit Science* 10, 157–166.

Maertens, L., Cavani, C. and Petracci, M. (2005a) Nitrogen and phosphorus excretion on commercial rabbit farms: calculations based on the input-output balance. *World Rabbit Science* 13, 3–16.

Maertens, L., Cornez, B., Vereecken, M. and Van Oye, S. (2005b) Efficacy study of soluble bacitracin (Bacivet S®) in a chronically infected epizootic rabbit enteropathy environment. *World Rabbit Science* 13,165–178.

Maertens, L., Lebas, F. and Szendrö, Zs. (2006) Rabbit milk: a review of quantity, quality and non-dietary affecting factors. *World Rabbit Science* 14, 204–230.

Morisse, J.P., Maurice, R. and Boilletot, E. (1985) La dureté du granulé chez le lapin. *Cuniculture* 12, 267–269.

Partridge, G., Daniels, Y. and Fordyce, R. (1986) The effects of energy intake during pregnancy in doe rabbits on pup birth weight, milk output and maternal body composition change in the ensuing lactation. *Journal of Agricultural Science Cambridge* 107, 679–708.

Pascual, J.J., Cervera, C., Blas, E. and Fernández-Carmona, J. (2003) High-energy diets for reproductive rabbit does: effect of energy source. *Nutrition Abstract Reviews* 73, 27R–39R.

Payne, J., Rattink, W., Smith, T. and Winowiski, T. (1994) *The Pelleting Handbook*. Borregaard Lignotech, Sarpsborg, Norway.

Phost, H.B. (1963) Testing the durability of pelleted feed. *Feedstuffs* 35, 66–68.

Ramon, J., Gomez, E.A., Perucho, O., Rafel, O. and Baselga, M. (1996) Feed efficiency and postweaning growth of several Spanish selected lines. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*. Association Française de Cuniculture, Lempdes, France, pp. 351–353.

Rommers, J.M., Meijerhof, R., Van Someren, G. and Kranenburg, M. (1996) Effecten van het bijvoeren van granen aan vleeskonijnen. *NOK-kontaktblad* 14, 121–128.

Rommers, J., Maertens, L. and Kemp, B. (2006) New perspectives in rearing systems for rabbit does. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Sciences*. COST and ILVO, Melle, Belgium, pp. 39–52.

Rosell, J. and González, F.J. (2007) Resultados de Gestión Técnica 2006. *Cunicultura*, October 2007, 189, 285–287.

Sánchez, W.K., Cheeke, P.R. and Patton, N.M. (1984) The use of chopped alafalfa rations with varying levels of molasses for weanling rabbits. *Journal of Applied Rabbit Research* 7, 13–16.

Schlolaut, W. (1995) *Das große Buch vom Kaninchen*. DLG-Verlag, Frankfurt am Main, Germany, pp. 219–226.

Tudela, F. and Lebas, F. (2006) Modalités du rationnement des lapins en engraissement. *Cuniculture Magazine* 33, 21–27.

Xiccato, G., Trocino, A. and Nicodemus, N. (2006) Nutrition of the young and growing rabbit: a comparative approach with the doe. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Sciences*. COST and ILVO, Melle, Belgium, pp. 239–246.

Xiccato, G., Trocino, A., Fragkiadakis, M. and Majolini, D. (2007) Enquête sur les élevages des lapins en Vénétie: résultats de gestion technique et estimation des rejets azotés. In: *Proceedings 12èmes Journées Recherche Cunicole, Le Mans*. ITAVI, Paris, France, pp. 167–169.

15 Nutrition and the Climatic Environment

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15.1 General Aspects of Environment

Biometeorology is the study of the relationship between the environment and living organisms. In homeothermic animals, the goal is to maintain a stable body core temperature under most conditions. The thermoregulatory system employs all of the systems of the body and integrates their activities into appropriate and coordinated reactions. This chapter considers one specific area of this by focusing on nutrition.

The variables that define an environment are temperature, humidity, light, altitude, smell and noise. Little is known about the last three, apart from some data relating levels of carbon dioxide or ammonia to some productive parameters of animals, which are essential in the control of the frequency of air exchange in intensive farms. The reciprocal problem of the contaminating effect of the animals themselves on the atmosphere and soil also appears to be very interesting, and numerous studies have been undertaken on species other than rabbits that show a relationship between nutrition and the production of ammonia, methane, phosphorus and sodium.

Lighting schedules are programmed to control reproduction and, together with feed restriction, can marginally improve feed efficiency in growing rabbits. This is a controversial idea because the effect depends on the age of the rabbit, length of feeding and time of feeding. Feeding usually occurs in the late afternoon and at night, so feed must be available at these times (Maertens, 1992).

Ambient temperature and humidity are the variables that most affect nutrition. Both directly influence the energy equilibrium of the animal, changing the flow of heat between the animal and the environment. In particular situations for species other than rabbits, correlations have been found between different functions of temperature and humidity that permit the most reliable to be selected: for example, the temperature humidity index (THI), wet bulb temperature, radiant temperature, equivalent temperature and effective temperature. The higher THI value in humid tropical climates compared to that found in temperate and subtropical areas elicits a more stressful environment for rabbit production (Ogunjimi *et al.*, 2008). Other indices define the environment in terms of the animal's response, including operative temperature, iso-ambient lines, thermoneutral zone (TNZ) and comfort zone.

Air temperature alone may be acceptable in laboratory conditions, but it becomes an inadequate measure of the degree of thermal stress on animals exposed to the natural environment (Young, 1977). Unfortunately, insufficient information is available to express all the climatic variables in one unit, such as the effective ambient temperature or others, and mean air temperature is employed instead as the usual index.

The lack of research work on rabbits limits the discussion of climatic variables solely to the effect of temperature, daily minimum temperature (Fuquay, 1981) or daily average temperature. This can lead to substantial errors where other parameters have a significant effect, for example wind, solar radiation or ambient humidity. Taking these factors into account, the dry temperature is the most important and representative index, and humidity (wet bulb temperature) can also be used when its incidence has been measured. Future research will perhaps define a THI similar to that used for other non-sweating species.

The environment is sometimes defined by the season of the year and even by the particular system of production, such as building interior versus open air. This type of loose definition adds even more imprecision to the conditions and makes it impossible to replicate observations or experimental work, although it may have practical value for specific zones or purposes.

Another difficulty in measuring temperature, in terms of minimum, maximum or mean, results from diurnal and seasonal fluctuations. Many experiments have been designed with animals in climate chambers at a constant temperature, making comparisons with animals exposed to a changing environment of limited relevance. In a naturally fluctuating environment, animals appear to partially overcome adverse effects by taking advantage of the more favourable parts of the cycle. It follows that a constant temperature is less well tolerated than a cyclic one, and the NRC (1981) suggested that the effect should be similar to that of a constant temperature equivalent to the mean of the cycle.

As a consequence of the small and fragmentary amount of work undertaken on rabbits to date, a review of experimental data raises more questions than it solves. This chapter tries to include the most valuable published work describing a general approach to the complex nutrition–environment relationship.

Interest in the subject is not recent, but has increased lately because it is a crucial element in the nutrition of domestic animals in cold climates and even more in subtropical and tropical environments.

Tables of nutrient requirements have been developed for rabbits from work carried out in intensive production systems in temperate countries under moderate conditions relatively free of thermal stress, where animals can realize their full genetic potential. Application of these data can lead to considerable errors in feeding animals exposed to acute cold or heat stress, because the physiological responses involve changes in voluntary water intake, feed intake, maintenance energy requirements and level of production.

It seems that some corrections need to be introduced, depending upon the environment to which animals are acclimatized or temporarily exposed.

15.2 Thermoneutral Zone

Homeothermic animals maintain a constant core temperature, compensating for heat loss through morphological, physiological and behavioural mechanisms. The definition of thermal neutrality accepted by the International Union of Physiological Sciences is: ‘The range of ambient temperature as an expression of thermal environment within which metabolic rates are at minimum and temperature regulation is achieved by non-evaporative physical processes alone’ (Bligh and Johnson, 1973). Including a phrase such as ‘in normal productive animals’ should make the concept of TNZ more acceptable to farmers. It implies that a non-stressful situation means not only normal body temperature and no shivering, sweating or panting, but also that feed intake does not change. Net energy for productive purposes is the difference between metabolizable energy (ME) intake and heat production, which in turn involves maintenance needs and the heat increment (HI) of food.

Therefore, level of nutrition and production of heat increase together, lowering critical temperatures with maximum energy retention for a given energy intake expected to be in the range between them (Fig. 15.1). HI contributes to the maintenance of body temperature in cold conditions, but compromises the heat balance in hot environments.

In the TNZ range the animal invokes mainly postural and vasomotor mechanisms to conserve or dissipate body heat. Rabbits take on a ball posture at <10°C to decrease their surface area for conduction or radiation losses. The spread posture at 30°C allows more sensible heat to be dissipated.

The energy-demanding behaviours of rabbits include maternal feeding and digging burrows. Many animals dig burrows underground to protect themselves in both tropical countries and the Arctic. Different types of burrows are connected with survival of rabbits in arid areas of New South Wales, as described by Hall and Myers (1978). Some systems in New Mexico (Gentry, 1983) and Tunisia have been designed to exchange conventional cages or burrows for concrete burrows. In these deep pits, temperatures remain up to 9°C lower than outside (Finzi *et al.*, 1989).

Vasoconstriction of peripheral blood vessels helps the rabbit to keep warm in cold conditions, so that metabolism need not increase to offset heat loss. In particular, the amount of heat conserved in the ears (with an area of 0.026 m^2 in adults) is considerable. McEwen and Heath (1973) reported that heat loss was 0.2 W $°C^{-1}$ linear over the range of 0–30°C ambient temperature, about half of the theoretical loss if the ears were maintained at core body temperature. Responses to cold stress rely on more carbohydrates being utilized and fat tissue being easily mobilized. Long-term adaptation to cold involves increased insulation.

In a hot environment rabbits have to dissipate metabolic heat when the thermal gradient between them and the ambient temperature is small or even negative. First, they use the least expensive heat loss procedure of skin vasodilation (Kruk and Davydov, 1977). When this becomes insufficient, a decrease in food intake, which decreases HI, and evaporation of water from the respiratory tract are the mechanisms used to lose excess heat.

The lower and upper critical temperatures should be assessed from metabolic

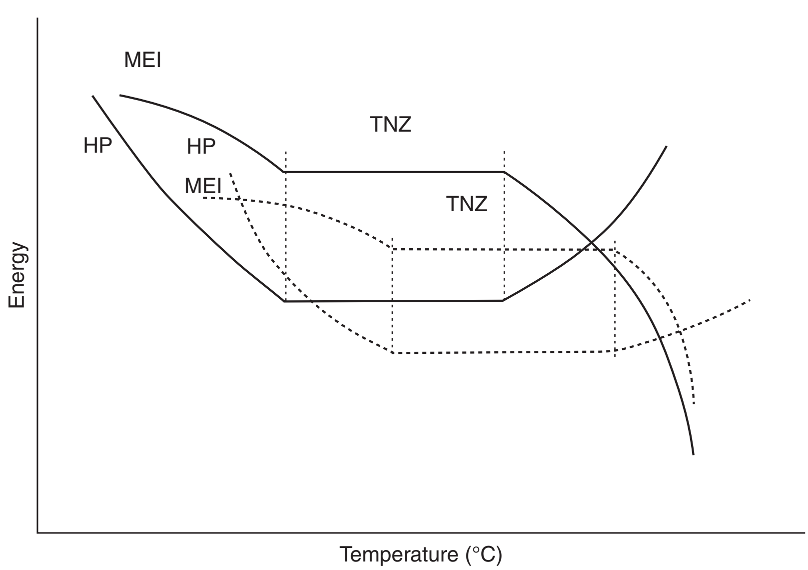

Fig. 15.1. Schematic representation of heat production and energy intake. — Non-acclimatized animals; … heat-acclimatized animals. HP, heat production; MEI, metabolizable energy intake; TNZ, thermal neutral zone.

Table 15.1. Heat production (kJ kg^{-1} body weight$^{0.75}$).

Source	Temperature °C								
	0	5	10	15	20	25	30	35	40
Johnson *et al.* (1958)[a]	–	–	660	–	630	580	575	570	675
Johnson *et al.* (1958)[b]	–	–	–	–	660	–	620	570	640
Gonzalez *et al.* (1971)[c]	–	613	520	428	398	364	352	421	–
McEwen and Heath (1973)[d]	352	–	312	–	300	–	273	–	–
Nichelmann *et al.* (1974a)[e]	–	–	570	520	490	425	400	400	485
Scheele *et al.* (1985)[f]	–	–	–	396	–	–	331	–	–
Sanz *et al.* (1989)[g]	–	–	–	–	550	520	440	400	–
Jin *et al.* (1990)[h]	–	–	–	–	488	–	378	–	–

[a]Fed females, 4 kg, reared at 9°C. (Values are approximate. Calculated from the original figure.)
[b]Fed females, 4 kg, reared at 28°C. (Values are approximate. Calculated from the original figure.)
[c]Fasting animals (assuming 3 kg live weight).
[d]Fasting animals, 3.9 kg.
[e]Fasting 42-day-old animals, approximately 1 kg live weight (points interpolated from the original response).
[f]Fed growing rabbits.
[g]Ten-day-old sucking rabbits (interpolating original values).
[h]Fed growing rabbits, approximately 1.6 kg (mean of two diets).

heat production figures. The great variability between published data may be due more to the experimental procedure than to any question related to breed (Table 15.1). The type of chamber, method of measurement, restlessness and adaptation of animals to the temperature and individual differences change the limits of the TNZ.

The TNZ given by several studies between the 1940s and 1960s varied from 20°C to 30°C. Later research has found heat production to decrease from 5°C to 35°C (Gonzalez *et al.*, 1971), 10°C to 30°C (Kluger *et al.*, 1973), 0°C to 30°C (McEwen and Heath, 1973), 18°C to 28°C (Scheele *et al.*, 1985) and 20°C to 30°C (Jin *et al.*, 1990).

The upper critical temperature has been estimated by all of the cited studies to be <30°C, when physiological reactions such as feed intake, respiration rate, panting and body temperature were examined, but lack of information for intermediate values from 20°C to 30°C makes it difficult to reduce the uncertainty. Most studies have found appreciable differences in heat production between 10°C and 20°C and, therefore, the lower critical temperature should be between these values. From a survey of the literature, McEwen and Heath (1973) calculated a value of approximately 15°C for the lower critical temperature.

Heat production has also been measured by Johnson *et al.* (1958) in fed mature New Zealand White rabbits exposed to temperature levels from 10°C to 40°C. Heat production decreased steadily up to 35°C and appeared to increase after this. From the data of heat production, feed intake and thyroid activity, the authors suggested an upper critical temperature of around 24°C.

These results have been confirmed in a series of experiments conducted by Nichelmann *et al.* (1973a, 1974a). They showed that heat production had the shape of a parabola, with the point of inflection at 25°C for adults, 30°C for 42-day-old rabbits and 35°C for 10-day-old pups. The TNZ lay between 15°C and 25°C for animals aged 80 days. This range coincides approximately with some estimates carried out on commercial farms. The shape of the response was not so clear in the work of Gonzalez *et al.* (1971), who reported a relatively constant heat production between 15°C and 30°C.

Thereafter, cooling of the animals is based upon evaporation, the rabbit being particularly ineffective at this process compared with other species. Relatively low ratios of surface to skin vaporization and respiratory tract evaporation to heat production indicate that the rabbit has little ability to dissipate heat. Panting alone, even at 400 breaths min^{-1} at 35°C, did not prevent an elevation of rectal temperature of 1.4°C (Gonzalez *et al.*, 1971). In stressed non-acclimatized animals at 38°C, Johnson *et al.* (1958) reported that evaporation accounted for 0.30 of the total heat produced by adult animals housed at 35°C (0.40 was recorded at 40°C by Nichelmann *et al.* (1973b)), of which 0.60 was lost through panting; no increase was observed from 20°C to 30°C in young animals acclimatized for 7 days (Nichelmann *et al.*, 1974b). Total evaporative heat loss has been estimated to be 0.6, 1.26 and 2.0 W kg^{-1} at each ambient temperature of 15°C, 30°C and 35°C. Using the latent heat of evaporation as 0.7 W g^{-1} h^{-1}, a 4 kg doe should evaporate around 7 g water h^{-1} at 30°C.

The ears are a means of dissipating heat. The heat exchange coefficient is 9.1 W m^{-2} $°C^{-1}$, about four times the coefficient for the whole animal (Kluger *et al.*, 1973). In a wind of 60 m s^{-1}, fully dilated ears can lose twice as much heat as ears in non-forced convection. However, most rabbits die after a few days' exposure to 40°C.

Stressed animals have lower productivity in the short term. The problem can be solved in temperate or even cold climates, where highly productive breeds can be satisfactorily reared in heated or insulated buildings. However, the production of meat and milk, and reproduction, suffer in this increasing order as a consequence of hot climates.

A lower basal or resting heat production reported in animals continuously exposed to environments of around 30°C would be of value in adaptation to hot environments. Alliston and Rich (1973) suggested that, following an 18-day acclimation period, there was a positive relationship between thermoregulatory efficiency and pregnancy. The results of Johnson *et al.* (1958) suggested that the influence of rising environmental temperature was less in New Zealand White rabbits reared at 28°C than in those reared at 9°C, as rectal minus air temperature, pulse rate and respiration rate were lower.

The heat production of newborn rabbits housed in metabolism cages and subjected to several ambient temperatures has been determined both for 10-day-old animals suckling normally (Nichelmann *et al.*, 1974a) and 2 h after birth for fasting animals (Cardasis and Sinclair, 1972). These studies demonstrated a cold-induced increment of heat production. Kits were able to raise their basal level of heat production at 35°C, followed by temperature drops to 30°C, 20°C or 10°C; peak metabolism was achieved at around 15°C at 10 days.

Neonatal animals cannot maintain body temperature if unfed or if the ambient temperature drops abruptly (Hill, 1961; Rödel *et al.*, 2008), although they tend to group together and curl up. The metabolic responses to both cold and fasting are the result of complex interactions (Schenk *et al.*, 1975) that bring about a higher mortality rate compared with normal suckling rabbits.

Survival time and rate of weight loss of fasted newborn rabbits are strongly influenced by temperature, which is known to stimulate thermogenic activity of brown fat. The probability that large or small littermates survive could be related to the different lipid stores (Dawkins and Hull, 1964). Cardasis and Sinclair (1972) placed newborn rabbits at 35°C, 30°C and 25°C, and reported that smaller animals survived for fewer hours. Young animals with a birth weight of >50 g were able to maintain metabolic rate and body temperature for longer.

Heat production of newborn rabbits increases during the first days of life. A 10-day-old rabbit coordinates movement and already has improved insulation, gaining hair and tissue fat. The use of nest boxes with bedding, where young rabbits huddle, thereby reducing body-surface exposure, means that the optimal temperature range becomes wider with increasing age: 20–30°C at 20 days of life (Rafai and Papp, 1984). Practical observations have concluded that a 20°C temperature indoors leads to high viability in farms (Delaveau, 1982), but maternal behaviour significantly affects the proper kindling and subsequent survival of litters under extreme climatic conditions in open sheds (Platukin and Konokhov, 1972).

15.3 Heat Stress

Exposure to high ambient temperatures induces rabbits to try to balance the excessive heat load by using different heat dissipation pathways. If such means are not sufficient then physiological traits deteriorate, including depression in feed intake, efficiency and utilization, disturbances in water, protein, energy and mineral metabolism balances, enzymatic reactions, hormonal secretions and blood metabolites.

In the late 1930s it was recognized that a high ambient temperature increases body temperature (Lee, 1939). The stress at ≥30°C seems to be very high; rectal temperature starts to increase at 27°C, rises abruptly at 32°C, together with a sudden decrease in feed consumption (Johnson *et al.*, 1958), and reaches 42.5°C at 35°C (Nichelmann *et al.*, 1973a). Above 35°C, rabbits can no longer regulate their internal temperature and heat prostration sets in.

A method to measure the severity of heat stress using the THI value has been proposed for rabbits under a subtropical climate and the values obtained are then classified as follows: <27.8 = absence of heat stress, 27.8–28.9 = moderate heat stress, 28.9–30.0 = severe heat stress and ≥30.0 = very severe heat stress. When comparing these values to those obtained for sheep and cattle (≥25.6 = very severe heat stress), it is evident that rabbits tolerate higher climatic stress than large mammals (Marai *et al*, 2002).

Thermal stress directly affects reproduction, health and nutrition, and all of these interact with each other. The overall result for animals exposed to thermal stress is always a reduction in productivity, which varies according to the severity of the stress and the acclimatization of the animal. Multiple factors cause the response of rabbits to a hot climate to be reduction in food intake, litters per year and offspring per litter. Normal production figures, as estimated by several experts, could be four or five litters, 20–25 animals weaned yearly and <20 g live weight gain day^{-1} for growing animals (Colin, 1991, 1995).

Depressed feed intake and increased water consumption are the most important reactions to heat exposure. At 30°C rabbits consume only 60–70% of the feed intake recorded at 20°C and at 35°C the feed intake is decreased by 28–17%. In contrast, water requirements increase by 50% as the temperature rises from 18°C to 38°C. Blood metabolites such as glucose, serum total protein, serum total lipids and cholesterol decrease in rabbits exposed to heat stress conditions, which may be correlated to the decrease in energy metabolism during heat exposure.

Moreover, the increased energy requirement during panting, with the additional rise in body temperature, seems to be non-linear, as opposed to the apparent linear increase in cold conditions. In severe heat stress, feed intake is so depressed that the maintenance requirements cannot be properly calculated.

It is important to predict the future resistance to thermal stress of animals that are likely to suffer it, and the selection of breeders must be based on an index linked to the production of heat, because this decreases in previously acclimatized animals. Probably the most useful indices are based on rectal temperature, such as the Iberia test (Rhoad, 1944), which is used in beef cattle and is also of proven validity in rabbits (Alliston and Rich, 1973; Finzi *et al.*, 1988). Other indices may measure biochemical factors (Amici *et al.*, 1995) or certain nutritional parameters, such as nitrogen retention, body fluids or fat (Kamal and Johnson, 1971).

Very little work to date has been designed to anticipate and understand the degree of re-adaptation or recovery of animals that have suffered thermal stress. Such re-adaptive behaviour can negate or change the conclusions obtained from short-term observations. Age, physiological condition and duration and intensity of stress should affect recovery to the original level of production, which in female breeding rabbits is possible after 10 months at 30°C (Fernández Carmona *et al.*, 1994c).

15.4 Nutritional Value of Feedstuffs and Environment

The nutritional value of rabbit feedstuffs is expressed in terms of digestible energy (DE) or ME. These biological values depend not only on the composition or quality of the feed, but also on the digestive or metabolic processes in the animal itself.

In moderate climates the quality of forages can vary greatly because of variety, maturity and harvesting. With regards to lucerne, the most frequent fibrous ingredient in rabbit diets, García *et al.* (1995), studying a Spanish variety, reported a wide range of values for dry matter (DM), energy and amino acid digestibility.

The composition and nutritive value of tropical forages are extremely diverse. Digestible DM was found by Raharjo *et al.* (1988) to range from 123 to 463 g kg^{-1} for grasses and from 281 to 494 g kg^{-1} for non-woody legumes. Similarly, Carew *et al.* (1989) evaluated 30 plant species collected in Nigeria: whole plants contained 66–284 g crude protein kg^{-1} and 45–407 g crude fibre kg^{-1}; results from the leaves of three tree species were 33–109 g crude protein kg^{-1} and 45–216 g crude fibre kg^{-1}.

In tropical countries, the nutritive value of forages is relatively low, with high indigestible fibre. This problem has been linked to the four-carbon atom compounds, rapid lignification associated with environmental temperature and a low leaf to stem ratio. Some factors such as oxalates, cyanides, tannins and alkaloids limit the efficiency of nutrient utilization. The low nitrogen digestibility detected in some plants, such as *Leucaena leucocephala* (Harris *et al.*, 1981) and *Robinia pseudoacacia* (Raharjo *et al.*, 1990), is apparently due to protein–tannin complexes.

Most systems operating in tropical countries are based on small subsistence-level farms, where forage provides a major part of the requirements of rabbits. Forage alone cannot support high performance in either growth or lactation, and this disadvantage occurs in addition to the low quality mentioned above. In fact, Raharjo *et al.* (1988) reported daily intakes of tropical grasses ranging from 10 to 28 g of DM; ingestion of leaves, shrubs and woody legumes was generally higher, but rabbits lost weight on all diets. This poor performance derives not only from the lower quality of forage, but also from the low feed intake of any unpelleted ingredient or diet (Harris *et al.*, 1983; Schlolaut, 1987).

Supplementation of pelleted diets with potential energy sources, including roots, tubers, fruits and grain by-products, has generally demonstrated that 0.50–0.75 of pellets can be replaced by green forages, by-products or roots without a significant reduction in growth performance (Pote *et al.*, 1980; Sanchez *et al.*, 1984; Partridge, 1988; Abdel-Samee *et al.*, 1994). Certainly the variety of feeding methods does not allow any general conclusion and many green feeds, milled grains, roots and protein supplements can be supplied to rabbits, based on seasonal availability and economic factors.

There is little information about whether climate affects the DE or ME values of feedstuffs, in addition to its influence on composition. Sanz *et al.* (1973) reported that increasing the temperature to 34°C adversely affected the coefficient of digestibility of a restricted-fed and balanced diet, but other studies (Kasa *et al.*, 1989) have failed to detect any significant change. Short-term estimates of digestibility could be altered by transient changes in rate of digesta passage and gut volume, related to sudden changes in ambient temperature.

The ME of a feed is affected by urinary losses and methane production, and is independent of its DE value. Although during exposure to extreme conditions there could be a shift in feed and tissue protein metabolism, the urinary losses increase and the ME value would decline (Gray and McCracken, 1974, in pigs). Usually the protein retention process is considered to be relatively independent of temperature.

No information is available for rabbits, where determined ME values are not abundant, and thus at present no recommendation can be made on how to adjust rabbit diets for the influence of temperature on DE or ME values.

15.5 Nutrient Allowances and Environment

The environment affects rate of intake and maintenance requirements. In a mildly cold situation, improved efficiency may be observed if voluntary intake increases more than the corresponding energy needs for heat production. In warm environments animals expend less energy on maintenance, and these savings might improve production or energy efficiency, despite the decreased feed intake. However, it appears unlikely that these theoretical approaches are borne out in practice, because most data show that increased heat requirements during cold conditions are not entirely compensated for by the consequent increased energy intake. Conversely, livestock exposed to high temperatures increase heat production while decreasing voluntary feed intake. Even in a situation where a thermal balance is maintained by reduced activity of the animal, these lower maintenance needs do not balance the lower intake.

Results showing improved efficiency can generally be explained by the composition of the animal product, mobilization of fat tissues to replace the energy deficit or the measurement of effective ambient temperature.

The adverse effect of temperature on efficiency and production should be minimized by adjusting nutrient levels. Assuming that maintenance needs for protein are not influenced by thermal stress, the protein/energy ratio is increased during both cold and heat stress, resulting in excess protein being used as an energy source. The practical approach in cold conditions is to increase dietary energy levels.

The consequences of hot environments on feed intake, which means less protein being ingested and reduced growth, have generally resulted in recommending higher levels of protein in warm climates or seasons. The addition of some amino acids, particularly lysine, has alleviated the effect of heat on pigs and poultry. It may be observed that equal daily intakes at different temperatures have resulted in comparable live weight gains.

In fact, levels of protein in the diet should be corrected according to body gains or milk protein yield, rather than being changed only as a result of the expected intake. The predicted performance of pigs and poultry is used to tabulate the adjustments for environmental stress, but insufficient information is available for rabbits.

Estimations of ME requirements based on heat production figures have been used for pigs and poultry exposed to different temperatures, but it would appear that no similar studies have been undertaken with rabbits.

High-energy diets have been reported to overcome the lower energy intake in hot environments when fed to cattle, pigs and poultry. Diets with a minimal HI should be beneficial; Jin *et al.* (1990) found that evaporative losses in 1.9-kg rabbits at 30°C were higher with a diet containing 231 g neutral detergent fibre (NDF) than for one based on 165 g NDF kg^{-1}. However, feed intake in that experiment was also higher, making it difficult to establish the relationship between fibre and stress.

Attempts to overcome the expected poor intake would require the use of high-fat, low-fibre diets. While the range of fibre is relatively narrow in feeding rabbits, the limit of fat incorporated into a diet seems to be controlled only by the physical structure of the pellet. Very few experiments have related the composition of rabbit diets to production outside the TNZ. However, some results suggest that the response of rabbit females at high temperature is especially poor when they are fed a low-energy diet (Simplicio *et al.*, 1991), and significantly improved when a high-energy diet is supplied (Simplicio *et al.*, 1991; Fernández Carmona *et al.*, 1996).

At this point it may be emphasized that two different ways of enhancing performance

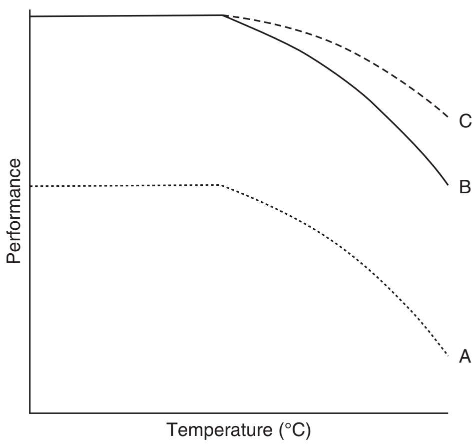

Fig. 15.2. Relationship between performance and diets. Response to diet B is always higher than diet A; response to diet C is only higher than diet B under extreme conditions.

through dietary improvement can be considered (Fig. 15.2). The first may be defined as a general improvement of performance, but it is questionable whether this is really what is wanted. Had the previous diet not been deficient, it might have promoted a similar response, although there is usually scope to improve a diet. In fact, most diets are formulated using a least-cost programme, assuming optimal, non-maximal performance. The second approach can respond to actual attempts to obtain a better diet for severe climatic conditions. It should be noticed that a different response caused solely by a difference in temperature implies a significant diet–temperature interaction.

Increased voluntary intake in cold conditions tends to overcome any marginal deficiency in nutrients, although not in energy. Compared with a given diet in TNZ, a smaller proportion of dietary protein is needed for the above reasons, and more protein is utilized as an additional energy source. In view of their HI, proteins may temporarily exert a favourable effect in a cold environment. For many years a high-fat diet has been shown to be the most effective means of maintaining body temperature, coincident with the needs arising from the two-stage adaptation of rabbits to cold: (i) cold stress with depletion of fat reserves; and (ii) cold adaptation linked to lipid deposition.

The increment of ME requirement corresponds exactly to the extra heat produced. From data on heat production, ME requirements can be estimated as 0.15–0.20 of the requirements for a growing animal at 15–20°C (Table 15.1).

The HI of roughage has been useful for feed-restricted or low-productive cattle. Using indirect calorimetry trials on growing rabbits, Ortiz *et al.* (1989) reported that the DE value overestimated by 5% the net energy value expected when the acid detergent fibre level increased from 180 to 240 g kg^{-1}. Fibre could then be considered for rabbits as a moderately effective means of enhancing heat production, and conversely of limiting the total net energy intake. No reference work is available for cold-stressed rabbits in any of the subjects mentioned here.

Obviously, there is broad scope for research on the interaction between protein and energy allowances and the environment in rabbits fed *ad libitum* in hot or cold conditions. Virtually no attempts have been undertaken to test inputs other than energy and nutrients other than protein. There are some references to the addition of probiotics in hot climates, with little emphasis on the problem of temperature. Adding disodium or dipotassium carbonate has proved to be effective at high temperatures (Bonsembiante *et al.*, 1989; Fayez *et al.*, 1994), probably preventing the action of hyperventilation on the acid–base balance.

The performance of rabbits in tropical countries is likely to be maximized by providing free access to drinking water at all times (Thwaites *et al.*, 1990). Water-restricted rabbits, besides saving water output in faeces and urine, show a significant reduction in DM intake. Kits <21 days of age seem to learn how to drink water at high temperatures (McNitt and Moody, 1992). A water to food intake ratio of about 2 has been recorded for adult rabbits fed *ad libitum* by Prud'hon (1976) at 20°C, similar to that published by Jin *et al.* (1990) in growing females and Kasa *et al.* (1989) in fattening rabbits. There is a rise in the ratio of water intake to DM intake up to 2.4 between 20°C and 30°C. Drinking cool water has sometimes been recommended in hot situations, but it should be noted that, besides the practical difficulty, the cooling effect is very small compared with the loss of heat through water vaporizing from the respiratory tract.

15.6 Effect of Heat Stress on Breeding Does and Litters

The effect of heat stress has been measured in experimental conditions on mating, fertility, embryo survival and litter size at birth (Howarth *et al.*, 1965; Alliston and Rich, 1973; Shafie *et al.*, 1979; Abo-Elezz, 1982; Kamar *et al.*, 1982; Tramell *et al.*, 1988; Marai *et al.*, 2006). Poor productive performance of does has also been found in commercial farms: Masoero and Auxilia (1977) reported a decline in mating and fertility at 25°C. Summertime has brought about poor results in mating, numbers of live offspring, mortality and size of weaning litter (Sittmann *et al.*, 1964; Pagano-Toscano *et al.*, 1990). The worst overall productive results are mostly obtained in hot conditions (summer in southern European countries). In a rabbit mortality survey, Rosell (1996) found respiratory disorders to be the main cause of death in females, and certainly in winter the mortality of lactating rabbits can increase and respiratory pathology may become severe (Battaglini *et al.*, 1986; Costantini and Castellini, 1990; Mori and Bagliacca, 1990). Reproductive traits in hot countries are far removed from European Standards (Cardelli, 1993), although some advances have been reported (Baselga and Marai, 1994).

High ambient temperature appears to act on reproduction both directly and through the depression of voluntary feed intake (Fig. 15.3). Experimental research, where feed intake during heat stress was

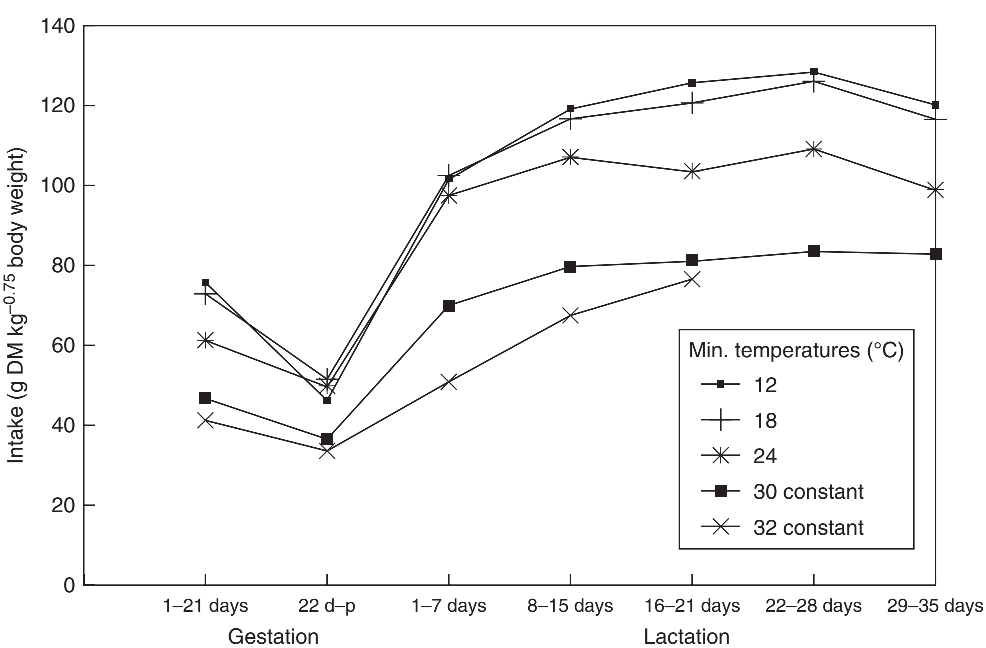

Fig. 15.3. Feed intake of does at different minimum (min.) temperatures (data for 32°C constant from Wittorff *et al.* (1988); other data from Fernández Carmona *et al.* (1994b)). Regression line for original data for 22 days of gestation to partum (22d–p) and lactation: intake g dry matter (DM) kg$^{-0.75}$ body weight = 81.72 + 10.45 × W – 0.048 × T^2 (standard error = 15.8, R^2 = 0.71; W = 1–6 weeks starting from 22 days gestation to partum; T, temperature (°C)).

recorded, has confirmed low performance during summer (Mendez *et al.*, 1986) or during a transitory temperature rise (Maertens and De Groote, 1990) and in a controlled environment (Rafai and Papp, 1984; Papp and Rafai, 1988; Wittorff *et al.*, 1988; Simplicio *et al.*, 1991; Fernández Carmona *et al.*, 1995).

The work of Rafai and Papp (1984) examined daily milk output and food intake for temperatures between 5°C and 35°C. At 25°C a noticeable reduction in feed intake started, and subsequently milk, litter weight and doe weight were affected. Later work (Papp and Rafai, 1988) showed that does kept at 35°C die within 72 h.

Angora rabbits suffer considerable stress when shorn at temperatures below 20°C (Schlolaut, 1987) and very low wool yield and quality have been reported at temperatures of ≥30°C (Stephan, 1980; Kong *et al.*, 1987). Cheng *et al.* (1991) recorded in summer, with a 23.1°C monthly average temperature, 5.6% less intake and 10.6% less wool yield than in spring. Shorter intervals between shearing can be employed in high-temperature conditions. Optimal temperatures for hair growth have been estimated at about 25°C after shearing, and not above 15°C before it (Schlolaut, 1987).

Cold seasons affect the mortality of suckling rabbits (Ferraz *et al.*, 1991), but Lukefahr *et al.* (1983) did not observe this relationship. A survey of the literature (Partridge, 1988) gave a mortality rate in pre-weaning rabbits from 0.15 to 0.30. Around 0.80 of pups died as a result of chilling and starvation during the first days of life. The data demonstrated that survival increased up to 0.957 when nestboxes with a low-voltage heated floor were provided. In a study of a European rabbit population living in a field enclosure, Rödel *et al.* (2008) found that the temperatures inside the subterranean nest were positively correlated with soil temperature (from 3°C to 21°C) and litter size. They concluded that under colder soil temperature conditions, the thermal benefits of a greater number of littermates outweighs the negative consequences of competition for milk, leading to an environment-dependent shift in the optimal litter size for individual growth in this species.

The possibility that modification of diets formulated for normal environments can alleviate thermal stress by avoiding nutrient deficiencies or increasing voluntary intake has hardly been explored. One logical course of action would seem to be to increase the DE of diets with more cereal or by adding fat. Although under normal conditions does compensate for different diet density through corresponding changes in feed intake (Maertens and De Groote, 1988; Fraga *et al.*, 1989), long-term experiments carried out by Mendez *et al.* (1986), Simplicio *et al.* (1991) and Cervera *et al.* (1993) showed that some added fat elicits a better response from does, perhaps related to high milk-fat output (Christ *et al.*, 1996; Pascual *et al.*, 1999).

Barreto and de Blas (1993) did not detect any improvement during summer in Madrid with diets varying from 8.9 to 11.9 MJ DE g^{-1} DM, the last containing 35 g lard kg^{-1}. In an experiment connected to this one, low-energy diets gave a poorer response at 30°C constant temperature, while no statistical difference was found between the high-energy diets (Table 15.2). However, a diet containing 100 g fat kg^{-1} at 30°C promoted better weight gains in litters than a normal control diet (Cervera *et al.*, 1997). It has yet to be established whether this is a specific effect of fat or of energy intake.

A relationship between the HI of food and the nutrition of does can only be found in a study by Fernández Carmona *et al.* (1995), where 57 New Zealand crossbred does were fed on two iso-energetic (DE) diets, with 121 and 193 g crude fibre kg^{-1} DM. Feed intake results showed the second diet to be less efficient, suggesting that does compensated for higher HI in terms of milk production.

Sanz *et al.* (1989) reported that milk intake was similar in rabbits aged 1, 10 or 20 days, reared at several temperatures between 20°C and 36°C. Suckling rabbits start to ingest solid food at about 18 days of age, and a measurable amount at 21 days. Fernández Carmona *et al.* (1991) reported that rabbits kept at 30°C ingested less DM in

Table 15.2. Effect of diet and housing on daily feed intake of does and litter weight at 28 days.

	Diet DE (MJ kg^{-1} DM)			
	12.9[a]	11.3	10.4	9.0
30°C constant[b]				
Intake (g DM)	185	181	162	163
Litter weight (kg)	2.65	2.32	1.64	1.38
Traditional building[c]				
Intake (g DM)	283	277	304	320
Litter weight (kg)	3.80	3.42	3.24	3.16

DE, digestible energy; DM, dry matter.
[a]Includes 35 g tallow kg^{-1}.
[b]Simplicio *et al.* (1991).
[c]Cervera *et al.* (1993).

a 35-day lactation than those at normal temperatures. The composition of pellets seems to be unimportant at these ages, at least from the results of Fernández Carmona *et al.* (1991), who reported no differences in ingestion between diets at 30°C and at normal temperatures. This agrees with the findings of Blas *et al.* (1990), who evaluated a diet with 100 g skimmed milk kg^{-1}.

15.7 Effects of Heat Stress on Males

High ambient temperatures have adverse effects in bucks, potentially producing temporary sterility, decreasing libido, delaying age at first mating and reducing semen quality and quantity. The effects of heat stress may be due to a decrease in testosterone concentration and spermatogenesis (Zeidan *et al.*, 1997) and become more pronounced when relative humidity is high (Marai *et al.*, 2002).

Semen characteristics and volume should be impaired in summer because of a low sperm concentration, and a decrease in the volume of seminal plasma occurs through secretion of testosterone, which is lowest in summer (Chiericatto *et al.*, 1995). Sperm motility, cell concentration, total number of spermatozoa in an ejaculate and incidences of abnormalities and dead sperm show poorest values when temperatures are higher.

The management of rabbit males during the growing and rearing periods seems to affect their subsequent performance and semen production. During a hot rearing season, Pascual *et al.* (2004) found that feed intake decreased, energy and protein requirements were not covered and perirenal fat thickness of males, semen production and sperm concentration were lower 2 months later when semen collection started, although a high-energy diet alleviated such effects. Similarly, zinc supplementation has been found to reduce the depression of semen production that is usually observed in autumn in Mediterranean countries (Mocé *et al*, 2000), but increasing vitamin and mineral content had no effect (Lavara *et al.*, 2000).

15.8 Effect of Heat Stress on Growing Rabbits

The effect of temperature on the growth of weaned rabbits during the fattening period has often been measured, confirming the fact that voluntary feed intake varies according to whether conditions are cold or warm. DM intake in terms of metabolic weight is shown in Fig. 15.4, where a regression equation has been calculated from the original data, discarding diets with a high DE content. From published works by Zicarelli *et al.* (1979), Stephan (1980), Mori and Bagliacca (1985), Bordi (1986), Casamassima *et al.* (1988) and Samoggia *et al.* (1988), a temperature range from 13°C to 20°C can be assumed to be suitable, though some authors have widened this range. Not everyone agrees, but it is generally accepted that a reduction in intake occurs at 25°C, and perhaps above 22°C (Casamassima *et al.*, 1988;

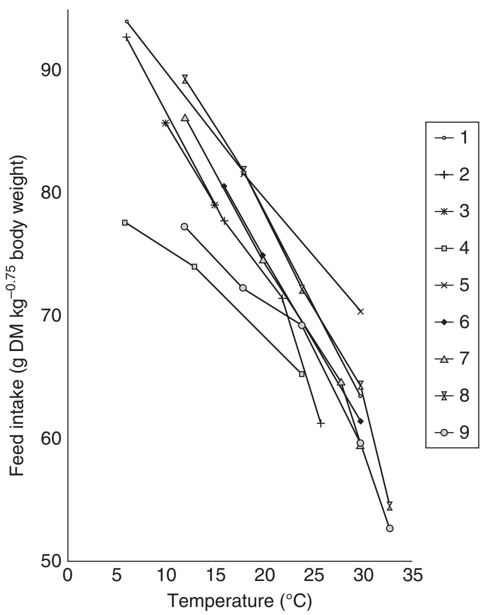

Fig. 15.4. Feed intake of growing rabbits. 1, Stephan (1980) (constant temperatures); 2, Lebas and Ouhayoun (1987) (mean temperatures); 3, Casamassima *et al.* (1988) (minimum temperatures); 4, Nizza *et al.* (1995), 12.5 MJ DE kg^{-1} (mean temperatures); 5, Borgida and Duperray (1992) (mean temperatures); 6, Fernández Carmona *et al.* (1994a) (minimum temperatures); 7, Boiti *et al.* (1992), Chiericatto *et al.* (1995) (nearly constant temperatures); 8, Cervera *et al.* (1997), 11 MJ DE kg^{-1} (minimum temperatures); 9, Cervera *et al.* (1997), 12.4 MJ DE kg^{-1} (minimum temperatures).

Table 15.3. Prediction of growth for 1.5-kg rabbits at two different temperatures.

	18°C	30°C
Feed intake (g DM $kg^{-0.75}$ body weight)[a]	80	60
Energy intake (kJ DE)[b]	1188	891
Maintenance requirements (kJ DE)	745[c]	633[d]
Growth allowances (kJ DE)	443	258
Growth (g day^{-1})[e]	37	21

DM, dry matter; DE, digestible energy.
[a]Approximate, from Fig. 15.4.
[b]$1.5^{0.75}$ = 1.35; diet 11 MJ DE kg^{-1} DM.
[c]552 kJ DE $kg^{-0.75}$ body weight (de Blas *et al.*, 1985).
[d]15% lower requirements at 30°C.
[e]Approximately 12 kg DE g^{-1} live weight gain.

Fernández Carmona *et al.*, 1994a) and certainly impaired growth is assured around 30°C (Table 15.3). Ogunjimi *et al.* (2008) showed that there is high correlation between both rabbit weight gain or feed efficiency and the thermal comfort level of the habitat measured by THI value.

This outcome is less predictable when seasons of the year are considered, because of the usually large degree of variation in temperature and humidity; regardless of this, a substantial number of controlled experiments have recorded lower intakes and growth rates in summer than during the rest of the year (Masoero and Auxilia, 1977; Simplicio *et al.*, 1988). It is hard to establish consistent differences between the results for winter, autumn and spring. Samoggia *et al.* (1988) found higher figures for intake and feed efficiency in winter, but other studies have been far from consistent.

Open-air systems tend to be slightly worse in terms of feed efficiency than indoor systems (Blocher *et al.*, 1990), but opposing results have also been published (Crimella *et al.*, 1996). It seems that a large rabbit farm should be provided with some means of regulating the internal environment, because the stress factors are strongly reinforced by a significant presence of pathogenic microbes and chemical pollutants in the air. Conversely, microbe colonies are non-existent in open-air systems, but climatic stress factors cannot be closely controlled.

The interaction between feeding and temperature remains almost unknown. A reduction of 25% in feed intake, comparable with the percentage observed in hot climates, should be balanced by about the same increment of dietary nutrients. Both Simplicio *et al.* (1988), increasing DE by some 10%, and Borgida and Duperray (1992), increasing protein and lysine, found no improvement in average daily gain in rabbits kept at 30°C. Neither increasing protein from 130 to 200 g kg^{-1} nor increasing total digestible nutrients from 57% to 62% improved the average daily gain when these diets were fed to slow-growing rabbits in tropical conditions (Deshmukh and Pathak, 1991).

Rabbits fattened at mean temperatures of 6°C, 16°C, 22°C and 26°C and fed on a diet with 210 g crude protein kg^{-1} (Lebas and Ouhayoun, 1987) have been found to gain about 5 g more at each temperature than those fed with 157 g crude protein kg^{-1}. When comparing low- and high-fat diets, while gains at moderate temperatures of 12°C and 18°C were similar, at 24°C, 30°C and 33°C the use of high-fat diets slightly improved growth performance as a consequence of small differences in DM intake (Cervera *et al.*, 1997). At a temperature of 30°C it also led to around 50% more dissectible fat (Plá, 1999).

Carcass yield, carcass fat and the efficiency of energy for fattening alter the significance of live weight gain as the sole predictor of growth performance. Slower growth leads to lighter and leaner carcasses, so that any diet should produce less carcass fat at high ambient temperature.

References

Abdel-Samee, A.M., El Gendy, K.M. and Ibrahim, H. (1994) Rabbit growth and reproductive performance as influenced by feeding desert forage (*Acacia saligna* and *Atriplex nummularia*) at North Sinai. *Egyptian Journal of Rabbit Science* 4, 25–36.

Abo-Elezz, Z.R. (1982) The effect of direct solar radiation during pregnancy on the postnatal growth of rabbits. *Beitrage Tropischen Lanwirtschaft Veterinarmedizin* 20, 69–74.

Alliston, C.W. and Rich, T.D. (1973) Influence of acclimation upon rectal temperatures of rabbits subjected to controlled environmental conditions. *Laboratory Animal Science* 23, 62–67.

Amici, A., Finzi, A., Mastroiacomo, P., Nardini, M. and Tomassi, G. (1995) Functional and metabolic changes in rabbits undergoing continuous heat stress for 24 days. *Animal Science* 61, 399–405.

Barreto, G. and de Blas, J.C. (1993) Effect of dietary fibre and fat content on the reproductive performance of rabbit does bred at two remating times during two seasons. *World Rabbit Science* 1, 77–81.

Baselga, M. and Marai, I.F.M. (eds) (1994) *Cahiers Options Mediterréennes. Vol. 8: Rabbit Production in Hot Climates*. Centre International de Hautes Etudes Agronomiques Méditerranéennes, Paris, France.

Battaglini, M., Penella, E. and Pauselli, M. (1986) Influenza del mese di parto sulla produttivitá del coniglio. *Rivista di Coniglicoltura* 8, 35–39.

Blas, E., Moya, A., Cervera, C. and Fernández-Carmona, J. (1990) Utilización de un pienso con leche en gazapos lactantes. *Avances en Alimentación y Mejora Animal* 30, 155–157.

Bligh, J. and Johnson, K.G. (1973) Glossary of terms for thermal physiology. *Journal of Applied Physiology* 35, 941–96l.

Blocher, F., Kohl, P.F. and Strehler, J.F. (1990) Un engraissement en plein air: résultats techniques et economiques. *Rivista di Coniglicoltura* 17, 144–149.

Boiti, C., Chiericato, G.M., Filotto, U. and Canali, C. (1992) The effect of high environmental temperature on plasma testosterone, cortisol, T3 and T4 levels in the growing rabbits. *Journal of Applied Rabbit Research* 15, 447–455.

Bonsembiante, M., Chiericato, G.M. and Bailoni, L. (1989) Risultati sperimentali sull'impiego del bicarbonato di sodio in diete per conigli da carne allevati in condizioni di stress termico. *Rivista di Coniglicoltura* 9, 63–70.

Bordi, A. (1986) Aspetti fisioclimatici dell'allevamento del coniglio. *Rivista di Coniglicoltura* 23, 36–41.

Borgida, L.P. and Duperray, J. (1992) Summer complementary feding of rabbits. *Journal of Applied Rabbit Research* 15, 1063–1070.

Cardasis, C.A. and Sinclair, J.C. (1972) The effects of ambient temperature on the fasted newborn rabbit. 1. Survival time, weight loss, body temperature and oxygen consumption. *Biology of the Neonate* 21, 330–346.

Cardelli, M. (1993) Rabbit production in developing countries. *Rivista di Coniglicoltura* 30, 34–39.

Carew, S.N., Aoyoade, J.A. and Zungwe, E.N. (1989) Composition of plants fed to rabbits in Benue State of Nigeria. *Journal of Applied Rabbit Research* 12, 169–170.

Casamassima, D., Manera, C. and Mugnozza, G.S. (1988) Influenza del microclima sulla produtivita del coniglio. *Rivista di Coniglicoltura* 25, 31–35.

Cervera, C., Fernández Carmona, J., Viudes, P. and Blas, E. (1993) Effect of remating interval and diet on the performance of female rabbits and their litters. *Animal Production* 56, 399–405.

Cervera, C., Blas, E. and Fernández Carmona, J. (1997) Growth of rabbits under different environmental temperatures using high fat diets. *World Rabbit Science* 5, 71–75.

Cheng, C., Shimin, L., Li, Z. and Zhuang, X. (1991) The effect of nutrition, temperature and physiology on wool production of the Angora rabbit. *Journal of Applied Rabbit Research* 14, 89–92.

Chiericatto, G.M., Boiti, C., Canali, C., Rizzi, C. and Ravarotto, L. (1995) Effects of heat stress and age on growth performance and endocrine status of male rabbits. *World Rabbit Science* 3, 125–13l.

Christ, B., Lange, K. and Jeroch, H. (1996) Effect of dietary fat on fat content and fatty acid composition of does milk. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 3. Association Francaise de Cuniculture, Lempdes, France, pp. 135–138.

Colin, M. (1991) La cuniculture des pays méditerranéens. *Cuni-Sciences* 7, 73–96.

Colin, M. (1995) La cuniculture Sud-Américaine. Le Bresil. *World Rabbit Science* 3, 85–90.

Costantini, F. and Castellini, C. (1990) Effetti dell'habitat e del management. *Rivista di Coniglicoltura* 27, 31–36.

Crimella, C., Biffi, B. and Luzi, F. (1996) Allevamento plein-air: attualitá e prospettive. *Rivista di Coniglicoltura* 33, 15–23.

Dawkins, M.J.R. and Hull, D. (1964) Brown adipose tissue and the response of new born rabbits to cold. *Journal of Physiology, London* 172, 216–238.

de Blas, C., Fraga, M.J. and Rodriguez, J.M. (1985) Units for feed evaluation and requirements for commercially grown rabbits. *Journal of Animal Science* 60, 1021–1028.

Delaveau, A. (1982) Mortalité des lapereaux entre la naissance et le sevrage. 2. Resultats experimentaux. *Dossiers del'Elevage* 5, 69–84.

Deshmukh, S.V. and Pathak, N.N. (1991) Effect of different dietary protein and energy levels on growth performance and nutrient utilization in New Zealand White rabbits. *Journal of Applied Rabbit Research* 14, 18–24.

Fayez, I., Marai, M., el-Masry, K.L. and Nasr, A.S. (1994) Heat stress and its amelioration with nutritional, buffering, hormonal and physical techniques for New Zealand White rabbits maintained under hot summer conditions of Egypt. In: Baselga, M. and Marai, I.F.M. (eds) *Cahiers Options Mediterréennes. Vol. 8: Rabbit Production in Hot Climates*. Centre International de Hautes Etudes Agronomiques Méditerranéennes, Paris, France, pp. 475–487.

Fernández Carmona, J., Cervera, C. and Sabater, C. (1991) Efecto del pienso y de una temperatura ambiente alta sobre la ingestión de pienso de gazapos lactantes y recien destetados. In: *Proceedings of the XVI Symposium Nacional de Cunicultura*. Asociación Española de Cunicultura, Castellón de la Plana, Spain, pp. 79–81.

Fernández Carmona, J., Cervera, C. and Blas, E. (1994a) Efecto de la inclusión de jabón cálcico en el pienso y de la temperatura ambiental sobre el crecimiento de conejos. *Investigación Agraria: Producción y Sanidad Animales* 9, 5–11.

Fernández Carmona, J., Cervera, C. and Blas, E. (1994b) Feed intake of does and their litters in different environmental temperature. In: Baselga, M. and Marai, I.F.M. (eds) *Cahiers Options Mediterréennes. Vol. 8: Rabbit Production in Hot Climates*. Centre International de Hautes Etudes Agronomiques Méditerranéennes, Paris, France, pp. 145–149.

Fernández Carmona, J., Cervera, C. and Blas, E. (1994c) Readapted does from high to normal ambient temperature. In: Baselga, M. and Marai, I.F.M. (eds) *Cahiers Options Mediterréennes. Vol. 8: Rabbit Production in Hot Climates*. Centre International de Hautes Etudes Agronomiques Méditerranéennes, Paris, France, pp. 469–470.

Fernández Carmona, J., Cervera, C., Sabater, C. and Blas, E. (1995) Effect of diet composition on the production of rabbit breeding does housed in a traditional building and at 30°C. *Animal Feed Science and Technology* 52, 289–297.

Fernández Carmona, J., Cervera, C. and Blas, E. (1996) High fat diets for rabbit breeding does housed at 30°C. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 1. Association Francaise de Cuniculture, Lempdes, France, pp. 167–170.

Ferraz, J.B.S., Johnson, R.K. and Eler, J.P. (1991) Breed and environmental effects on reproductive traits of Californian and New Zealand rabbits. *Journal of Applied Rabbit Research* 14, 177–179.

Finzi, A., Kuzminsky, G. and Morera, P. (1988) Evaluation of thermotolerance parameters for selecting thermotolerant rabbit strains. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 11. Sandor Holdas, Hercegalom, Hungary, pp. 388–394.

Finzi, A., Tani, A. and Scappini, A. (1989) Tunisian non-conventional rabbit breeding systems. *Joumal of Applied Rabbit Research* 12, 181–184.

Fraga, M.J., Lorente, M., Carabaño, R.M. and de Blas, J.C. (1989) Effect of diet and remating interval on milk production and milk composition of the doe rabbit. *Animal Production* 48, 459–466.

Fuquay, J.W. (1981) Heat stress as it affects animal production. *Journal of Animal Science* 52, 164–174.

García, J., Pérez-Alba, L., Alvarez, C., Rocha, R., Ramos, M. and de Blas, J.C. (1995) Prediction of the nutritive value of lucerne hay in diets for growing rabbits. *Animal Feed Science and Technology* 54, 33–44.

Gentry, J.W. (1983) Raising rabbits in domes. *Journal of Applied Rabbit Research* 6, 89.

Gonzalez, R.R., Kluger, M.J. and Hardy, J.D. (1971) Partitional calorimetry of the New Zealand White rabbit at temperature 5–35°C. *Journal of Applied Physiology* 31, 728–734.

Gray, R. and McCracken, K.J. (1974) Utilization of energy and protein by pigs adapted to different temperature levels. In: *Proceedings of the 6th Symposium on Energy Metabolism*. EAAP publ. No. 14, Stuttgart University, Germany, p. 161.

Hall, L.S. and Myers, K. (1978) Variations in the microclimate in rabbit warrens in semi-arid New South Wales. *Australian Journal of Ecology* 3, 187–194.

Harris, D.L., Cheeke, P.R., Patton, N.M. and Brewbaker, J.L. (1981) A note on the digestibility of leucaena leaf in rabbits. *Journal of Applied Rabbit Research* 4, 99.

Harris, D.L., Cheeke, P.R. and Patton, N.M. (1983) Food preference and growth performance of rabbits fed pelleted versus unpelleted diets. *Journal of Applied Rabbit Research* 6, 15–17.

Hill, J.R. (1961) Reaction of the newborn animal to environmental temperature. *British Medical Bulletin* 17, 164–167.

Howarth, B., Alliston, C.W. and Ulbera, L.C. (1965) Importance of uterine environment on rabbit sperm prior to fertilization. *Journal of Animal Science* 24, 1027–1032.

Jin, L.M., Thomson, E. and Farrell, D.J. (1990) Effects of temperature and diet on the water and energy metabolism of growing rabbits. *Journal of Agricultural Science, Cambridge* 115, 135–140.

Johnson, H.D., Cheng, C.S. and Ragsdale, A.C. (1958) *Environmental Physiology and Shelter Engineering*. Research Bulletin 648, Agricultural Experiment Station, University of Missouri, USA.

Kamal, T.H. and Johnson, H.D. (1971) Total body solids as a measure of a short stress in cattle. *Journal of Animal Science* 32, 306–311.

Kamar, G.A.R., Shafie, M.M., Hassanein, A.M. and Borady, A.M. (1982) Effects of modification in environmental conditions on the reproductive activity of Giza rabbit does. In: *Proceedings of the 6th International Conference on Animal and Poultry Production*, Vol. 1. Zagazig, Egypt, pp. 316–328.

Kasa, W., Thwaites, C.J., Jianke, X. and Farell, D.L. (1989) Rice bran in the diet of rabbits grown at 22° and 30°C. *Journal of Applied Rabbit Research* 12, 75–77.

Kluger, M.J., González, R.R. and Stolwijk, J.A.J. (1973) Temperature regulation in the exercising rabbit. *American Journal of Physiology* 224, 130–135.

Kong, P.L., Ping, F.Z., He, S.Q., Yuan, M.T., Huang, T.X. and Li, G.M. (1987) Effect of temperature on wool yield and quality in German Angora rabbits. *Chinese Journal of Rabbit Farming* 3, 22–25.

Kruk, B. and Davydov, F. (1977) Effect of ambient temperature on thermal sensitivity of POAH area in the rabbit. *Journal of Thermal Biology* 2, 75–78.

Lavara, R., Mocé, E., Andreu, E., Pascual, J.J., Cervera, C., Viudes de Castro, M.P. and Vicente, J.S. (2000) Effect of environmental temperature and vitamin supplementation on seminal parameters from a rabbit line selected by high growth rate. *World Rabbit Science. Proceedings of the 7th World Rabbit Congress* 8 (Suppl. 1, Vol. A), 167–172.

Lebas, F. and Ouhayoun, J. (1987) Incidence du niveau protéique del'aliment, du milieu et de la saison sur la croissance et les qualités boucheres du lapin. *Annales de Zootechnie* 36, 421–432.

Lee, R.C. (1939) The rectal temperature of the normal rabbit. *American Journal of Physiology* 125, 521–529.

Lukefahr, S.D., Hohenboken, W.D., Cheeke, P.R. and Patton, N.M. (1983) Doe reproduction and preweaning litter performance of straight-bred and crossbred rabbits. *Journal of Animal Science* 57, 1090–1099.

Maertens, L. (1992) Rabbit nutrition and feeding: a review of some recent developments. *Journal of Applied Rabbit Research* 15, 889–913.

Maertens, L. and De Groote, G. (1988) The influence of the dietary energy content on the performance of post-partum breeding does. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 3. Sandor Holdas, Hercegalom, Hungary, pp. 42–52.

Maertens, L. and De Groote, G. (1990) Comparison of feed intake and milk yield of does under normal and high ambient temperature. *Journal of Applied Rabbit Research* 13, 159–162.

Marai, I.F.M., Habeeb, A.A.M. and Gad, A.E. (2002) Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livestock Production Science* 78, 71–90.

Marai, I.F.M., Askar, A.A. and Bahgat, L.B. (2006) Tolerance of New Zealand White and Californian doe rabbits at first parity to the sub-tropical environment of Egypt. *Livestock Science* 104, 165–172.

Masoero, G. and Auxilia, M.T. (1977) Evoluzione della produttivita del coniglio nel curso di un anno. In: *Annali dell'Istituto Sperimentale per la Zootecnia*, Vol. 10. Istituto Sperimentale per la Zootecnia, Torino, Italy, pp. 93–111.
McEwen, G.N. and Heath, J.E. (1973) Resting metabolism and thermoregulation in the unrestrained rabbit. *Journal of Applied Physiology* 35, 884–886.
McNitt, J.I. and Moody, G.L. (1992) A method for weaning rabbits pups at 14 days. *Journal of Applied Rabbit Research* 15, 661–665.
Mendez, J., de Blas, J.C. and Fraga, M.J. (1986) The effects of diet and remating interval after parturition on the reproductive performance of the commercial doe rabbit. *Journal of Animal Science* 62, 1624–1634.
Mocé, E., Aroca, M., Lavara, R. and Pascual, J.J. (2000) Effect of dietary zinc and vitamin supplementation on semen characteristics of high growth rate males during summer season. *World Rabbit Science. Proceedings of the 7th World Rabbit Congress* 8 (Suppl. 1, Vol. A), 203–210.
Mori, B. and Bagliacca, M. (1985) Al!evamento del coniglio: microclima e ritmo riproduttivo. *Rivista di Coniglicoltura* 22, 45–50.
Mori, B. and Bagliacca, M. (1990) Effetto dell'ambiente sulle produzioni cunicole. *Rivista di Coniglicoltura* 27, 17–21.
Nichelmann, M., Rohling, H. and Rott, M. (1973a) Der einfluss der umgebungstemperatur auf die hohe des energieumsatzes erwachsener kaninchen. *Archiv für Experimentelle Veterinarmedizin* 27, 499–505.
Nichelmann, M., Rohling, H. and Rott, M. (1973b) Der einfluss der umgebungstemperatur auf die warmeabgabe beim kaninchen. *Archiv für Experimentelle Veterinarmedizin* 27, 507–512.
Nichelmann, M., Rott, M. and Rohling, H. (1974a) Beziehungen zwischen energieumsatz und umgebungstemperatur beim kaninchen. *Monatshefte für Veterinarmedizin* 29, 257–261.
Nichelmann, M., Rott, M. and Rohling, H. (1974b) Untersuchungen zur evaporativen warmeabgabe beim kaninchen. *Monatshefte für Veterinarmedizin* 29, 261–266.
Nizza, A., Moniello, G. and Lella Di, T. (1995) Prestazioni produttive e metabolismo energetico di conigli in accrescimento in funzione della stagione e della fonte proteica alimentare. *Zootecnica e Nutrizione Animale* 21, 173–183.
NRC (1981) *Effect of Environment on Nutrient Requirements of Domestic Animals*. National Academy Press, Washington, DC, USA.
Ogunjimi, L.A.O., Ogunwande, G.A. and Osunade, J.A. (2008) Rabbit weight gain, feed efficiency, rectal temperature and respiration rate as affected by building thermal environment in the humid tropical climate of southwestern Nigeria. *Agricultural Engineering International: the CIGR Ejournal* 10, 1–14.
Ortiz, V., de Blas, J.C. and Sanz, E. (1989) Effect of dietary fiber and fat content on energy balance in fattening rabbits. *Journal of Applied Rabbit Research* 12, 159–162.
Pagano-Toscano, G., Zoccarato, I., Benatti, G. and Lazzaroni, C. (1990) Fattori ambientali e prestazioni delle coniglie. *Rivista di Coniglicoltura* 2, 23–29.
Papp, Z. and Rafai, P. (1988) Role of the microclimate in intensive rabbit production. IV. Effects of environmental temperature stressors in pregnant does, embryonic development and viability of young rabbits. *Magyar Allatorvosok Lapja* 43, 529–534.
Partridge, G.G. (1988) Research of nutrition, reproduction and husbandry of commercial meat rabbits at the Rowett Institute, 1971–1985. *Journal of Applied Rabbit Research* 11, 136–141.
Pascual, J.J., Cervera, C., Blas, E. and Fernández-Carmona, J. (1999) Effect of high fat diets on the performance, milk yield and milk composition of multiparous rabbit does. *Animal Science* 68, 151–162.
Pascual, J.J., García, C., Martínez, E., Mocé, E. and Vicente, J.S. (2004) Rearing management of rabbits males selected by high growth rate: the effect of diet and season on semen characteristics. *Reproduction Nutrition Development* 44, 49–63.
Plá, M. (1999) Carcass and meat quality of growing rabbits under high ambient temperature using high fat diets. In: Chioccioli, E. (ed.) *Cahiers Options Mediterranéennes Vol 41. 2nd International Conference on Rabbit Production in Hot Climates. Adana (Turkey)*. Institut Agronomique Méditerranéen, Zaragoza, Spain, pp. 93–98.
Platukhin, A.I. and Konokhov, S.A. (1972) Winter kindling in one rabbit sheds. *Krolikovdstvo-i-Zverovodstvo* 5, 15–16.
Pote, L.M., Cheeke, P.R. and Patton, N.M. (1980) Use of greens as a supplement to a pelleted diet for growing rabbits. *Journal of Applied Rabbit Research* 3, 15–19.
Prud'hon, M. (1976) Comportement alimentaire du lapin soumis aux temperatures de 10, 20 et 30°C. In: Lebas, F. (ed.) *Proceedings of the 1er Congres International Cunicole*. Communication no 14, Association Francaise de Cuniculture, Dijon, France, 7 pp.

Rafai, P. and Papp, Z. (1984) Temperature requirement of does for optimal performance. *Archiv für Experimentelle Veterinarmedizin, Leipzig* 38, 450–457.
Raharjo, Y.C., Cheeke, P.R. and Patton, N.M. (1988) Evaluation of tropical forages and rice by-products as rabbit feeds. *Journal of Applied Rabbit Research* 9, 201–211.
Raharjo, Y.C., Cheeke, P.R. and Patton, N.M. (1990) Effect of cecotrofy on the nutrient digestibility of lucerne and black locust leaves. *Journal of Applied Rabbit Research* 13, 56–61.
Rhoad, A.O. (1944) The Iberia heat tolerance test for cattle. *Tropical Agriculture (Trinidad)* 21, 162–164.
Rödel, H.G., Hudson, R. and von Holst, D. (2008) Optimal litter size for individual growth of European rabbit pups depends on their thermal environment. *Oecologia* 155, 677–689.
Rosell, J.M. (1996) Rabbit mortality survey. Necropsy findings in the field during the period 1989–1995. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol. 3. Association Francaise de Cuniculture, Lempdes, France, pp. 107–111.
Samoggia, G., Bosi, P. and Scalabrini, C. (1988) Ambiente zootecnico e performances produttive del coniglio da carne. *Rivista di Coniglicoltura* 25, 37–40.
Sanchez, W.K., Cheeke, P.R. and Patton, N.M. (1984) The use of chopped alfalfa rations with varying levels of molasses for weanling rabbits. *Journal of Applied Rabbit Research* 7, 13–16.
Sanz, E., Ortiz, V., de Blas, J.C. and Fraga, M.J. (1989) A note on the critical temperature of sucking rabbits. *Animal Production* 49, 333–334.
Sanz, R., Fonollá, J. and Aguilera, J. (1973) Estudios de digestibilidad en conejos sometidos a alta temperatura. Utilización de antitérmicos. *Revista de Nutrición Animal* 11, 167–172.
Scheele, C.W., van der Broek, A. and Hendricks, F.A. (1985) Maintenance energy requirements and energy utilization of growing rabbits at different environmental temperatures. In: Moe, P.W., Tyrrell, H.F. and Reynolds, P.J. (eds) *Energy Metabolism of Farm Animals*. Rowman and Littlefield, New Jersey, USA, pp. 202–204.
Schenk, H., Heim, T., Varga, F. and Goetze, E. (1975) Effects of cold and fasting on metabolism of serum free fatty acids in newborn rabbits. *Acta Biologica et Medica Germanica* 34, 613–623.
Schlolaut, M. (1987) Angora rabbit housing and management. *Journal of Applied Rabbit Research* 10, 164–168.
Shafie, M.M., Abdel-Malek, E.G., El-Issawi, H.F. and Kamar, G.A.R. (1979) Effect of environmental temperature on physiological body reactions of rabbits under sub-tropical conditions. *Egyptian Journal of Animal Production* 10, 133–149.
Simplicio, J.B., Cervera, C. and Blas, E. (1988) Effect of two different diets and temperatures on the growth of meat rabbit. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 3. Sandor Holdas, Hercegalom, Hungary, pp. 74–77.
Simplicio, J.B., Fernández-Carmona, J., Cervera, C. and Blas, E. (1991) Efecto del pienso sobre la producción de la coneja a una temperatura ambiente alta. *Investigación Agraria: Producción y Sanidad Animales* 6, 67–74.
Sittman, D.B., Rollins, W.C., Sittman, K. and Casady, R.R. (1964) Seasonal variation on reproductive traits of the New-Zealand rabbits. *Journal of Reproduction and Fertility* 8, 29–37.
Stephan, E. (1980) The influence of environmental temperatures on meat rabbits of different breeds. In: Camps, J. (ed.) *Proceedings of the 2nd World Rabbit Congress*, Vol. 1. Asociación Española de Cunicultura, Barcelona, Spain, pp. 399–409.
Thwaites, C.J., Baillie, N.B. and Kasa, W. (1990) Effects of dehydration on the heat tolerance of male and female New Zealand White rabbits. *Journal of Agricultural Science, Cambridge* 115, 437–440.
Tramell, T.L., Stallcup, O.T. and Harris, G.C. (1988) Effect of high temperature on certain blood hormones and metabolites on reproduction in rabbit does. *Journal of Applied Rabbit Research* 12, 101–102.
Wittorff, E.K., Heird, C.E., Rakes, J.M. and Johnson, Z.B. (1988) Growth and reproduction on nutrient restricted rabbits in a heat stressed environment. *Journal of Applied Rabbit Research* 11, 87–92.
Young, B.A. (1977) Effect of cold environments on nutrient requirements of ruminants. In: Fonnesbeck, F.V., Harris, L.E. and Kearl, L.C. (eds) *Proceedings of the 1st International Symposium. Feed Composition, Animal Nutrient Requirements and Computerization of Diets*. Utah Agricultural Experiment Station, Logan, Utah, USA, pp. 491–496.
Zeidan, A.E.B., Marai, I.F.M. and Abd El-Kariem, Z.A. (1997) Effects of intratesticular injection of gonadotropin-releasing hormone on reproductive performance of low fertile male rabbits under Egyptian summer conditions. In: *Proceedings of 1st International Conference on Animal Production and Health*. Dokki, Egypt, pp. 557–566.
Zicarelli, L., Nizzi, A. and Perrucci, G. (1979) Influenza della temperatura ambientale su alcuni parametri produttivi del coniglio da carne. *Rivista Acta Medica Veterinaria* 25, 79–84.

16 Nutritional Recommendations and Feeding Management of Angora Rabbits

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16.1 Introduction

The Angora rabbit produces 1.0–1.4 kg year^{-1} of pure fine animal fibre without grease or plant material contamination, named 'Angora wool'. This represents some 0.30 of its live weight, the highest keratin production to live-weight ratio found in any fibre-producing animal. In sheep, goat or camelids, this figure is generally <0.10.

The capacity of the Angora rabbit to convert food to keratin requires that particular attention be given to its nutrition. There are two important nutritional objectives:

1. To provide all the nutrients the rabbit needs to realize its genetic potential for wool production.
2. To avoid any disorder that may reduce the life-time performance of the animal.

Individual productive longevity (3–4 years on average) is an important economic parameter in the Angora production system.

There is a considerable paucity of information on Angora rabbit nutrition compared with published work on the production of meat from rabbits or wool from sheep. This applies to the genetics and physiology of Angora wool growth, as well to other areas of study such as pathology. In practice, producers have observed that the nutrient requirements of Angora rabbits bred for wool production are similar to those of breeding does kept for meat production and consequently have used this knowledge as a basis for diet formulation. Nevertheless, some specific modifications are necessary.

For a long time, Angora rabbits were fed in the same way as rabbits kept for meat production on a mixture of cereals (oats, barley or wheat), lucerne hay and fresh forages such as cabbage or fodder beet. Since the 1960s, complete diets based on pelleted concentrates have been used extensively in rabbit meat systems; Angora rabbit farmers, however, continued with the traditional feeding method through the 1970s, while Angora wool yields remained <850 g year^{-1}. To improve wool yields, a mixture of 0.75 traditional feed and 0.25 supplementary feed pellets was subsequently used in some practical systems (Rougeot and Thébault, 1984). Other producers began using pelleted concentrates alone for Angora breeding does. By the beginning of the 1980s, as the genetic potential for wool production exceeded 1 kg per animal year^{-1}, the use of specific pelleted diets formulated for Angora production became general practice as feed quality and safety (absence of induced disorders) were also improved. Schlolaut (1985) quantified the production advantages of concentrate feeds. Taking the Angora wool yield obtained with these as 1.00, mixed feeding (raw products plus cereals) and hay-based feeding reduced the yearly wool production to 0.85 and 0.72, respectively.

16.2 Nutritional Requirements

This chapter considers the nutrient requirements of Angora wool-producing females, since males are not frequently employed because of their lower wool production (5–10% less). Animals producing Angora wool are assumed to be adults with no production other than wool. For breeding does or growing animals, the recommendations are those proposed for meat production rabbits (see Chapters 10 and 14).

16.1.1 Consequences of daily variations in wool production

The amount of hair covering the body plays an important role in thermal insulation and heat loss. In France, Angora rabbits are de-fleeced every 3 months and are consequently completely or relatively naked and without thermal protection for 2–3 weeks. Vermorel *et al.* (1988) demonstrated a large increase in heat production just after the harvest (Tables 16.1 and 16.2). To reduce heat loss, some form of protection is often provided, either in the form of a woollen jacket (‘jersey rabbits’) or by leaving a strip of fleece on the back (‘strip rabbits’). Such techniques are less common with the German strain because the animals are shorn (i.e. a few millimetres of stubble is always left above the skin), which limits heat loss. In addition, German Angora rabbits have a higher proportion of down in the fleece, which improves thermal insulation. Nevertheless, whatever the Angora strain, the period of 2–3 weeks after harvesting is when energy requirements for thermal regulation are at their highest.

A further source of variation for nutrient requirements is the hair growth rate (i.e. the rate of keratin synthesis). The highest growth rate is observed during the fourth week after harvesting (31.7 g week^{-1}), with a reduction in the weekly wool output after this period (Fig. 16.1). Between weeks 4 and 14, the wool output is halved.

According to these data, nutrient and energy requirements appear to be maximum for energy, protein and sulphur amino acids (SAA, the main components of keratin) in the first month following fleece harvesting. The weekly requirements vary during the 3 months between two consecutive harvests (Table 16.3) and have been summarized by Rougeot and Thébault (1984).

16.1.2 Nutrient recommendations

As previously mentioned, Angora rabbits are now fed with balanced pelleted feeds. The desirable composition of such feeds has

Table 16.1. Skin temperature and total and net radiative heat flow of Angora rabbits before and after de-fleecing, with or without a strip of hair on the back or a jersey jacket (means of six different spots measured during the 2 days following harvest ± standard deviations) (from Vermorel *et al.*, 1988).

			‘Strip rabbits’		‘Jersey rabbits’	
	Before harvest	After complete de-fleecing	On the hair strip	Naked areas[a]	With woollen jacket	Without woollen jacket
No. of animals	6	6	9	9	6	6
Skin temperature at 10°C	38.8 ± 0.4	36.5 ± 0.6	–	37.9 ± 0.6	31.9 ± 0.5[b]	36.4 ± 0.7
Total radiative heat flow at 15°C (W m^2)	422 ± 5	513 ± 12	416 ± 7	519 ± 7	479 ± 6	515 ± 7
Net radiative heat flow at 15°C (W m^2)	23 ± 6	176 ± 7	35 ± 6	187 ± 12	24 ± 12	177 ± 7

[a]Means of values obtained on the thigh, thorax and abdomen.
[b]Temperature on the jersey jacket.

Table 16.2. Heat production (kJ kg^{-1} W$^{0.75}$ h^{-1}) of Angora rabbits before de-fleecing (at 10°C) and after de-fleecing, with or without a strip of hair or a jersey jacket (for 2 days) and after harvesting the strip of hair or removing the jersey jacket (for 2 days) (means ± standard deviations) (from Vermorel *et al.*, 1988).

	'Strip rabbits'				'Jersey rabbits'			
			Strip of hair				Jersey jacket	
Environmental temp.	*n*	Before de-fleecing	With	Without	*n*	Before de-fleecing	With	Without
15°C	2	18.1 ± 2.0^{a}	20.8 ± 0.2^{a}	29.9 ± 1.3^{b}	–	–	–	–
10°C	4	16.7 ± 0.3^{a}	23.2 ± 2.4^{b}	32.6 ± 1.5^{c}	5	17.6 ± 1.0^{a}	28.9 ± 1.7^{b}	32.8 ± 2.0^{c}
5°C	3	16.3 ± 2.6^{a}	25.5 ± 3.3^{b}	35.6 ± 2.7^{c}	–	–	–	–

[a,b,c]For the same type of animals and the same environmental temperature, values with different superscripts are significantly different (P <0.05 or P <0.01).

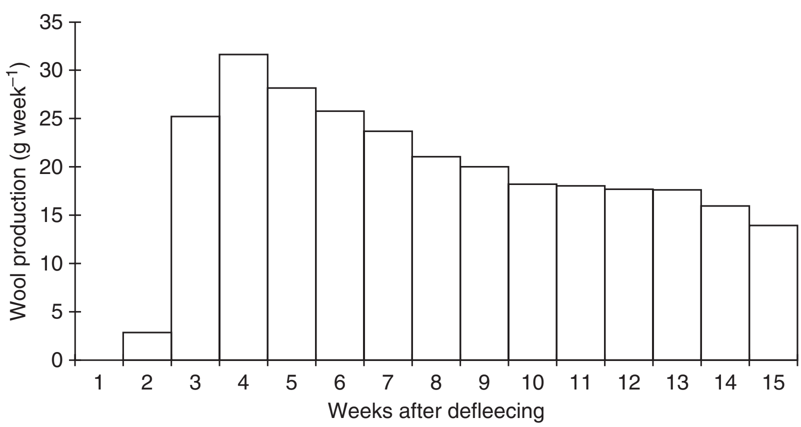

Fig. 16.1. Variations in wool production (g week^{-1}) between two harvests. From Rougeot and Thébault (1984).

Table 16.3. Monthly variations in nutrient and energy requirements for French Angora rabbits between two wool harvestings (recalculated from Rougeot and Thébault, 1984).

	Month 1	Month 2	Month 3
Crude protein (g)	190	175	160
Fat (g)	37	34	31
Crude fibre (g)	205	190	170
Sulphur amino acids (g)	10	9	8
Digestible energy (MJ)	12.6	11.6	11.5

been the object of specific experiments. The recommendations proposed by different authors are a combination of the results of a critical analysis of the available 'Angora' data and of the recommendations proposed for meat rabbits. Table 16.4 presents the recommendations of German and Chinese authors, where available, and the recommendations proposed by the authors of the current chapter.

16.1.3 Energy

Recommendations for dietary digestible energy (DE) content are in the same range for German (Schlolaut, 1985), Chinese (Liu *et al.*, 1992) and French authors (Rougeot and Thébault, 1984; Charlet-Lery *et al.*, 1985). Nevertheless, German and Chinese data are not very precise since the variation between the proposed minimum and maximum

Table 16.4. Nutrient recommendation for adult Angora rabbits.[a]

Nutrients	Unit kg^{-1}	Source of recommendations: Germany[b]	China[c]	Current work
Digestible energy	MJ	9.6–10.9	10.0–11.7	10.5
	Kcal	2,300–2,600	2,400–2,800	2,500
Lipids	g	20	30	30
Crude fibre	g	140–160	120–170	140
Crude protein	g	150–170	150–160	160
Digestible protein	g	–	110	122
Lysine	g	5	7	7
Methionine + cystine	g	7	7	8
Arginine	g	6	7	6
Minerals				
Calcium	g	10	–	8
Phosphorus	g	3–5	–	4
Sodium	g	2.5	–	3
Potassium	g	7	–	13 maximum
Chloride	g	4	–	4
Sulphur	mg	–	–	400
Magnesium	mg	300	–	300
Iron	mg	50	–	50
Copper	mg	10	–	50
Zinc	mg	50	–	50
Manganese	mg	10	–	10
Vitamins				
A	IU	6,000	–	10,000
D_3	IU	500	–	800
E	mg	20	–	40
K	mg	1	–	1

[a]As-fed basis with 890 g dry matter kg^{-1}.
[b]Schlolaut (1985).
[c]Liu *et al.* (1992).

represents 13–17% of the minimum. The Hungarian recommendations are also in the same range: 10.7 MJ kg^{-1} (Tossenberger and Henics, 1988; Henics *et al.*, 1989). The recommendation is accordingly 10.5 MJ kg^{-1} of feed on an as-fed basis.

According to Charlet-Lery *et al.* (1985), metabolizable energy (ME) represents 0.95 of DE content; in addition, the same authors have indicated that energy utilization by Angora rabbits, as DE or ME, is independent of the season or the time since the previous wool harvest.

16.1.4 Protein

Among German, Chinese and French data, there is agreement on dietary protein requirements, which are in the order of 160 g kg^{-1}. On the other hand, the Hungarian recommendations are higher at 196 g kg^{-1} (Tossenberger and Henics, 1988; Henics *et al.*, 1989). However, this latter recommendation is based on an experiment in which protein and SAA were studied simultaneously and where the lowest protein level tested was 175 g kg^{-1} with a relatively low SAA content. Thus, this recommendation for a very high concentration of protein seems unrealistic and has not been retained.

16.1.5 Crude fibre

No specific study has been published on dietary fibre content as a possible source of variation in Angora wool production. The recommendations of various authors are easily calculated from the analysis of practical diets employed. The available recommendations are currently expressed only in terms of the level of crude fibre. However, one of the roles of dietary fibre is to remove hair swallowed by the rabbit from the digestive tract. To achieve this objective, a significant proportion of dietary fibre must be non-digestible; a minimum level of lignin seems reasonable to reduce fibre digestibility and, to achieve this latter objective, a value of 40 g acid detergent lignin (according to the Van Soest methodology) kg^{-1} may be proposed.

16.1.6 Amino acids

Lysine

German recommendations for dietary lysine in Angora rabbit production are 5 g kg^{-1} diet, significantly lower than the 7 g kg^{-1} suggested by Chinese data; however, neither of these figures is based on direct experiments. Lysine is not an important component of keratin, but it does play a significant role in body protein turnover and assists the animal in restoring its live weight following the body weight loss observed after de-fleecing. Therefore, a level of 7 g of lysine kg^{-1} feed is recommended for Angora rabbits.

Methionine and cystine

Several studies have been undertaken in Germany (Schlolaut and Lange, 1983), Hungary (Henics *et al.*, 1990) and France (Lebas and Thébault, 1990) on the requirements for SAA. From this last work (Table 16.5), it has been concluded that, for a level of wool production >1000 g $year^{-1}$, SAA intake is an important limiting factor. Practical recommendations for SAA are 8 g kg^{-1} diet on an as-fed basis. A more recent study (F. Lebas and R.G. Thébault, unpublished data) indicates that efficient SAA supplementation can be achieved with either D,L-methionine or L-cystine. Under some conditions, a slight advantage can be attributed to cystine supplementation. Nevertheless, for economic reasons SAA supplementation, if necessary, is recommended in the form of D,L-methionine.

The Hungarian SAA recommendation is 9 g kg^{-1} diet (Henics *et al.*, 1990), but this figure was obtained after a comparison of only two SAA dietary levels: 5.6 and 9.0 g kg^{-1}. Because the highest level examined in the French study (8.8 g SAA kg^{-1}; Lebas and Thébault, 1990) failed to induce any improvement in wool production above that achieved with 8.0 g kg^{-1}, the Hungarian recommendation of 9 g kg^{-1} leads to a significant SAA wastage and is not considered practical.

Other amino acids

No specific evaluation has been undertaken for the other amino acids. The current die-

Table 16.5. Mean relative performances of Angora rabbits receiving sulphur amino acid (SAA) supplementation at different dietary levels (adjusted by co-variance analysis to an initial live weight of 4.128 kg). Fleece weight 1.00 = 264.2 g per harvest, feed intake 1.00 = 15.57 kg between two harvests. Other nutrients are as recommended in Table 16.4 (from Lebas and Thébault, 1990).

	Dietary SAA level (g kg^{-1})				Residual	
Performance	5.6	6.4	7.2 (control)	8.0	coefficient of variation	Statistical probability
Number of harvests	69	62	76	60	–	–
Fleece weight	0.948[a]	1.008[b]	1.000[b]	1.056[c]	0.135	0.002
Feed intake	0.991	1.000	1.000	1.006	0.043	NS
Feed efficiency[d]	0.951[a]	1.005[bc]	1.000[b]	1.049[c]	0.127	0.004
Live weight	0.990	1.020	1.000	1.006	0.07	NS

NS, not significant.

[a,b,c]Values with different superscripts are significantly different (P <0.05 or P <0.01).

[d]Calculated as g wool produced per g feed intake.

tary recommendation for arginine (7 g kg^{-1}) is based only on the actual content observed in adequate Angora diets. In the absence of further information, the recommendations for growing rabbits are suitable.

16.1.7 Minerals and vitamins

As for most of the amino acids, the current recommendations for minerals (Table 16.4) are derived from the observed composition of Angora diets and from knowledge of the mineral requirements of growing and adult meat rabbits.

The German recommendation for vitamin A (6000 IU kg^{-1}; Schlolaut, 1985) is lower than the recommendation of 10,000 IU kg^{-1} proposed in France by Rougeot and Thébault (1984). Hungarian experimental results (Table 16.6) indicate clearly that 5000 IU kg^{-1}, which is very close to the German recommendation, is not adequate for Angora wool production (Kovácsné-Virányi, 1990). By comparison with meat rabbit reproduction, it can be assumed that the maximum level employed in the Hungarian experiments is too large and the proposed recommendation is 10,000 IU vitamin A kg^{-1}, which is the same as for most meat rabbits. A complementary experiment included in the same Hungarian publication demonstrated that β-carotene can sometimes completely replace the supply of vitamin A, but the two experiments were not precise enough to support any calculation of the transformation of β-carotene into vitamin A.

It is important to note that dietary vitamin D levels should not exceed 800 IU kg^{-1}. Adult females that are not reproducing, lactating or growing are susceptible to heart valve and kidney calcification with D hypervitaminosis (Thébault and Allain, 1995).

16.2 Feeding Management

As mentioned in the introduction, in practice Angora rabbits are fed balanced pelleted feeds (3–5 mm pellet diameter). In addition, they must have permanent access to clean fresh water. Daily water intake is about 0.33 l per animal day^{-1}, with a large variation between animals and season. Significant mortality can be observed if insufficient water is available during a hot period. Dietary roughage, supplied once or twice a week as straw or hay or *ad libitum* as straw bedding, is not essential for health or wool production in the Angora rabbit (Rougeot *et al.*, 1980). However, when straw is fed, average daily intake falls from 19 to 13 g between the first and third months following the harvest. Greater variations are observed between individual rabbits (e.g. straw intakes from 43 to only 3 g day^{-1}) without any apparent effects on wool production.

16.2.1 Feed restriction

Preliminary studies showed that feed restriction decreases wool production by 14.7% (Rougeot and Thébault, 1977) or 9.2% (Schlolaut and Lange, 1983). In both of these studies, however, feed restriction was severe and no account was taken of the variability in hair growth rate between harvests. More recently, Lebas and Thébault (1988) have shown that feed intake can be reduced by 61% in winter

Table 16.6. Relative effect of dietary supplementation with vitamin A or β-carotene on the quantity of hair produced by a surface of 14 cm^2 of skin shaved once a week during 8 consecutive weeks (from Kovácsné-Virányi, 1990). Value for the control, 1.000 = 1.17 g.

	Control (5000 IU vitamin A kg^{-1})	Vitamin A + 15.000 IU kg^{-1}	β-Carotene + 45 μg kg^{-1}
No. of rabbits	5	7	7
Hair production	1.000^{a}	1.132^{b}	1.055^{ab}

a ≠ b (P = 0.05).

and 26% in summer with an adapted feed restriction (1200 g week^{-1}) during the first month following harvest without any adverse effects on wool production (Fig. 16.2 and Table 16.7).

However, the Angora rabbit seems unable to regulate daily intake and some does are able to consume >400 g day^{-1} and exceptionally 500 g day^{-1} during the first 2 weeks following harvest. This can cause nutritional disorders (e.g. enterotoxaemia), which occur when pellets are fed *ad libitum*.

A restricted feeding regime, as described in the next section, has been developed using the pattern (Fig. 16.1) of weekly hair production over the 3 months of hair growth between harvests (Rougeot and Thébault, 1984). This has now been adopted in commercial practice.

- First month: 1200 g per animal week^{-1}.
- Second month: 1100 g per animal week^{-1}.
- Third month: 1000 g per animal week^{-1}.

The weekly ration must be distributed equally over 6 days a week as Angora rabbits are not able to self-regulate their feed intake.

16.2.2 One fasting day a week

A fasting day is essential when fibres are long or when hair losses are observed. Angora rabbits, like most mammals, lick their fleece when grooming. Hair is swallowed, representing 0.3–0.4 g day^{-1} during the last month between harvests (Charlet-Lery *et al.*, 1985). As the rabbit is unable to vomit, long hair mixed with feed material is retained in the

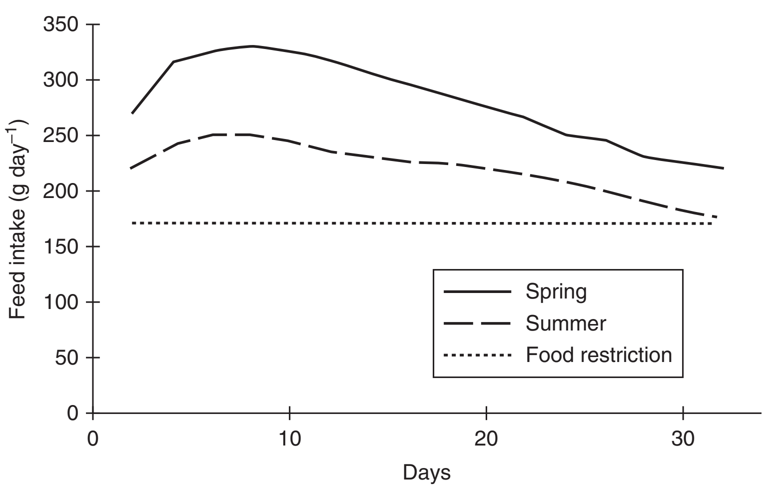

Fig. 16.2. Change in daily feed intake of Angora rabbits restricted-fed or fed *ad libitum* during the 5 weeks following de-fleecing, in spring and summer (from Lebas and Thébault, 1988).

Table 16.7. Live weight (g) 5 weeks after the last de-fleecing and wool production at the next harvest of Angora rabbits (mean ± standard error of the mean) with or without feed restriction during two different seasons (from Lebas and Thébault, 1988).

		Ad libitum	Feed restriction	Statistical probability
Live weight (g)	Spring	4457 ± 106	4244 ± 47	0.014
	Summer	4234 ± 73	4127 ± 44	0.08
Wool production (g)	Spring	258.3 ± 8.7	259.8 ± 6.6	NS
	Summer	235.9 ± 6.4	246.5 ± 8.7	NS

NS, not significant.

stomach. It rapidly forms a stomach hair ball (trichobezoar), which blocks the pylorus and prevents gastric emptying. The animal stops feeding and will die. Plitt-Hardy and Dolnick (1948) described this phenomenon and Rougeot and Thébault (1977) observed the death of five out of 11 females fed a pelleted diet *ad libitum*. Autopsy revealed the presence of a trichobezoar in the stomach of each animal. Feed restriction and fasting on 1 day week^{-1}, when only straw or bulky forage is available, will facilitate voiding of ingested hair in hard faeces. On the day following the fast, hard faecal pellets connected to each other are often observed.

16.3 Conclusions

As Angora rabbits are housed in individual cages, it is very easy to control their feeding regime. When traditional raw feeds such as hay and cereals are used, no specific nutritional problems occur. Angora rabbits that have been selected for wool production cannot, however, achieve their genetic potential on such a regime. In addition, when hay is floor-fed inside the cage or even distributed in a feeding rack, vegetable matter tends to 'contaminate' the fleece, which drastically reduces its commercial value. For these reasons, most commercial Angora rabbits are fed a specific pelleted balanced diet. Pelleted concentrates have the advantage that nutritional characteristics are precise and constant, feed storage is minimum and labour costs for feeding are reduced. Some precautions are necessary when using a complete pelleted diet. Finally, water must be supplied *ad libitum* using an automatic watering trough. To avoid wastage and nutritional disorders, a restricted feeding regime adapted to variations in both feed requirements and hair growth should be adopted. Fasting once a week will avoid the formation of a stomach hair ball, which, should it occur, is invariably fatal.

References

Charlet-Lery, G., Fiszlewicz, M., Morel, M.T., Rougeot, J. and Thébault, R.G. (1985) Variations annuelles de l'état nutritionel de la lapine angora durant les pousses saisonnières des poils. *Annales de Zootechnie* 34, 447–462.

Henics, Z., Tossenberger, J. and Gombos, S. (1989) [Digestion, and wool production of Angora rabbits depending on the energy, protein and methionine content of feeds]. *1. Nyultenyéstési Tudományos Nap, Kaposvar (Hungary) 26 Május 1989*, pp. 80–99.

Henics, Z., Biróné Németh, E., Szendrö, Z. and Tossenberger, J. (1990) [Effect of diet's sulphur amino acid content on wool production of males, female and castrated males Angora rabbits]. *2. Nyultenyéstési Tudományos Nap, Kaposvar (Hungary) 16 Május 1990*, pp. 68–74.

Kovácsné-Virányi, A. (1990) [Effect of vitamin A deficiency on the wool production of Angora rabbit]. *2. Nyultenyéstési Tudományos Nap, Kaposvar (Hungary) 16 Május 1990*, pp. 91–96.

Lebas, F. and Thébault, R.G. (1988) Influence of *ad libitum* feeding 5 weeks after plucking on wool production in Angora rabbits. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 3. Sandor Holdas, Hercegalom, Hungary, pp. 249–253.

Lebas, F. and Thébault, R.G. (1990) Besoins alimentaires en acides aminés soufrés chez le lapin Angora. In: *5èmes Journées de la Recherche Cunicole, Paris*. Communication no. 49, ITAVI, Paris, France.

Liu, S.M., Zang, L., Chang, C., Pen, D.H., Xu, Z., Wang, Y.Z., Chen, Q., Cao, W.J. and Yuan, D.Z. (1992) The requirements of digestible energy, crude protein, and amino acids for Angora rabbits. *Journal of Applied Rabbit Research* 15, 1615–1622.

Plitt-Hardy, T.M. and Dolnick, E.H. (1948) Angora rabbit wool production. US Department of Agriculture Circular no. 785, USDA, Washington, DC, USA, pp. 1–22.

Rougeot, J. and Thébault, R.G. (1977) Formation de trichobezoards chez le lapin Angora nourri *ad libitum* avec un aliment aggloméré. *Revue Médecine Vétérinaire* 153, 655–660.

Rougeot, J. and Thébault, R.G. (1984) *Le Lapin Angora, sa Toison, son Élevage*. Le Point Vétérinaire, Maisons Alfort, France.

Rougeot, J., Colin, M. and Thébault, R.G. (1980) Définitions de conditions expérimentales pour l'étude des besoins nutritionnels du lapin: nature de la litière et présentation du lest alimentaire. *Annales de Zootechnie* 29, 1–11.

Schlolaut, W. (1985) *Abrégé de Production Cunicole*. Deutsche GTZ GmbH, Eschborn, Germany, pp. 215–216.

Schlolaut, W. and Lange, K. (1983) Untersuchungen über die beeinflussung quantitiver merkmale der wolleistung beim Angorakanichen durch geschlecht, alter, fütterungstechnik und methioningehalt des futters. *Züchtungskunde* 55, 69–84.

Thébault, R.G. and Allain, D. (1995) Dietary requirements and feeding management of Angora rabbits. In: Laker, J.P. and Russel, A.J.F. (eds) *The Nutrition and Grazing Ecology of Speciality Fibre Producing Animals*. Occasional Publication No. 3, European Fine Fibre Network, Aachen, Germany, pp. 71–84.

Tossenberger, J. and Henics, Z. (1988) Effect of energy, protein and sulfur amino acids on wool production of Angora rabbits. In: *Proceedings of the 4th World Rabbit Congress, Budapest*, Vol. 3. Sandor Holdas, Hercegalom, Hungary, pp. 274–280.

Vermorel, M., Vernet, J. and Thébault, R.G. (1988) Thermoregulation of Angora rabbit after plucking. 2. Heat loss reduction and rewarming of hypothermic rabbits. *Journal of Animal Physiology and Animal Nutrition* 60, 219–228.

17 Pet Rabbit Feeding and Nutrition

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17.1 Introduction

The predecessor of the modern rabbit – whose Latin name, *Oryctolagus cuniculus*, literally means ‘hare-like digger of underground passages’ – is known to have originated, based on Upper Pleistocene fossil records, in the Iberian peninsula. It appears to have been confined to Spain for some time after the last Ice Age. Interestingly, the rabbit was a symbol of fertility in ancient Egypt. The rabbit/hare hieroglyph has been translated as the verb ‘to be’ and the Pharoah Unas (2375–2345 BC) is named using this hieroglyph. Stone Age cave drawings clearly indicate its presence and Phoenician traders reported large numbers of wild rabbits around 1100 BC. The spread of rabbits out of Spain during this period was probably hindered by the Pyrenees and the dense forestation of the rest of Europe. With the gradual deforestation of Europe the rabbit spread and diversified into Algeria, Morocco, the Azores, the Mediterranean and Russia, and then to Northern Europe. This initial spread was devoid of any true domestication.

Partial domestication was undertaken by the Romans around AD 1, with rabbits breeding in the wild and only being caught for fattening purposes. This partial domestication extended across the Mediterranean, with rabbit becoming a regular meat by AD 600, which is relatively recent compared with other species (Zeuner, 1963). The evidence for this timing is further supported by the depiction of the rabbit on the coins of the period AD 120–130 and in Roman art.

The first housed breeding and controlled feeding of rabbits appears to have been in French monasteries, in which rabbits were kept in large leporaria, between the 6th and 10th centuries. Subsequently, rabbits became more commonplace such that by the 16th century selection for tameness, size and variety was clearly evident. The rabbit does not feature in the art of the domestic animal until the appearance of a white rabbit in 1530 in the painting *Madonna of the Rabbit* by Titian.

While there are some records and evidence for the development of the domestic rabbit in mainland Europe, no such material as to the existence of the rabbit in Britain appears to be available until after the Norman Conquest. The rabbit was commonplace in Britain by the 14th century and captive by the 17th century, but only truly domesticated by the end of the 18th century. It was not, however, until the mid-19th century that the pet and show rabbit began to appear (Sandford, 1986). As the rabbit is very adaptable and is today distributed over the five continents, it is surprising that it was so late in becoming a domesticated species and is only now a significant pet.

Although it is extensively farmed in China, Italy, France and Spain (Costachescu and Hoha, 2006), the rabbit has become an increasingly popular and important pet in certain areas, in particular Germany, the UK, the USA and Canada (Santomá *et al.*, 1989).

The number of pet rabbits in the UK has steadily risen over the last few years, with current estimates in the range of 1.0–1.4 million, making the rabbit the third most popular pet (excluding fish) and present in almost 0.7 million pet-owning households (PFMA, 2009). The US pet rabbit population has similarly risen, with estimates in the region of 5 million rabbits in around 2.2 million households in 2002 (Grannis, 2002) and 6.17 million rabbits in 2 million households in 2007 (AVMA, 2007).

The pet rabbit has a life expectancy of 8–12 years. It is a small and pleasant animal to handle, and can be kept as easily by the elderly and handicapped as by the child or fit adult. It has always been regarded as the 'classic' pet for children, but, with the advent of the house rabbit, has more recently begun to fulfil a similar position in the eyes of the pet-keeping public as the cosseted cat. The rabbit's place as a significant pet in small-animal veterinary practice is well established (Hartmann *et al.*, 1994; Malley, 1994, 1995, 1996), even to the extent of there being a lecturer's post dedicated to rabbit medicine and surgery at the University of Edinburgh Veterinary School. More than 50 breeds of rabbits are now recognized, ranging in weight from 1 to >10 kg. Rabbit fanciers divide them into two categories referred to as 'fur' and 'fancy', with the smaller breeds (e.g. Netherland Dwarf and Mini-lop) dominating as the most popular pets.

Much of the detailed information on the nutrient requirements and feeding of the rabbit are derived from studies and publications that are concerned with the rabbit as a research animal (NRC, 1977) or farmed for meat or fur (Lebas, 1980, 1987; Schlolaut, 1982). There are, however, some studies specific to the pet animal (Hartmann *et al.*, 1994; Schwabe, 1995; Haffar, 1996) as well as some useful veterinary and specific texts (Sandford, 1996; Brooks, 2004). In assessing the nutrient requirements of the pet rabbit, reference to the basic principles of these and similar published information has therefore been made. Appropriate adaptations of values for nutrient requirements have been determined using the more recent articles as a check for the feeding of the smaller rabbit, at maintenance, as a pet.

Considerations have also been given to the particular requirements that arise from the fact that the owner of the pet rabbit may wish to supplement the commercial diet with fresh material and the problems that may subsequently arise through mal-feeding practices. It is strongly recommended that when designing diets for pet rabbits or considering the pet rabbit's specific needs, consideration be given to the welfare of the rabbit. It is therefore desirable for manufacturers to supply additional on-pack or supportive information to assist the pet owner in maintaining animal health and well-being.

17.2 Feeding Management

17.2.1 General considerations

It must be emphasized that keeping of rabbits will not be successful unless attention to detail is paid in terms of housing, the behaviour of the rabbit and the feed quality and balance of the diet, as well as the most suitable feeding practices for the rabbit. The rabbit is a social creature and requires companionship, ideally from another rabbit, and exercise on a daily basis. The rabbit also benefits from mental stimulus; if this is unavailable from other rabbits or human interactions then it should be provided with appropriate 'toys'. Studies using Norway spruce sticks (Jordan *et al.*, 2008) have, for example, been shown to provide behavioural enrichment.

The pet rabbit should be given adequate opportunity to feed in a quiet place. Feed should be provided on a regular basis (e.g. in the early morning and evening to reflect the rabbit's crepuscular behaviour) and with appropriate recognition of individual habits and changes to the routine (e.g. in the summer rabbits tend to eat more at night, when it is cooler). While rabbits dislike dust in their food, it is important to ensure that the rabbit consumes all of the components of a coarse or flake-mix proprietary product to ensure a balanced diet. Removal of any dusty or fouled portion of the diet is important, but must not be such that the rabbit continually leaves the same component each day. It is sometimes suggested that the rabbit should have non-forage components of food withheld once each week to ensure that the digestive system is clear of furballs and to encourage the consumption of essential roughage, usually in the form of hay.

Rabbits like variety in their diet and mixtures are often considered more palatable than single-component diets. However, the importance of avoiding selective feeding and the consequential provision of an imbalanced diet and resultant health problems must be emphasized. For simplicity, and with modern production processes, palatability can be maintained with a single pelleted complete compound diet to which the only additional provision required is hay and fresh drinking water. With the exception of long fibre, it is not wise to dilute such a complete compound feed. Changing the diet to provide variety may also inadvertently result in obesity or digestive upsets. This comes about as a result of the rabbit consuming the diet for pleasure rather than nutritional needs, with an excess consumption of compound feed compared to forage. Sudden or inappropriate dietary changes often cause diarrhoea, which if inappropriately managed may lead to dehydration and death or predispose the rabbit to fly-strike.

With home-made diets, variety often consciously or unconsciously offsets inadequacies in one or more of the foods. It is important to offer only clean and dust-free food. Where a cereal or bran that is prone to dustiness is included, this can be moistened with a little warm water to a crumbly texture to encourage intake. All foods must be free from mould and frost and green foods must be fresh. Detailed considerations of each foodstuff are given later in this chapter. The rabbit will selectively take concentrates if the palatability of roughage is variable (Van Soest, 1982); however, this behaviour will readily result in a scour from the consumption of too much protein or starch relative to hay. A simple yet effective treatment for this is to remove the feed and provide warm water and hay. A well-fed rabbit masticates its food extensively, whereas this practice does not occur to any great extent when the rabbit is hungry. This forms a good practical guide to regulating food quantities.

Good-quality hay should form the basis of any rabbit feeding programme and is essential not only for appropriate nutrition (Meredith, 2000), but also for maintaining behavioural norms (Mulder *et al.*, 1992) and good physiology (e.g. tooth wear).

17.2.2 Feeding guide

It is generally agreed that the energy requirement of the pet rabbit can be estimated with the following equation:

Maintenance energy in Kcal ME = body weight$^{0.75}$ $\times 100$

ME, metabolizable energy

Typically a multiplier of 1.35 is used for early gestation; of 2 for late gestation and growth; and 3 for lactation. However, considerable between-animal variation should be expected (Tobin, 1996).

A guide to typical daily feed intakes for adults at maintenance is around 0.030–0.035 of body weight.

Practically, adult animals at maintenance need to be controlled-fed, based initially on the ideal weight for the breed and then on body condition. A suitable body condition scoring method and advice on how to estimate the body condition score of the rabbit can be found at http://www.pfma.org.uk/pet-ownership/pet-size-o-meter.htm. Adjustments to feeding rates should be in the order of ±10% every 14 days. It is probably better to alter the amount of non-forage food as rabbits are efficient utilizers of diet and the obese animal may maintain weight even on high-fibre foods (as opposed to forage).

After weaning, reduced rations should be given for a few days followed by feeding to condition. Growing young rabbits should be fed to appetite until 10 weeks of age and then at maintenance amounts.

Responsible manufacturing would imply that suitable daily feeding guidance is provided on-pack. This should be based on the above equation for requirements and the appropriate energy value for the feed. Diets containing 10.5 MJ kg^{-1} are the norm and may be fed up to 25 g kg^{-1} body weight along with free-choice good-quality hay. However, due to variations between rabbits these values should only be used as a guide and owners are encouraged to feed to condition.

Fresh foods can be fed to appetite in adults, again making sure that excessive weight gain does not occur. A good approach is to provide only as much as will be consumed by the next meal. Hay or similar fresh long fibre must be supplied on a free-choice basis. Roots, if fed, should be given as lumps rather than slices or small pieces. It is worth remembering that an average adult rabbit can eat an amount of root equivalent to the size of a tennis ball.

If feeding extensively on a home-grown diet, one meal of grains and one of greens or roots should be provided each day, with dust-free hay and water offered on a free-choice basis. It should be noted that 'green foods' and roots do not affect bulk and thus influence digesta transit in the intestine as hay (long fibre) does.

Any changes to the dietary composition should be made gradually, over a 7- to 10-day period. Sugary treats, bread and other table scraps are inappropriate feeds for rabbits.

17.2.3 Housing

Rabbits should be housed in hutches or pens sited in a quiet part of the garden or yard. Small wooden hutches are preferable, with some protection from the weather in winter. The housing should have a darkened area for sleep and an open area for exercise and feeding. The exercise area should be large enough for the rabbit to stretch out fully and run, as opposed to just hopping. Exercise is important for the overall physiological and psychological health of the rabbit. If the hutch is within an outbuilding then it is important to avoid drafts, yet to provide adequate ventilation and light.

The 'house rabbit' is now widely popular. Established in the USA in the 1980s, the loose-housed, house-trained indoor rabbit makes an affectionate and intelligent companion. Indeed, there is some evidence to suggest that because of the social nature of rabbits, the behaviour patterns of house rabbits are more natural than those kept in a hutch. In addition, such methods of keeping encourage exercise. Even with the house rabbit, however, penning may be beneficial on certain occasions.

17.2.4 Feeding equipment

Feed containers should be heavy in construction as rabbits are prone to throwing them about. Earthenware or galvanized steel, the latter hung on the door, seem to be the most effective. The best designs accommodate a turned-in lip to prevent the rabbit from scratching out the feed. Containers must be <75 mm deep or the rabbit will find access a problem. The inclusion of a fine wire sieve in the bottom to remove dust from feed pellets can be beneficial if feed replacement occurs only once daily.

Water is frequently provided via nipple or automatic drinkers in commercial situations and such arrangements are ideal. For the domestic situation, however, heavy earthenware pots or bottle drinkers are more practical. If a pot is provided within the cage or pen then precautions to prevent fouling by the rabbit are essential. A bottle drinker in a frame outside the pen and a small trough inside or a 6 mm diameter drinking tube overcome such problems with ease.

The provision of free-choice roughage in the form of quality meadow hay is essential. The best method of supply is in a hay rack, which not only saves space but also keeps the hay fresh and free from fouling. In pens with a wire mesh roof, the hay can be put on top of the pen and rabbits will happily pull it through.

17.3 Physiology/Anatomical Considerations

Within the 50 or more breeds of domestic rabbit there are four basic fur types: (i) normal with fur length 30 mm in length; (ii) rex 12 mm; (iii) satin; and (iv) the one wool-producing Angora, which has 120-mm-long, fine fur. Typical pet breeds weigh in the range of 1–5 kg, although larger breeds >10 kg as mature adults are occasionally selected (Sandford, 1986).

The rabbit is a true non-ruminant herbivore grazer. Its dentition differs from that of the rodent, with which it is sometimes mistakenly categorized, having two pairs of upper incisor teeth whereas rats and mice have only one. The upper and lower teeth, which continually grow, meet and grind each other down with use. Herbivores by their nature consume high-fibre plant-based diets, which present their own unique problems in terms of digestive efficiency.

Herbivores in general have developed in different ways to produce a digestive reservoir, which permits an increase in the efficiency of utilization of their fibrous diets (Cheeke, 1988). The rabbit has a very large non-compartmentalized and non-distensible stomach. In the adult this has a very low pH <2.0, exerting an antimicrobial effect. The weanling rabbit does not exhibit this low stomach pH and is thus more prone to bacterial infestation and upset (Meredith, 2008).

The stomach, which empties into a relatively narrow-diameter small intestine, comprises about 15% of the volume of the gastrointestinal tract. It functions largely as a food reservoir and is usually never empty. The cardiac sphincter is well developed and anatomically arranged to prevent vomition. The pylorus is very muscular.

The rabbit also possesses a well-developed, coiled, thin-walled caecum. This is the largest organ of the gastrointestinal tract, having ten times the capacity of the stomach, and provides substantial microbial digestion. The colon is characterized by sacculations (haustra) and bands. The horse utilizes the caecum and colon for insoluble fibre (lignocellulose) fermentation. The rabbit, however, rapidly eliminates insoluble fibre from the gut, using this fraction of the diet primarily as a motility modifier rather than a nutrient.

Relying on the bulk in the stomach to effect intestinal passage of digesta, this high voluntary feed intake, some four times higher *pro rata* than that of a 250 kg steer (Santomá *et al.*, 1989) is associated with a low gut retention time of 17.1 hours in the rabbit compared with, for example, cattle at 68.8 hours. The high voluntary feed intake, together with the re-utilization of gut content by reingestion of caecal material (referred to as caecotrophy), supports the rabbit's high nutrient requirement per unit of body weight and improves feed utilization. This approach allows the rabbit to consume a large amount of roughage while avoiding being weighed down by the storage of bulk fibre, which is a good approach considering the small size and high metabolic rate of the rabbit. The rabbit exhibits frequent feeding, with estimates ranging from 30 times per day of 2–8 g intake over 4–6 min per occasion (Prud'hon *et al.*, 1975), up to 4–6 hours in total.

The nature of the dietary fibre component, pectic constituent concentration, degree of lignification of neutral detergent fibre (NDF) and particle size best characterize the influence of the source of fibre (García *et al.*, 2000) on the rabbit.

Designing a safe and efficacious diet for the rabbit must thus give considerable consideration to the fibre profile of the daily ration. Unfortunately, 'fibre' measurement is still a developing science and many measurement methods that would be helpful in rabbit diet design are not routinely available in commercial laboratories. It is therefore beholden upon the nutritionist to factor a margin of safety into the daily diet and ration design. Thus, while it would appear to be entirely possible to produce a complete processed diet for pet rabbits, because of the importance of fibre and forage to the health (gut, teeth) and well-being (behaviour) of the rabbit, even when a complete diet is appropriately designed and subsequently stated on-pack, it is always considered good practice to encourage the pet rabbit owner to offer good quality long fibre in the form of hay and to feed to condition.

A note on-pack to this effect could be considered as critical as the one stating that the rabbit must always have access to fresh, clean drinking water.

Diseases of the rabbit linked to inappropriate dietary fibre intake are gastric trichobezoars, chronic soft stools (sticky-bottom syndrome), diarrhoea, gut motility stasis, obesity and dental problems.

17.3.1 Caecotrophy

At 3–8 h after feeding the rabbit produces a soft, mucus-coated faecal pellet that is swallowed whole, without chewing, directly from the anus. The arrival of the pellet at the anus induces a reflex action to consume the pellet. The normal faecal pellet is harder and is passed both during the day and night. A 2.5–3.0 kg typically rabbit passes 150 faecal pellets day^{-1}.

The soft faecal pellet results from the separation of digesta on the basis of solubility and particle size in the hind gut. Peristaltic action removes larger particles (>0.5 mm) of predominately ligno-cellulose through to the colon, which are subsequently excreted as hard pellets. Antiperistalsis moves the smaller particles (0.1–0.2 mm) and soluble components into the caecum, where fermentation occurs (Bjornhag, 1987). Once consumed soft faeces remain in the stomach of the rabbit for 6–8 h, where they are protected from digestive attack by the mucosal cover. Microorganisms within the soft faecal pellets continue fermentation with the production of lactic acid. These pellets provide microbial protein, which accounts for between 0.15 and 0.25 of total amino acid and between 0.09 and 0.15 of the digestible energy (DE) requirement of the rabbit (Lebas, 1989). In addition, the pellets provide all of the B-group and K vitamins and a certain amount of volatile fatty acids (VFAs).

17.3.2 Digestive efficiency

Rabbits digest fibre poorly (mean coefficient of total tract digestibility of lignin 0.10–0.15, cellulose 0.15–0.18, hemicellulose 0.25–0.33; Gidenne, 2003) because of selective separation and rapid excretion. However, rabbits require generous amounts of fibre to ensure intestinal motility and minimize disease. In crude fibre (CF) terms, a diet with <140 g kg^{-1} CF will almost always result in digestive upsets, while a diet with >250 g kg^{-1} CF may result in increased incidences of caecal impaction and mucoid enteritis (Fraser, 1991).

Fibre influences the digestibility of the diet and alters the analysis of caecal contents. A diet devoid of fibre results in a coefficient of apparent digestibility of organic matter of 0.90; this declines in a linear fashion to 0.40 when the diet contains 350 g kg^{-1} CF. Increasing the CF of the diet increases the CF of the caecal contents and decreases the protein content (Carabaño *et al.*, 1988). The enzyme profile of the digestive system of the rabbit is similar to that of other non-ruminants and thus the digestive efficiency of non-cell-wall constituents is comparable.

17.4 Raw Materials

17.4.1 General considerations

As with any pet animal, the key to feeding lies in the provision of a well-balanced diet. The range of feeds upon which the rabbit can survive is wide and varied. This means that the pet rabbit owner can tailor the feeding from purchased compounds to entirely home-made diets from the garden to meet the budget and individual circumstances. The creation of an entirely home-grown complete diet does, however, demand a knowledge of both the requirements of the rabbit and the nutrient content, risks and benefits of the plants in the garden. Conversely, the many commercially available compound feeds now offer a simple and convenient alternative package, with only the additional requirement of water to provide complete and balanced nutrition. Such diets have steadily increased in presence and popularity since their introduction in the 1950s. Some compound diets will require the addition of hay to supply a complete diet. In general, the recommendation that hay should be supplied on a free-choice basis as a rule of good husbandry of the pet rabbit should be included in the feeding instructions.

Information on feed analyses is found in Aitken and King-Wilson (1962), Tobin (1996) and Wiseman (1987).

Table 17.1 gives a typical schedule for the feeding of 'classic' home-made diets based on cereals, bran and forages, fed fresh in the summer and dried in the winter and supplemented with hay, beet and carrots.

17.4.2 Raw material groups

The raw materials used to feed the rabbit can be grouped into succulents (greens and roots), roughages, concentrates (grains and proteins) and compounds. The last is not strictly a raw material group, but general considerations will be covered.

Succulents

If feeding roots and greens, it should be remembered that their nutritional value will vary with season, age at harvest, soil type, weather and storage, and that this will inevitably affect the nutrient supply to the rabbit and possibly the overall balance of the diet. There are many green foods suitable for the pet rabbit and a few general principles apply to them all:

- Greens and roots should ideally be fed fresh.
- If they have to be stored prior to feeding then they should not be left in a heap as they quickly ferment and this can be fatal to young rabbits.
- Introduce greens and roots gradually. Any new food should be introduced in this way, not only to minimize digestive upsets but also to avoid excessive feed selection and therefore perceived palatability. A sudden change of diet may lead to inappetence and the animal may starve itself rather than eat the new diet. Force-feeding by starvation to achieve diet acceptance is not effective in the rabbit.
- Fresh cut grass is favoured. However, lawn mowings should not be used as these will begin to inappropriately ferment almost before the rabbit can consume them.
- Wilted greens are acceptable, provided they are not yellow in colour or mouldy in any way.
- Plants to avoid include those with bulbous roots, *Lobelia*, lupins, potato leaves and tomato haulm.
- Rabbits will *not* in themselves reject poisonous plants. A further peculiarity of the rabbit is that it is unable to vomit and therefore cannot expel unwanted material or poisons if consumed.

GREENS. Kale chicory, kohlrabi, carrot leaves, endive, spring greens, spinach and watercress are very acceptable to rabbits, although the leaves of the cabbage (*Brassica*) family should only be fed in small quantities to minimize the intake of the glucosinolate goitrogens, which impair the uptake of iodine by the thyroid. Leafy vegetables are rich sources of minerals and vitamins.

Hedge or cow parsley (*Anthriscus sylvestris*), dandelion (*Taraxacum officinale*), coltsfoot (*Tussilago farfara*), sow thistle (*Sonchus*), plantain (*Plantago*) and knapweed (*Centaurea* species, especially *C. nigra*) are similarly useful succulents. However, if fed to excess the dandelion may result in a condition known as 'red-water', which is a kidney complaint.

Lettuce should be fed in strict moderation as its milky juice, lactucarium, is soporific and similar in action to opium. Wild varieties are somewhat worse than cultivated in their effects.

Rhubarb, clover and lucerne may be fed in small amounts. All are prone to inducing bloat.

Elder keeps flies away, but is not recommended as a food.

Some plants are thought to act medicinally. For example, shepherd's purse (a white-flowered, hairy cornfield weed with a triangle

Table 17.1. An illustration of the availability of succulent foods each month to the pet rabbit feeder throughout the year (adapted from data in Sandford, 1973).

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Carrots	Carrots	–	–	–	Chicory	Chicory	Chicory	Chicory	–	Carrots	Carrots
Cabbage and kale	Kale (thousand head)	–	–	Oats, clover and lucerne	Oats, clover and lucerne	Oats, clover and lucerne	Oats, clover and lucerne	–	Cabbage and kale (marrow stem)	Cabbage and kale	Cabbage and kale
Swede	Swede	–	–	–	–	–	Green maize	Green maize	Swede	Swede	Swede
Mangolds	Mangolds	Mangolds	Mangolds	–	–	–	–	Kohlrabi	Kohlrabi	Kohlrabi	Kohlrabi
Fodder and sugarbeet	Fodder and sugarbeet	Fodder and sugarbeet	–	–	–	–	–	–	Fodder and sugarbeet	Fodder and sugarbeet	Fodder and sugarbeet
Cereals	Cereals	Cereals	Cereals	Cereals	Cereals	Cereals	Cereals	Cereals	Cereals	Cereals	Cereals
Hay	Hay	Hay	Hay	Hay	Hay	Hay	Hay	Hay	Hay	Hay	Hay

of cordate pods) and blackberry, raspberry and strawberry leaves are all considered beneficial in cases of scouring in the rabbit.

The most useful and successful greenfeed is grass and the products of grass (hay and dried grass pellets). Traditionally, young grass has been used to improve growth and the general plane of nutrition. Similarly, lucerne (alfalfa), a modest protein source that is rich in fibre, calcium, carotene and vitamin E, is a common material in rabbit diets.

ROOTS. Carrots, swedes, turnips, parsnips and fresh sugarbeet (dried beet, although low in sugar, will require soaking before use) are all useful feeds. They are high in moisture (750–950 g kg^{-1}) and low in protein, starch and fibre and a poor source of vitamins. Most of the dry matter (DM) fraction is in the form of sugars; thus, they should be limited to a small proportion of the total feed. They should never be fed frozen or thawed, mouldy or rotten.

Potatoes are somewhat higher in DM than other roots and are primarily a starch source; cooking to a mash has the advantage of reducing the non-protein nitrogen alkaloid (solanidine) and substantially reduces the risk of gastrointestinal disturbances.

The Jerusalem artichoke is a good food all year round, with the leaves and stems as a summer food and the roots in the winter. The plant is related to the sunflower and its roots are similar in nutrient content to potatoes. One of the advantages is that the root is impervious to frost and can therefore be left in the ground all year round. When harvested it must be washed prior to feeding.

Beetroot is considered to be a good appetite stimulant.

Roughages

Good hay – as explained in the previous section – is vital, with meadow hay before flowering being the best. True clover hay may be too coarse and of doubtful value, although a proportion of clover in the meadow is considered beneficial. Good hay smells sweet with no sense of mustiness and a faint tang of tobacco. Hay should be stored prior to use; new-season hay (i.e. cut within the last 3–4 months) will often result in scouring in rabbits. Lucerne hay and pea haulm are also currently popular and are favoured by rabbits; however, lucerne is higher than grass hay in protein and calcium and, while appropriate during the growth period, should be limited or avoided in the adult. Hay from well-grown stinging nettles and dried carefully makes a pleasant change and is protein-rich, though rabbits will not eat fresh nettles (Netherway, 1979).

Straw, although traditionally used for bedding, is consumed by rabbits and is a useful ingredient in mixed feeds and compound diets in the form of alkali-treated (sodium hydroxide) straw pellets.

Concentrates

GRAINS. Oats are generally considered to be the best grain. The order of preference is oats, barley, maize and then wheat. Little or no information on rye, triticale and others is found in the literature for pet rabbits. Whole plump oats are preferable to crushed oats as, with the latter, the rabbit will pick out the kernel and leave husk. Rolling the oat so as to just crack the skin is an alternative preparation for use in flake mixes. Wheat has a tendency to be pasty and is not generally fed alone. Wheat bran is a very popular and useful raw material and was traditionally fed in quantity to domestic rabbits in the early part of the last century (Netherway, 1979). It was the only foodstuff of all rationed materials to be allowed to the rabbit breeder during post Second World War rationing in the UK (Sandford, 1986). Low-starch wheat feed is a similarly useful material. Maize, although excellent, may be relatively expensive compared to other cereals in certain countries.

Cereals in general are good energy sources, typically containing 80–120 g kg^{-1} protein. The primary deficiencies in cereals are in lysine, vitamins A and D and calcium. The phosphorus content, although frequently twice the level of calcium, is of limited availability as approximately 0.5 is in the phytate form.

PROTEINS. Both beans and peas are useful, with peas being preferred. Only old-season beans should be used as new-season crops tend to heat when ground. Both are rich in protein (200 g kg^{-1}) with a high lysine content which complements the cereals, although they are similar to cereals in terms of being low in calcium and high in phosphorus.

Many legumes contain antinutritional factors such as trypsin inhibitors, tannins and phytohaemagglutinins. While heat treatment can reduce these factors, the inclusion of such materials in the diet should be limited. Beans of the genus *Phaseolus* (kidney, navy and butter beans) are toxic when fed raw and should not be used.

For commercial feeds both the Mediterranean carob and African locust bean are very palatable and popular favourites. Sunflower seeds are also popular and visually attractive in flake mixes. Oil cakes (peanut, sunflower, sesame and soya), usually in decorticated form, can form an important part of a mixed feed, but should never be fed alone. Linseed, which has traditionally been used in a mash as a laxative and to achieve a 'bloom' on the coat, is now recognized as an important source of n-3 fatty acids as well as protein.

Animal proteins are unnecessary for the pet rabbit. Although they have been considered important in farmed situations, they are now illegal for use in rabbit diets throughout the European Union.

Compounds

The design of a pelleted diet allows for a wide range of raw materials to be selected for their nutritional qualities rather than visual appeal. This also means that less reliance needs to be placed on any single ingredient in the diet, producing a more consistent approach to meeting the nutrient requirements of the rabbit. Furthermore, the inclusion of supplements to balance the diet together with vitamins and trace minerals in a palatable form is easy. Diets do not necessarily need to be complicated in their raw material composition, as confirmed by Cheeke (1988) who used a combination of only five ingredients, although relying heavily on lucerne, oats and a mineral and vitamin supplement. There are, however, some general restrictions on the inclusion of certain raw materials that should be taken into account in the design of the complete compound feed (Table 17.2).

Pellet hardness and the homogeneity of the mix are critical to the construction of the diet. Dusty pellets are poorly accepted by the rabbit and a non-uniform mix of components will exacerbate selective feeding and result in dietary imbalance. A wide range of technologies is employed in the production of prepared compound feeds for rabbits, which include conventional pelleted diets and mixes combining either steamed or micronized flakes of cereal, pulses and legumes, together with pellets or extrusions. Many diets are now solely based on either soft or crisp extruded complete diets. All of these formats are proven to be acceptable to the rabbit. Flaked mixes can be coated with oil, molasses or glucose syrup to aid palatability and reduce dust. The amounts of these coatings do not, if applied appropriately to a well-designed diet, provide either excess calories in the case of fat or excess total sugar (>6%) in the case of molasses or glucose syrups. The basic techniques for the processing of such diets have been reviewed by Tobin (1996).

In addition to conventional ingredients, purified fibre sources are now available for compound feeds. These include refined lignins, celluloses and materials with prebiotic properties such as mannans and fructose oligosaccharides.

Compound feeds that are designed for *ad-libitum* feeding should contain sufficient energy (>9.3 MJ DE kg^{-1}) to allow the rabbit to regulate its intake based on energy consumption. Very high or very low (<9.0 MJ DE kg^{-1}) energy diets require a twice-daily restricted feeding regime, which will be explained in the feeding instructions. Restricted feeding can be used to reduce growth rates below the theoretical maximum genetic potential by up to one-third (Schultz *et al.*, 1988). This may be desirable in pets, where growth rate is relatively unimportant.

Table 17.2. A guide to typical raw material constraints in compounds for pet rabbits (based on data from Santomá *et al.*, 1989; Maertens, 1992; and industry experience).

Ingredient	Suggested maximum inclusion (g kg^{-1})	Comments
Barley	300	–
Wheat	250	All wheat products 300 g kg^{-1}
Oats	350	May be lower for processing reasons
Maize	250	–
Wheat feed	300	All wheat products 300 g kg^{-1}
Bran	250	May be lower for processing reasons
Oat feed	250	May be lower for processing reasons
Dried distillers' grains	100	Some sources may contain high copper levels, thus restricting use to 50 g kg^{-1}
Peas	100	50 g kg^{-1} more common
Field beans	50	Old crop to avoid heating on grinding
Sunflower extractions	1000	Commercially, a 300 g kg^{-1} limit is more practical
Soya (dehulled)	200	Some believe this should have no upper limit
Full-fat soya	250	Typically no more than 125 g kg^{-1} is used
Citrus pulp	75	–
Grass nuts	1000	No limit on grass inclusion, either as dried grass or hay
Lucerne	300	Ensiled lucerne may result in decreased food intake
Sugarbeet	200	May consider lower value if molasses is included in the process
Soya hulls	250	–
Molasses	50	Consider contribution from sugarbeet
Wheat straw	150	Either plain or sodium hydroxide-treated. Up to 250 g kg^{-1} has been used successfully
Maize gluten feed	200	Maize gluten-60 to no upper limit
Locust beans	1000	Carob has similar inclusions
Grape seed	100	May depress feed intake at higher inclusion rates

Pelleted feeds are best produced to a finished size of 3–4 mm in diameter by 10 mm in length. It is important not to exceed 5 mm in diameter as this has been shown to increase wastage of the diet, while a pellet that is much shorter than 10 mm is not well accepted by the rabbit (Lebas, 1987). It is possible to feed compounds as meals, providing the grist is large enough and product essentially dust-free. However, this imposes certain constraints on the method of water supply, which is likely to be a problem for a pet (Lebas, 1987).

17.4.3 Water

Water is perhaps the most often neglected raw material (or, perhaps more correctly, nutrient). A fresh supply of clean, high-quality drinking water should always available. The popular belief that rabbits that are fed fresh greens do not need water is not entirely without foundation (Schwabe, 1995), but should not be considered as a safe practice for the domestic pet rabbit. A loss of 0.10 body water results in death. In the summer, rabbits in a hutch may lose up to 28 g h^{-1} in direct sunlight and 3.5 g h^{-1} in shade (Sandford, 1986). In winter, it is often wise to provide warm water to encourage intake and prevent digestive upsets.

The various estimates provided in the literature for the water intake of the rabbit vary widely. Netherway (1979) reported that a 4–5 kg rabbit on dry diet will drink 0.25 l 24 h^{-1}, whereas Sandford (1986) suggested a range of between 0.42 and 0.57 l 24 h^{-1} for rabbits on a dry diet. A similar doe with a litter of seven or eight kits at 3 weeks of age will drink 0.5–0.625 l 24 h^{-1}, while the litter on their own in a hutch will drink up to 1.5 l 24 h^{-1}. A good basis for maintenance would be 120 ml kg^{-1} body weight.

17.5 Nutrient Requirements

The suggested values presented below are based on a review of the literature, commercial diet design practice and general experience.

17.5.1 Protein

While there are a number of excellent reviews on the protein requirements of the rabbit (NRC, 1977; Lebas, 1989), scant consideration has been given to the effect of varying protein quality on the pet rabbit. The fact that commercial rabbits have successfully been reared on diets based on simple mixtures of plant proteins suggests that both protein quantity and quality are of little importance to the pet. There are, however, suggestions that excess dietary protein results in scours (NRC, 1966; Lebas, 1989).

The protein digestibility capacity of the rabbit is maximized early in life, at around 4 weeks of age (Lebas *et al.*, 1971). In the adult rabbit the ability to digest protein appears to be related to the dietary protein source, with protein concentrates, cereals and forages having a coefficient of apparent digestibility of 0.80, 0.67, 0.55, respectively. The fermentation within and contents of the caecum are critical to the supply of protein to the rabbit, as only 0.35 of total protein digestion occurs in the small intestine (Gidenne, 1988).

Ammonia is the main end product of nitrogen catabolism as well as the main nitrogenous source for the microbial population in the caecum. Ammonia is between 6.0 and 8.5 mg 100 ml^{-1} caecal contents with normal diets (Carabaño *et al.*, 1988). If caecal ammonia concentrations are limiting for microbial growth, as in very low-protein diets, then urea supplementation is effective; urea is metabolized and absorbed before reaching the caecum, resulting in increased urinary nitrogen (Santomá *et al.*, 1989). Consequently, as caecotrophy provides approximately 0.18 of the total protein intake for the rabbit, although varying with dietary constituents, excessively low-protein diets are to be avoided. Consideration of the protein quality of the rest of the diet is probably important even for the pet rabbit, but more in terms of health and tissue repair than for production considerations.

The contribution of the soft faeces increases protein digestibility in the rabbit by a factor between 1.05 and 1.2 (Fraga and de Blas, 1977). This may explain the better utilization of protein from forages in rabbits than in other non-ruminant species.

In terms of specific amino acids, lysine is considered the first limiting, followed by methionine (Santomá *et al.*, 1989). Colin (1975) suggested that cystine can meet the total methionine plus cystine requirement. Given the typical raw material base of the pet rabbit diet, a shortfall in sulphur amino acids is likely to be the first concern. The NRC (1977) and Lebas (1987) suggested that the rabbit requires ten essential amino acids, while Cheeke (1987) suggested that glycine should be also considered essential.

It is concluded from a review of the literature, based on adult maintenance recommendations, that a protein content for the domestic pet rabbit should be in the range 120–160 g kg^{-1}. The minimum amino acid constraints on this should be 5–6 g kg^{-1} for lysine and 5–7 g kg^{-1} for methionine plus cystine. Arginine should be in the region of 8–9 g kg^{-1}, but this is unlikely to require constraining in typical formulations and some have argued that the rabbit is capable of some arginine synthesis (Santomá *et al.*, 1989).

17.5.2 Fibre

Fibre is critical to the rabbit for health and well-being. Many chemical compounds are included in the broad definition of fibre.

Their relative proportions in the diet will affect the way in which the rabbit responds to the diet. The nature of the fibre is important both in terms of its chemical (soluble versus insoluble fibre) and physical (long unground fibre versus short ground fibre) characteristics. Reviews of the nature and characterization of fibre have been published (Van Soest and McQueen, 1973; Sunvold and Fahey, 1994; Fahey, 1995).

Cell wall constituents (indigestible fibre; lignocellulose, estimated by acid detergent fibre (ADF) analysis) are important in providing bulk to the diet. This is reflected by the high CF values of complete diets. Too little indigestible dietary fibre (IDF) increases mortality as a result of a malfunctioning of the digestive system and the proliferation of certain undesirable microflora and pathogenic bacteria (Gidenne, 1987), particularly in growing rabbits (Perez *et al.*, 1994; Chao and Li, 2007). Carabaño *et al.* (1988) demonstrated that this was the result of an increase in retention time with diets containing low levels of IDF by using a diet with <120 g CF kg^{-1}. Increases in the caecal content and a reduction in the rate of caecal content turnover was observed.

The presence of adequate IDF in the diet of the rabbit maintains intestinal transit times, whereas the presence of an appropriate amount of soluble fibre (hemicellulose, estimated by NDF plus pectins minus ADF) is important for satisfactory fermentation in the caecum. Gidenne *et al.* (1986) demonstrated that the production of a diet based on beet pulp, higher in soluble fibre, increased retention time in the rabbit when compared with an iso-CF diet based on lucerne. Grape residue has the reverse effect (Santomá *et al.*, 1989). Thus, for the complete compound rabbit diet, it is important to consider the relative proportions of digestible fibre and starch to IDF or long fibre when using beet, sunflower hulls, rice bran, olive pulp and grape cake. One approach is to include a minimum amount of conventional raw materials such as lucerne, straw and wheat bran and exclude the CF contribution from beet and other digestible sources, or fix a minimum IDF nutrient constraint.

Because fibre influences transit time, and transit time is rapid in the rabbit, the energy supply from CF is less than for other species. In conventional diets this may be 0.05 of the DE. Consequently fibre digestion is relatively poor in the rabbit, although coefficients of apparent digestibility in the region of 0.55–0.7 have been reported, which may be explained by the extent of the lignification of cell wall material in the diet (Lebas, 1989).

The degree of grinding of the fibre fraction of the diet, including the IDF fraction, is an important consideration in the design of the complete compound rabbit diet as it can exert similar physical effects on intestinal motility as those resulting from insufficient IDF (Pairet *et al.*, 1986; Bouyssou *et al.*, 1988). The finer the grinding, the greater the digesta retention time and caecal content (Lebas and Laplace, 1977; Candau *et al.*, 1986), with resulting digestive upsets. Screen sizes of 1 mm induce such digestive upsets, especially if the diet is marginal in IDF content (Pairet *et al.*, 1986; Auvergne *et al.*, 1987). However, diets ground on 2–7 mm screens do not produce such problems (Lebas *et al.*, 1986; Lebas and Franck, 1986). There is general agreement that screen sizes for complete compound feeds would be 2 mm.

The fine fibre material and soluble components of the diet that enter the caecum are fermented mainly to VFAs between 34.5 and 351 μmol g DM, predominantly acetic (0.73), butyric (0.17) and propionic (0.08) acids. Energy is the limiting factor for the caecal microbial population. The VFAs produced may contribute between 0.12 and 0.40 of the DE of the adult rabbit (Hoover and Heitman, 1972; Marty and Vernay, 1984). Thus, maintaining caecal fermentation and gut health is critical to diet design. To promote a beneficial microbial caecal population and enhance the production of butyrate with its associated immune-stimulation properties, the inclusion of a fructose oligosaccharide is now popular in pet rabbit formulations (P. Bruneau, Lichfield, 1998, personal communication). This has the added advantage that the shift in VFA proportions enhances intestinal motility, thereby reducing the importance of a minimum inclusion of IDF.

As with all diet designs, a balance of fibre type to other nutrients is important and this is perhaps more so in the case of the small pet rabbit. For example, with the Netherland Dwarf it is possible that a high-fibre diet with a concomitantly low DE of <8.1 MJ kg^{-1} (as-fed) may result in insufficient intake to provide sufficient energy for maintenance, although this problem may not arise for larger breeds (>3.5 kg, e.g. Mini Lop and New Zealand White).

If the diet is designed so that the CF levels are <100–120 g kg^{-1} then a decrease in intestinal transit time occurs. This increases the caecal volume, resulting in a decrease in the carbohydrate supply for energy. This increases (in relative terms) the proportion of energy from protein sources. At the other extreme an increase in the fibre content of the diet, such that insufficient DE can be consumed, also results in a concomitant rise in the proportion of energy derived from protein and has the effect of promoting proteolytic bacteria and ammonia production. This inevitably leads to digestive problems. If, however the increase in fibre (150–160 g kg^{-1}) is associated with a reduction in the protein to DE ratio then digestive upsets will be avoided.

A similar increase in caecal content and caecal impaction as occurs with an increase in the fibre protein to DE ratio can also occur with an excess mineral load in the diet, such as when a clay binder is used in excess to enhance pellet quality (Grobner *et al.*, 1985). De Blas *et al.* (1986) suggested that sufficient IDF is provided with 100–110 g CF kg^{-1}. However, to achieve 90 g kg^{-1} IDF it is suggested that 130–140 g CF kg^{-1} diet is necessary in many formulations. Exceeding a maximum CF of 160 g kg^{-1} can lead to increased mortality in young rabbits (Lebas, 1989).

Given that rabbits thrive on diets of hay and grass, it may be considered appropriate to formulate a fibre intake similar to these materials. However, to exercise this approach a better measurement of the fibre fraction of the diet is required. This has been reviewed by Gidenne (2003).

- Total dietary fibre: this represents all water-soluble non-starch polysaccharides.
- Water-insoluble cell wall content: this represents the pectic substances of hemicellulose, cellulose and lignin.
- NDF: equates to most of the hemicellulose and lignin, and all of the cellulose.
- ADF: equates to cellulose and most of the lignin. Consequently, NDF minus ADF represents the hemicellulose content of the diet.
- Acid detergent lignin: nearly all lignins.
- CF: this is the term declared on pack and represents 20–90% of the lignin and 30–100% of cellulose, depending upon the plant ingredient being analysed.

For a complete diet, CF ranges as wide as 140–250 g kg^{-1} fibre may be found. It is suggested that a minimum of 140–160 g kg^{-1} CF should be adopted, or preferably a more detailed assessment of the fibre fraction should be used with a minimum of 170–200 g ADF kg^{-1}, NDF in the region of 300–400 g kg^{-1} and a ratio of (NDF + pectins) – ADF to ADF of ≤1.3. Alternatively, for a complementary feed a minimum source of IDF should be supplied through the addition of traditional materials such as hay or straw to the daily ration of compound feed.

17.5.3 Fat

It is generally thought that plant materials meet the essential fatty acid requirements of the rabbit, which can be achieved with a diet containing 25 g fat kg^{-1}. For the pet rabbit, where weight constraint is important, the major role of fat in the diet is for the supply of essential fatty acids. An adequate supply of fat is often found within the normal raw materials used in diet formulations. However, Cheeke (1974) reported that rabbits prefer diets coated with 50 g kg^{-1}

maize oil over those without fat addition. It is therefore suggested that, if added, the fat content should not exceed 50 g kg^{-1}.

17.5.4 Starch and energy

While starch is well-digested even at high levels (>600 g kg^{-1} of barley in the diet), starch levels >150 g kg^{-1} at the caecum will lead to undesirable fermentation patterns. The sensitivity to high-starch diets is controversial and appears to be much more apparent in the young weanling rabbit than in the adult. As discussed by Lebas (1989), if the relationship between starch and fibre is independent, a minimum fibre and maximum starch constraint (<140 g kg^{-1} in the young rabbit and 180–200 g kg^{-1} in the adult) is a sensible formulation consideration. There is evidence to suggest that the presentation of starch in terms of flakes, ground or cereal type is immaterial to the total amount (Santomá *et al.*, 1985; Seroux, 1986). However, if the initial starch in the dietary ingredients is processed in such a manner (e.g. extrusion) as to be readily digested and absorbed as simple sugar in the small intestine then this may not present a problem in the final diet.

While many of the equations used to predict the DE of diets overestimate the DE content of diets with high levels of digestible fibre and underestimate those with added fat, for the conventional pet rabbit diet it is suggested that the following equation provides a reasonable practical predictor of DE:

$$DE = (-1801 + 7.10CP + 12.01EE + 5.59NFE) * 0.004184$$

Where DE is MJ kg^{-1} (or kcal kg^{-1} without the correction factor of 0.004184); CP is crude protein; EE is ether extract; and NFE is nitrogen-free extract, each in g kg^{-1} as-fed.

Alternatively, a more recent equation (Villamide *et al.*, 2009) may be considered:

$$DE = 0.013CP + 0.036EE + 0.017NFC + 0.006NDF$$

Where DE is MJ kg^{-1}; CP is crude protein; EE is ether extract; NFC is organic matter minus crude protein, ether extract and NDF; and NDF is neutral detergent fibre, assayed with a heat-stable amylase and expressed exclusive of residual ash.

The literature provides some estimates of the DE of raw materials for rabbits. A selection is summarized in Table 17.3, together with data for NDF and ADF. It is interesting to note the similarity between these values and those generally used for pigs, even for forages.

In conclusion, the DE of the diet should be in the region of 9–10.5 MJ DE kg^{-1}. Based on typical values of coefficients of apparent digestibility for concentrates at 0.8, cereals and brans at 0.65–0.70 and forages at 0.45–0.65, the energy concentration of the diet per unit of digestible protein (DP) can be estimated at 98 kJ g DP^{-1}. Thus, for a typical diet of 102–110 g DP kg^{-1}, an energy level of 10.0–10.5 MJ DE kg^{-1} would be considered suitable.

17.5.5 Vitamins and minerals

Vitamins

As indicated, the B-group vitamin requirements of the pet rabbit are supplied in sufficient quantity from soft faeces (NRC, 1977; Harris *et al.*, 1983). For complete compound feeds it is usual to supplement this to a limited extent, together with up to 2 mg kg^{-1} vitamin K.

While fresh green foods may contain large amounts of carotene, the precursor of vitamin A, much of this is lost upon drying, storage and processing. It is thus advisable to supplement complete diets with vitamin A in the range of 5000–12,000 IU kg^{-1} (Roche, 1998). There is also some evidence to indicate that breeding rabbits benefit from an additional 30 mg carotene kg^{-1} diet, even when vitamin A is in plentiful supply (Tobin, 1996).

Vitamin E is usually provided at 40–70 mg kg^{-1}. Rabbits are sensitive to its deficiency, developing muscular dystrophy and myocardial dysfunction and showing an increased incidence of coccidiosis.

Table 17.3. A selection of values for the digestible energy (DE), neutral detergent fibre (NDF) and acid detergent fibre (ADF) content of some raw materials (data adapted from Wiseman, 1987; Santomá *et al.*, 1989; Maertens, 1992; Gidenne, 2003).

Raw material	DE (MJ kg^{-1})	NDF (g kg^{-1})	ADF (g kg^{-1})
Barley	12.5–12.8	175	55
Wheat	12.8–13.2	110	31
Oats[a]	11.2–11.7 (14.2)	280 (100)	135 (75)
Maize	13.2–13.7	95	25
Beet pulp	10.9–11.3	428	212
Soybean meal	13.7–14.1	124	65
Sunflower meal	9.3–10.1	383	270
Wheat straw	2.7–5.4	750	474
Lucerne	7.6–7.9	418	326
Bran	9.2–10.0	405	118
Grass	7.2	460	260
Peas	11.7	120	70
Soya hulls	7.5–8.4	588	426

[a]Values in parentheses are for naked varieties.

Minerals

The rabbit appears to be unable to regulate its uptake of calcium from the intestinal tract and the concentration of calcium in the complete diet must therefore be close to the requirement. However, it is important to consider the extent to which low-calcium fresh foods may be fed as part of the diet, leading to a reduction in plasma calcium and a possible excess of phosphorus. While rabbits tolerate wide calcium to phosphorus ratios, inverse ratios soon lead to problems.

Such imbalances of calcium to phosphorus have been implicated in dental problems in pet rabbits (Harcourt-Brown, 1996). However, Bucher (1994) indicated that the nature of the feed ingredients also exerts effects on incisor growth and attrition. The provision of too much calcium in the diet may lead to an increased incidence of urolithiasis. The deposition of calcium salts in the urine occurs due to the alkalinity of the urine, which is often enhanced by high levels of potassium from grass or lucerne consumption, contributing to the base excess. It is thus suggested that dietary calcium should be in the region of 5–10 g kg^{-1}. To minimize the risk of the deposition of excess calcium in soft tissues the vitamin D content of the diet should be in the region of 800–1200 IU kg^{-1}. While the rabbit may be tolerant of a wide range of calcium to phosphorus ratios, it is desirable to maintain the ratio between 1:1 and 2:1 in favour of calcium.

There is no reason to believe that the requirements for other minerals and trace elements are different from those indicated by the NRC (1977). However, 50 mg of supplementary zinc kg^{-1} should be considered to overcome the potential low bioavailability of zinc raw material in the diet when large amounts of phytate are present. There may be a benefit to the addition of the supplementary zinc in the form of a chelate or polysaccharide complex as there are indications that these sources are not involved in such interactions (Lowe and Wiseman, 1997).

17.5.6 Suggested diet specifications

On the basis of the foregoing discussion and the author's experience, the specifications given in Table 17.4 are suggested for diets suitable for the pet rabbit.

17.5.7 Nutritional ailments

When faced with an unwell pet, it is important to differentiate between symptoms and disease. Many so-called diseases, for

Table 17.4. Suggested nutrient constraints for pet rabbit diets.

Component and nutrient ($g\ kg^{-1}$)	Range	Nutrient ($mg\ kg^{-1}$)	Typical range
Protein	120–160	Vitamin A ($IU\ kg^{-1}$)[d]	5,000–12,000
Crude fibre[a]	140–200	Vitamin D ($IU\ kg^{-1}$)	800–1,200
ADF	170–n/a	Vitamin E	40–70
Starch[b]	0–140	Copper	5–10
Fat	20–50	Vitamin B_1	1–10
Digestible energy ($MJ\ kg^{-1}$)	9–10.5	Vitamin B_2	3–10
Lysine	5–n/a	Vitamin B_6	2–15
Methionine and cystine	5–n/a	Vitamin B_{12}	0.01–0.02
Calcium[c]	5–10	Folic acid	0.2–1.0
Phosphorus[c]	5–8	Pantothenic acid	3–20
Magnesium	3	Niacin	30–60
Zinc	50–100	Biotin	0.05–0.20
Potassium	6–7	Choline	300–1,500
Sodium chloride	5–10		

ADF, acid detergent fibre; n/a, not applicable.
[a]A more appropriate estimate for minimum fibre inclusion is 310 g kg^{-1} neutral detergent fibre (NDF) and 190 g kg^{-1} ADF for the young rabbit and 270 g kg^{-1} NDF and 170 g kg^{-1} ADF for the adult (Gidenne, 2003).
[b]The starch maximum only applies to diets for the very young rabbit. For the young adult pet, a 140–200 g kg^{-1} maximum constraint for the mature adult may be considered, providing the fibre constraints are exceeded.
[c]These levels allow for the fact that the pet rabbit may be used for breeding. Adult maintenance can be satisfied with levels in the region of 4 g phosphorus kg^{-1} and 6 g calcium kg^{-1}. Also note that >10 g kg^{-1} may be unpalatable to the rabbit (NRC, 1977).
[d]It may be necessary to include higher levels of certain vitamins to allow for losses during manufacture. These will be specific to the raw materials and production processes used and must be taken into account when designing the supplementary additions.

example scouring (diarrhoea), are not in themselves a disease but are actually a symptom of a disease. As far as the complete or complementary diet manufacture is concerned, nutritional deficiencies and metabolic disorders may predispose to these conditions. It is worth bearing in mind that there is nothing so potent for producing ill health as improperly constituted food or an inappropriate feeding strategy

In many cases it is not the design of the food that is at fault, but the way in which the diet is presented to, fed to or selected by the pet rabbit. Therefore, the feeding instructions and guide as to how to best utilize the food in the day-to-day feeding programme of the pet rabbit are as important in the design and manufacture of pet rabbit diets as the nutrient and raw material specifications. Death from malnutrition is rare, but excess bulk in the diet of young rabbits can result in starvation. This may arise when insufficient complete diet relative to hay is provided. Similarly, selective feeding of flake-mix-type diets has been reported to lead to deficiencies or excess of certain nutrients.

17.6 Conclusions

Diets for the pet rabbit can be produced from a wide selection of materials, ranging from those grown in the garden to complete commercial diets. The creation of entirely home-produced diets must be treated with caution unless there is extensive knowledge of both the requirements of the rabbit and the nutrient content of the ingredients. The science of nutrition and diet formulation requires education and considerable experience, and a haphazard approach is not usually successful. Furthermore, even the best designed and manufactured diets are only successful in providing appropriate nutrition to the pet rabbit if they are fed according to the instructions provided with them. The nutritionist is duty-bound to provide the best guidance possible in this area and the pet rabbit owner would be wise to follow instructions carefully and pay due care and attention to the finer points of management, including feeding to maintain an appropriate body weight.

The importance of fibre to the rabbit cannot be overstated. Dietary minimums should always be comfortably met and the pet owner should be clearly encouraged to feed additional fibre, even when complete diets that meet the daily nutritional needs are fed.

References

Aitken, F.C. and King Wilson, W. (1962) *Rabbit Feeding for Meat and Fur,* 2nd edn. CAB International, Wallingford, UK.

Auvergne, A., Bouyssou, T., Pairet, M., Bouillier-Oudot, M., Ruckebusch, Y. and Candau, M. (1987) Nature del'aliment, finesse de mouture et donnees anatomo-functionnelles de tube digestif proximal du lapin. *Reproduction Nutrition Development* 27, 755–768.

AVMA (American Veterinary Medical Association) (2007) *US Pet Ownership and Demographic Source Book*. Schaumburg, Illinois, USA.

Bjornhag, G. (1987) Comparative aspects of digestion in the hindgut of mammals. The colonic separation mechanism. *Deutsche Tierarztliche Wochenschriift* 94, 33–36.

Bouyssou, T., Candau, M. and Ruckebush, Y. (1988) Réponses motrices du côlon aux constituants pariétaux et à la finesse de mouture des aliments chez le lapin. *Reproduction Nutrition Development* 28, 181–182.

Brooks, D. L. (2004) Nutrition and gastrointestinal physiology. In: Quesenberry, K. and Hillyer, E. (eds) *Ferrets, Rabbits, and Rodents: Clinical Medicine and Surgery*, 2nd edn. WB Saunders, Pennsylvania, USA, pp. 155–160.

Bucher, L. (1994) Diet related influences on growth and attrition of incisors in dwarf rabbits. Doctoral thesis, Freie Universitat, Berlin, Germany.

Candau, M., Auvergne, A., Comes, F. and Bouillier-Oudet, M. (1986) Effect of the physical form and grinding fineness of feeds upon growth and cecal function in growing rabbits. *Annales de Zootechnie* 35, 373–386.

Carabaño, R., Fraga, M.J., Santomá, G. and de Blas, J.C. (1988) Effect of diet on composition of cecal contents and on excretion and composition of soft and hard feces of rabbits. *Journal of Animal Science* 66, 901–910.

Chao, H.Y. and Li, F.C. (2007) Effect of level of fibre on performance and digestion traits in growing rabbits. *Animal Feed Science and Technology* 144, 279–291.

Cheeke, P.R. (1974) Feed performances of adult male Dutch rabbits. *Laboratory Animal Science* 24, 601–604.

Cheeke, P.R. (1987) *Rabbit Feeding and Nutrition*. Academic Press Inc., Orlando, Florida, USA, p. 376.

Cheeke, P.R. (1988) Rabbit nutrition: a quiet growth area with great potential. In: Lyons, T.P. (ed.) *Biotechnology in the Feed Industry, Proceedings of Alltech's Fourth Annual Symposium*. Alltech, Lexington, Kentucky, USA, pp. 249–260.

Colin, M. (1975) Effects on growth of rabbit of l-lysine and dl-methionine supplementation of simplified plant diets. *Annales de Zootechnie* 24, 465.

Costachescu, E. and Hoha, G. (2006) Present tendency in breeding rabbits. *Lucr ri Stiintifice – Universitatea de Stiinte Agricole si Medicin Veterinar , Seria Zootehnie* 49, 713–718.

de Blas, J.C., Santoma, G., Carabano, R. and Fraga, M.J. (1986) Fibre and starch levels in fattening rabbit diets. *Journal of Animal Science* 63, 1897–1904.

Fahey, G.C. Jr (1995) Practical considerations in feeding dietary fibres to companion animals. In: Phillips, T. (ed.) *Pet Food Forum 95*. Watt Publishing, Mount Morris, Illinois, USA, p. 43.

Fraga, M.J. and de Blas, J.C. (1977) Influencia de la coprofagia sobre la utilización digestiva de los alimentos en el conejo. *Annales del Instituto Nacional de Investigaciones Agrarias* 8, 43–47.

Fraser, C.M. (1991) *Merck Veterinary Manual*, 7th edn. Merck & Co., Rahway, New Jersey, USA, pp. 1278.

García, J., Carabaño, R., Pérez-Alba, L. and de Blas, J.C. (2000) Effects of fibre source on caecal fermentation and nitrogen recycled through caecotrophy in rabbits. *Journal of Animal Science* 78, 638–646.

Gidenne, T. (1987) Effect of dietary lignin content on digesta composition and soft feces production in the young rabbit. *Annales de Zootechnie* 36, 85–90.

Gidenne, T. (1988) Ileal digestibility measures an canulated rabbit. In: WRSA (ed.) Proceedings of the 4th World Rabbit Science Association, volume 3. Budapest, Hungary, pp.345–350.

Gidenne, T. (2003) Fibres in rabbit feeding for digestive troubles prevention. *Livestock Production Science* 81, 105–117.

Gidenne, T., Poncet, C. and Gómez, L. (1986) Transit digestif des consituants de rations riches en fibres, distribuées à deux niveaux alimentaires chez la lapine adulte. In: Lebas (ed.) 4ème Journées de la Recherche Cunicole, Paris. Communication no.4, ITAVI, Paris, France.

Grannis, J. (2002) *US Rabbit Industry Profile*. USDA:APHIS:VS, Centers for Epidemiology and Animal Health, Fort Collins, Colorado, USA.

Grobner, M.A., Robinson, K.L., Cheeke, P.R. and Patton, N.M. (1985) Utilisation of low and high energy diets by dwarf (Netherland Dwarf), intermediate (Mini Lop) and giant (Flemish Giant) breeds of rabbits. *Journal of Applied Rabbit Research* 8, 12–18.

Haffar, A. (1996) Treating the pet rabbit. *Point Veterinaire* 28, 347–353.

Harcourt-Brown, F.M. (1996) Calcium deficiency, diet and dental disease in pet rabbits. *Veterinary Record* 139, 567–571.

Harris, D.J., Cheeke, P.R. and Patton, N.M. (1983) *Journal of Applied Rabbit Research* 6, 15–17.

Hartmann, K., Fischer, S. and Kraft, W. (1994) Small pet animals as patients in veterinary practice. 1. Descent, physiology, husbandry, feeding. *Tierarztliche Praxis* 22, 585–591.

Hoover, W.H. and Heitmann, R.N. (1972) Effects of dietry fibre levels on weight gain, cecal volume and volatile fatty-acid production in rabbits. *Journal of Nutrition* 102, 375–380.

Jordan, D., Gorjanc, G., Kermauner, A. and Stuhec, I. (2008) Wooden sticks as environmental enrichment. *World Rabbit Science* 16, 237–243.

Lebas, F. (1980) *Les Recherches sur l'Alimentation du Lapin: Évolution au Cours des 20 Dernières Années et Perspectives d'Avenir*. II Cong. Mund. Cunic, Barcelona, Spain.

Lebas, F. (1987) Nutrition of rabbits. In: Wiseman, J. (ed.) *Feeding of Non-Ruminant Livestock*. Butterworths, London, UK, pp. 63–69.

Lebas, F. (1989) Nutrient requirements of various categories of rabbits. In: Piva, G. and Wiseman, J. (eds) *Proceedings of the First International Feed Production Conference*. Piacenza, Italy, pp. 297–332.

Lebas, F. and Franck, T. (1986) Incidence du broyage sur la digestibilité de quatre aliments chez le lapin. *Reproduction Nutrition Development* 26, 335–336.

Lebas, F. and Laplace, J.P. (1977) Digestive transit in rabbit 6: Effect of feed pelleting. *Annales de Zootechnie* 26, 83–91.

Lebas, F., Corring, T., Courtot, D., Gueugneau, A., Sardi, G. and Cotta, Y. (1971) Équipement enzymatique du pancréas exocrine chez le lapin, mise en place et évolution de la naissance au sevrage. relation avec la composition du régime alimentaire. *Annales de Biologie Animale, Biochimie, Biophysique* 11, 399–413.

Lebas, F., Maitre, I., Seroux, M. and Franck, T. (1986) Influence du broyage des matieres premieres avantl'agglomeration de 2 aliments pour lapins, differant par leux taux de constityuants membranaires. In: *4emes Journées de la Recherche Cunicole, Paris*. Communication no. 9, ITAVI, Paris, France.

Lowe, J.A. and Wiseman, J. (1997) The effect of the source of dietary supplemental zinc on tissue copper concentrations in the rat. *Proceedings of the British Society of Animal Science* 67, p. 67.

Maertens, L. (1992) Rabbit nutrition and feeding: a review of some recent developments. *Journal of Applied Rabbit Research* 15, 889–913.

Malley, A.D. (1994) The pet rabbit in companion animal practice. 1. A clinician's approach to the pet rabbit. *Irish Veterinary Journal* 47, 9–15.

Malley, A.D. (1995) The pet rabbit in companion animal practice. 2. General clinical examination. *Irish Veterinary Journal* 48, 307–311.

Malley, A.D. (1996) The pet rabbit in companion animal practice. 4. Hematological and biochemical reference values. *Irish Veterinary Journal* 49, 354.

Marty, J. and Vernay, M. (1984) Absorption and metabolism of the volatile fatty acids in the hind-gut of the rabbit. *British Journal of Nutrition* 51, 265–277.

Meredith, A.L. (2000) Introduction to rabbit nutrition; advances in pet health through nutrition. In: *Proceedings of BSAVA/PFMA Nutrition Symposium*.

Meredith, A.L. (2008) Gastrointestinal disease in the rabbit. *Proceedings of the 33rd World Small Animal Veterinary Congress*. Dublin, Ireland, pp. 262–264.

Mulder, A., Nieuwenkamp, A.E., van der Palen, J.G., van Rooijen, G.H. and Beynen, A.C. (1992) Supplementary hay reduces fur chewing in rabbits. *Tijdschrift voor diergeneeskunde* 117, 655–658.

Netherway, M.E.P. (1979) How to feed rabbits. In: *Home Rabbit Keeping*. E.P. Publishing, Wakefield, UK, pp. 23–32.

NRC (1966) *Nutrient Requirements of Rabbits*, 1st revised edn. National Academy of Sciences, Washington, DC, USA.

NRC (1977) *Nutrient Requirements of Rabbits*, 2nd revised edn. National Academy of Sciences, Washington, DC, USA.

Pairet, M., Bouyssou, T., Auvergne, A., Candau, M. and Ruckebusch, Y. (1986) Stimulation physicochimique d'origine alimentaire et motricite digestive chez le lapin. *Reproduction Nutrition Development* 26, 85–95.

Perez, J.M., Gidenne, T., Lebas, F., Caudron, I., Arveux, P., Bourdillon, A., Duperray, J. and Messager, B. (1994) Dietary lignin in growing rabbits. 2. Consequences on growth-performance and mortality. *Annales de Zootechnie* 43, 323–332.

PFMA (Pet Food Manufacturers' Association) (2009) Statistics. Available from: PFMA.org.uk (accessed 25 January 2010).

Prud'hon, M., Cherubin, M., Goussopoulos, J. and Charles, Y. (1975) Evolution au cours de la croissance des caracteristiques de la consommation d'aliments solide et liquide du lapin domestique nourri ad libitum. *Annales de Zootechnie* 24, 289–298.

Roche (1998) *Roche Vitamin Supplementation Guidelines for Domestic Animals*. Roche, Paramus, New Jersey, USA.

Sandford, J.C. (1973) *The Domestic Rabbit*, 3rd edn. Crosby, Lockwood and Staples, London, UK, pp. 84–118.

Sandford, J.C. (1986) *The Domestic Rabbit*, 4th edn. Collins, London, UK.

Sandford, J.C. (1996) *The Domestic Rabbit,* 5th edn. Blackwell Science, Oxon, UK.

Santomá, G., Carabaño, R., de Blas, J.C. and Fraga, M.J. (1985) Ultilización de dietas con distintos cereales para conejos en cebo. *Annales del Instituto Nacional de Investigaciones Agrarias* 22, 75–82.

Santomá, G., de Blas, J.C., Carabaño, R. and Fraga, M.J. (1989) Nutrition of rabbits. In: Haresign, W. and Cole, D.J.A. (eds) *Recent Advances in Animal Nutrition*. Butterworths, London, UK, pp. 109–138.

Schlolaut, W. (1982) *The Nutrition of the Rabbit*. Roche Information Service, Basel, Switzerland.

Schultz, W.H., Smith, W.C. and Mougham, P.J. (1988) Amino acid requirements of the growing meat rabbit. *Animal Production* 47, 303–311.

Schwabe, K. (1995) Feed and water intake of different species of pets (rabbits, guineapigs, chinchilla, hamsters) offered different water supply (drinkers vs. succulent feeds). Thesis. Tierarztliche Hochschule Hannover, Hannover, Germany.

Seroux, M. (1986) Effect de la cuisson de l'amidon par floconnage des céréales sur les performances zootechniques des lapereaux sevrés In: *4emes Journées de la Recherche Cunicole, Paris*. Communication no. 10, ITAVI, Paris, France.

Sunvold, G.D. and Fahey, G.C. Jr (1994) The role of dietary fiber in the nutrition of dogs and cats. In: Phillips, T. (ed.) *Pet Food Forum 94*. Watt Publishing, Mount Morris, Illinois, USA.

Tobin, G. (1996) Small pets – food types, nutrient requirements and nutritional disorders. In: Kelly, N. and Wills, J. (eds) *Manual of Companion Animal Nutrition and Feeding*. British Small Animal Veterinary Association, Cheltenham, UK, pp. 208–225.

Van Soest, P.J. (1982) *Nutritional Ecology of the Ruminant*. Q&B Books, Corvallis, Oregon, USA.

Van Soest, P.J. and McQueen, R.W. (1973) The chemistry and estimation of fibre. *Proceedings of the Nutrition Society* 32, 123.

Villamide, M.J., Carabaño, R., Maertens, L., Pascual, J., Gidenne, T., Falcao-E-Cunha, L. and Xiccato, G. (2009) Prediction of the nutritional value of European compound feeds for rabbits by chemical components and *in vitro* analysis. *Animal Feed Science and Technology* 150, 283–295.

Wiseman, J. (1987) *Feeding of Non Ruminant Livestock*. Butterworths, London, UK.

Zeuner, F.E. (1963) The small rodents. In: *A History of Domesticated Animals*. Hutchinson & Co, London, UK, pp. 409–415.

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