NEW CONCEPTS AND OBJECTIVES FOR PROTEIN-AMINO ACID NUTRITION IN RABBITS: A REVIEW

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Abstract: In the European context, the new legislation to avoid mineral contamination and the ban on antibiotics as growth promoters has led to the definition of new objectives in respect of nitrogen supply. The present study summarizes the state of nitrogen nutrition in rabbits and reviews the role of protein and amino acids in rabbit health and the new nitrogen value of protein sources based on true ileal digestibility (TID) for future recommendations. The main sources of nitrogen for microbial growth are ammonia, urea and protein (endogenous and dietary). The surplus of nitrogen flow to the caecum increases mortality rates during fattening by favouring the growth of potential pathogenic bacteria. Accordingly, feeding strategies to reduce ileal nitrogen flow have been reviewed. A large reduction of dietary protein level might have negative consequences on growth performances and mortality. In order to formulate balanced low-protein diets, data on ileal and faecal amino acid digestibility of 14 raw materials is summarized. The use of this different unit for amino acid digestibility is also discussed.

Key Words: amino acids, ileal digestibility, microbial growth, intestinal health, nutrition, rabbits.

INTRODUCTION: A HISTORICAL PERSPECTIVE

Knowledge of nitrogen nutrition, including protein and amino acid requirements, is essential for formulating productive and cost-effective diets for domestic animals. In rabbits, there is a scarcity of knowledge as compared with what is reported for ruminant (cattle or sheep) or other non ruminant species (pigs or poultry). Also, rabbits are relatively less important than other livestock species, and combined with a slow rate of industrialization, this might have contributed to this fact, despite the historically extensive contribution of rabbit to the protein supply of the human diet.

From the 1940's to the early 1970's, the rabbit was considered a laboratory animal and consequently research focused on investigating the "qualitative" aspects of nitrogen and amino acids utilization. The role of caecotrophy and caecal metabolism on nitrogen digestion and body retention (Tacker and Brant, 1955, Yoshida and Kandatsu, 1960 and 1964; Yoshida *et al.*, 1968, 1971 and 1972; Hoover and Heitman, 1975; Proto, 1976), or the essentiality of some amino acids for growth (McWard *et al.*, 1967; Gaman and Fisher, 1970; Cheeke, 1971; Adamson and Fisher, 1971 and 1973) have been determined by several authors. From these studies, it was concluded that caecal microbiota are able to use non-protein

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compounds (such as urea) and that caecotrophy helps to improve N digestion and retention. However, these studies also affirm that this extra N cannot compensate for a low dietary protein level or the use of low quality protein sources (with unbalanced amino acid composition) to meet growth requirements.

Consequently, in the 1970's and 1980's, research focused on quantitative aspects in order to determine the optimal concentration of some amino acids (arginine, lysine and methionine) and the protein needs for productive purposes (growth) using practical instead of purified diets. Studies performed at INRA (Lebas *et al.*, 1973; Colin 1974, 1975 a and b) and those of Davison and Spreadbury (1975) and Spreadbury (1978) led to confirming the essentiality of these amino acids and the high requirements of arginine, in contrast to the ones observed in poultry. They also suggested that the optimal level of an amino acid depends on the balance with other amino acids and the level of energy in the diet, so the recommendation for lysine is given in grams per 1000 kcal of digestible energy. The above mentioned works constituted the base of the NRC recommendations in 1977 (NRC, 1977) (Table 1).

With respect to optimal dietary levels of protein for growth, in the early 1980's our research at Universidad Politécnica of Madrid focused on establishing the best nutritive unit for energy and protein (crude, digestible or net), and the requirements of these nutrients for growth. These studies considered a wide range of protein and energy levels, different slaughter weights (2.0, 2.25 or 2.5 kg) or weaning age (25 *vs* 35 d) (de Blas *et al.*, 1981 and 1985; Fraga *et al.*, 1983). From these results it was concluded that Digestible Energy (DE) to Digestible Protein (DP) ratio is a more reliable unit as it has a higher and direct impact on body nitrogen and energy retention than the dietary content of fibre, which is inversely related

Table 1: Protein and amino acid recommendations according to several authors (as-fed basis).

	NRC (1977)		INRA (1984)		de Blas and Mateos (1998)	
	Growing rabbits	Lactating does	Growing rabbits	Lactating does	Growing rabbits	Lactating does
Digestible energy (MJ/kg)	10.5	10.5	10.5	11.0	10.5	11.1
Crude protein (%)	16.0	17.0	16.0	18.0	15.3	18.4
Digestible protein (%)					10.7	12.9
Lysine:						
Total (%)	0.65		0.65	0.75	0.75	0.84
Digestible (%)					0.59	0.66
Sulphur aa:						
Total (%)	0.60		0.60	0.60	0.54	0.65
Digestible (%)					0.41	0.50
Threonine:						
Total (%)	0.60		0.55	0.70	0.64	0.70
Digestible (%)					0.44	0.48
Arginine (%)	0.60		0.90	0.90		
Histidine (%)	0.30		0.35	0.43		
Leucine (%)	1.10		1.05	1.25		
Isoleucine (%)	0.60		0.60	0.70		
Phenylalanine and Tyrosine (%)	1.10		1.20	1.40		
Tryptophan (%)	0.20		0.18	0.22		
Valine (%)	0.70		0.70	0.85		

with digestible energy. Hence, the optimal level for crude protein in a diet depends on its digestibility and the DE content. A recommended ratio of 23.5 kcal DE/g DP (or 10 g DP/MJ DE) was suggested to optimize growth rate and mortality.

The protein requirements for reproductive does were studied by several researchers in America and Europe (Partridge and Allan, 1982; Adams, 1983; Sanchez *et al.*, 1985; Partridge *et al.*, 1986; Parigi Bini *et al.*, 1990, 1991 and 1992; Xiccato *et al.*, 1992). These works determined higher requirements for protein (around 20%) to optimize reproductive performances than those needed for growth. The lack of specific studies on amino acid requirements for lactating or pregnant does has led several authors to consider analogous requirements as for growth (Lebas, 1988; Partridge, 1989) despite the fact that differences between the amino acid profile of milk and muscle protein (Moughan *et al.*, 1988; Partridge, 1989) may compromise any such analogy.

In the 1990's, the most important practical advance was the recommended nutritional needs of growing rabbits and does for the most frequent limiting amino acids (lysine, methionine and threonine) (Maertens and de Groote, 1988; Taboada *et al.*, 1994 and 1996; de Blas *et al.*, 1998) as presented by de Blas and Mateos (1998) (Table 1). It is noted that current recommendations are higher for lysine and threonine (from 12 to 23%). These differences might be explained by the higher productivity observed in newly developed genotypes, but also by differences in the digestive utilization of the diets used in the experiments where these recommendations were determined.

In fact, these studies led to a new unit for amino acid supply being considered, addressing the need to determine not only "crude" but also "digestible" (at faecal level) amino acid requirements. This unit leads to a better adjustment of dietary supply and requirements as it considers the high variability of protein digestibility observed in raw materials (Villamide *et al.*, 1998). The utility of digestible units for protein characterization was also largely recognized by the feed industry. However, the lack of information about the faecal digestible amino acids content of raw materials (at that time only data on alfalfa hay was available from García *et al.*, 1995) limited the practical use of this unit. This raised the question for a further digestible amino acid content evaluation by doing the balances at faecal or at ileal level. The preliminary results presented at the 7th Word Rabbit Congress (Carabaño *et al.*, 2000) suggested that faecal balance is not correct for characterizing the absorption of amino acids at ileal level and consequently for meeting the amino acid requirements. Consequently, studies have recently been carried out to characterize the digestible amino acid content in the main raw materials used in rabbit diets. In the following sections, the results of these studies will be summarized.

Hence, until the 21th century, the dietary supply of protein and amino acids had the primary and traditional objective of meeting rabbit requirements for production. As crude or total units have been widely used in practical diet formulation, excess protein is typical in commercial diets. Furthermore, the tendency in the last decade to increase the dietary fibre level and reduce starch to avoid digestive problems has favoured increased inclusion levels of alfalfa hay and cereal by-products, resulting in higher dietary protein levels than recommended (>15%). In the European context however, the new legislation to avoid mineral contamination and the ban of antibiotics as growth promoters has led to defining new objectives with respect to nitrogen supply. In all livestock species, the current trend is to maximize N retention by adjusting dietary protein levels accordingly to a balanced amino acid supply and thus avoiding any N excess in the diet. In rabbits, about 2/3 of ingested N is excreted (Maertens *et al.*, 2005; Xiccato *et al.*, 2006), and therefore a reduction in dietary protein level may help to minimize the N load passed on to the environment, while conforming to new environmental laws. Furthermore, this reduction may also help to control the growth of pathogenic bacteria that promote higher mortality in the postweaning period. Experience with pigs and poultry has confirmed the utility of this strategy, but has also brought up the important role of some essential or semi-essential amino acids on gut health.

Table 2: Ileal balance in adult rabbits fed alfalfa-based diets (adapted from Gidenne, 1992; Merino and Carabaño, 1992; Carabaño *et al.*, 2001).

	Intake (g/d)	Ileal flow (g/d)
Dry matter (DM)	150	60
Crude protein (CP)	24	9
Starch	22	0-2
Neutral detergent fibre (NDF)	52	42

In the following sections, current knowledge on the role of protein and amino acid nutrition in preventing digestive disorders and in productive traits will be reviewed.

THE ROLE OF NITROGEN IN MICROBIAL GROWTH

Caecum is the main reservoir for microorganisms in the intestinal tract (10^{10} - 10^{12} bacteria/g of caecal content; Penney *et al.*, 1986). The last segment of the small intestine (the ileum) and the proximal colon also contain a large proportion of total intestinal microbiota. Residues of intestinal digestion and the nutrients recycled through the blood are the potential substrates that allow the growth of microbiota. At the end of the ileum, fibre is the main component of the digesta (about 70% of total dry matter (DM)) while nitrogen is the second in importance (about 15% of total DM) (Table 2). This last figure may be a relatively poor indicator of nitrogenous components as potential substrate for microbial growth. However, taking into account the low fermentability of the fibre (30% of digestibility for neutral detergent fibre (NDF) components) and the high content of endogenous substances in nitrogen residues (about 65%, see the following sections), both components may equally contribute to maintaining resident intestinal microbiota.

There is little information about the qualitative and quantitative importance of nitrogenous components utilization by caecal microbiota. However, early studies on caecal metabolism indicate that microbiota is able to utilize the nitrogen that enters into the caecum. When germ-free animals are compared with conventional ones, studies (Yoshida *et al.*, 1972; Rerat, 1978) observed that caecal content 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 contrary, in conventional animals the caecum contains more ammonia and lower quantities (up to 10-fold) of endogenous components. Further studies confirmed the previous conclusions. Some of the most frequent isolated caecal bacteria (*Bacteriodes* spp.) are the most active genera in mucin digestion (Hill, 1986; Sirotek *et al.*, 2003). Also, Emaldi *et al.* (1979) observed that the main activities of caecal microbiota were in decreasing order: ammonia-user, ureolytic, proteolytic and cellulolytic. Figure 1 shows a tentative scheme of the caecal nitrogen metabolism. The proteolytic activity of caecal bacteria would be particularly responsible for total volatile fatty acid and ammonia production in the caecum. These end products are partially absorbed through the caecal and colon walls, and ammonia is also used by caecal bacteria as the main substrate for protein synthesis. However, the extent of these processes has not been quantified.

Another nitrogen source for microbial growth is the urea recycled into the caecum through the blood. When protein intake exceeds the requirements for body protein synthesis, it is catabolised, producing urea as an end product. Then urea is partially recycled to the caecum (Forsythe and Parker, 1985). As previously mentioned, one important urease activity has been detected in the caecum. The hydrolysis of urea produces ammonia that can be used for microbial growth to increase the ammonia concentration of caecal contents, provided that there is not enough energy for bacterial protein synthesis. Fraga (1998)

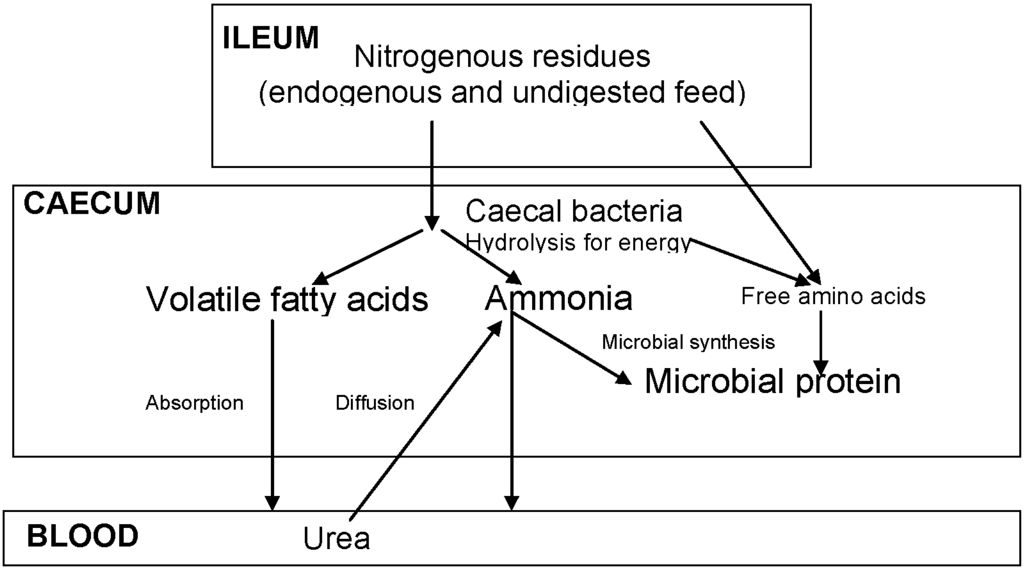

Figure 1: Caecal nitrogen metabolism.

observed a negative correlation (r=–0.88) between the DP/DE ratio and caecal ammonia concentration in a review involving 25 experimental diets.

THE ROLE OF ILEAL NITROGEN FLOW ON INTESTINAL HEALTH

The resident caecal microbiota seems to be able to flourish from nitrogenous substrates. However, when unbalanced diets are offered to the animals, some potential pathogens can also be favoured. According to de Blas *et al.* (1981), there is a quadratic relationship between mortality and DP/DE ratio, with a minimum for a ratio of 10 (Figure 2). Extreme diets with low (12%) or high crude protein (CP; 18%) content showed the highest mortality rates. The reason for this relationship was unknown because there was no control of the microbiota. Some genera with potential pathogenic effect, such as *E. Coli* or *Clostridia*, can use protein or amino acids as substrate for growth. So, an increase of the nitrogen flow into the caecum could favour these changes in microbial growth. Haffar *et al.* (1978) observed an increase of *Clostridium* in animals fed on diets containing excessive protein concentration.

This hypothesis has now been confirmed with the development of ileal digestibility techniques in the postweaning period (Blas *et al.*, 2000; Carabaño and Merino, 2003). Chamorro *et al.* (2007a) observed that a reduction of dietary CP level (by dilution of the diet with starch and without modifying ileal CP digestibility) from 18 to 16% led to a reduction of ileal CP flow (16%), the presence of potential pathogen bacteria (*Clostridium perfringens*) and mortality (10 points) due to Epizootic Rabbit Enteropathy (ERE) (Table 3).

Further reductions of protein (from 16 to 14% CP; unpublished data) followed the same tendency but the effects were of smaller magnitude, and the reduction of mortality was only observed in the postweaning period. The great contribution of endogenous losses at the ileal level may limit the reduction of N overflow. Large-scale studies conducted on French commercial farms confirmed the beneficial effect of a reduction (from 18 to 14%) in the dietary CP level on mortality (5 points) and morbidity due to different pathologies including ERE (Gidenne and García, 2006).

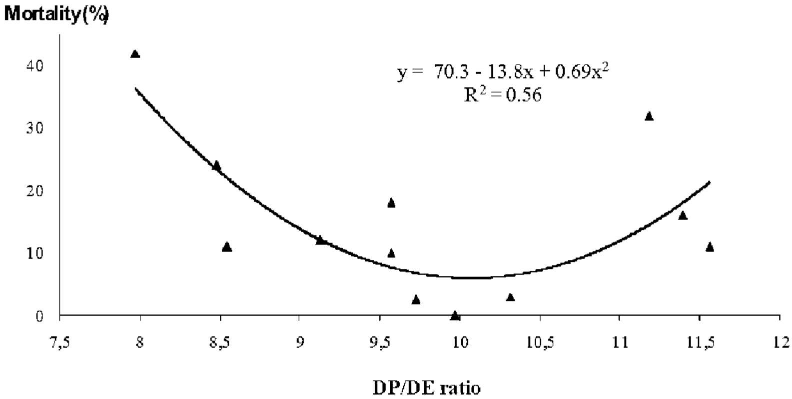

Figure 2: Relationship between mortality (%) and the DP/DE ratio in the growing period. (De Blas *et al.*, 1981).

Another way to reduce nitrogen flow is by including highly ileal-digestible sources in the diet. Gutierrez *et al.* (2003) observed that the source of protein affected CP ileal digestibility in diets with the same level of CP (18%) and faecal digestibility (80%). Concentrated (ethanol treated) soybean protein or sunflower meal gave higher ileal digestibility, lower ileal flow and lower mortality than soybean meal or a mixture of soybean meal with potato protein (Figure 3). The results of García-Ruiz *et al.* (2006) confirm the higher mortality of diets with soybean with respect to those with sunflower (11 *vs* 4%, respectively).

The dietary addition of proteases could also help to reduce nitrogen flow, but mainly in the postweaning period when animals have a limited enzymatic capacity to hydrolyse the protein (Dojana *et al.*, 1998). Accordingly, the results of García-Ruiz *et al.* (2006) showed that dietary supplementation with proteases was effective in the reduction of nitrogen ileal flow both for sunflower or soybean based diets. However, this reduction only improved the intestinal health in animals fed on the sunflower diets. The presence of anti-nutritive factors or allergenic compounds in soybean-based diets might exert an additional effect on mortality. The source of protein may also affect the mucosal integrity of the small intestine and its functionality by modulating local immune responses and possibly triggering an inflammatory response, as shown in piglets (Vente-Spreeuwenberg *et al.*, 2004). Limited works have been carried out on rabbits on this topic without any conclusive results. Cano *et al.* (2004) reported that rabbits fed a soybean meal-rich diet had a lower feed intake around weaning, associated with a higher serum anti-feed IgG, hypothesising that this provokes a sub-chronic inflammation process, and consequently increases the sensitivity of young rabbits to digestive diseases. Gutiérrez *et al.* (2000) observed that inclusion of animal plasma instead of soybean meal improved intestinal mucosal morphology in early-weaned rabbits. However, other studies did not find any differences in mucosal integrity (Gutiérrez *et al.*, 2003) or in the phenotypic distribution of lymphocytes in the duodenal lamina propria (Campín *et al.*, 2003) when soybean meal is included in the diet.

Table 3: Effect of the level of protein in isofibrous diets (30% NDF) on pathogenic flora and mortality in early (25 d) -weaned rabbits (Chamorro *et al.*, 2007a).

	18% CP	16% CP	SEM	*P*<
Ileal CP flow (g/d)	6.0	5.0	0.25	0.05
Frequency of animals with *C. perfringens* (%)	47.2	18.0	-	0.05
Fattening mortality (%)	21.2	11.0	-	0.05

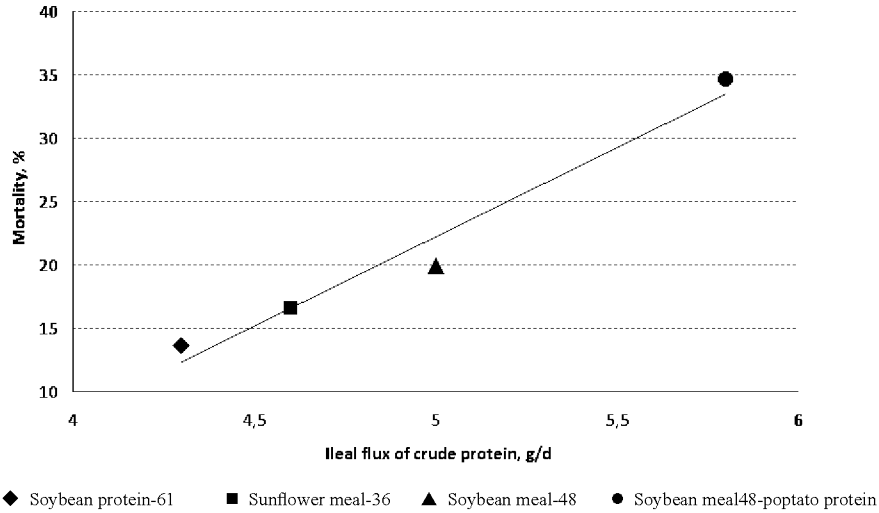

Figure 3: Effect of ileal flux of crude protein on mortality from 25 to 60 d of age (Gutiérrez *et al.*, 2003).

Endogenous nitrogen (e.g., digestive enzymes, mucoproteins, desquamated cells, urea) is another relevant source of protein for microorganisms in the gut, and in rabbits it may represent about 64% of the total ileal protein flow (García *et al.*, 2005; Llorente *et al.*, 2006 and 2007b). However, this contribution is variable and is mainly influenced by DM intake (DMI), but also by the diet composition, depending on fibre type and inclusion level or anti-nutritional factors (ANF). The relevance of this nitrogen supply for microbial growth (pathogen or saprophyte) and its consequences on mortality are unknown. The interpretation of actual results on this subject is difficult, mainly due to the effect of these dietary factors on other gut barrier mechanisms that also help to maintain intestinal health (Carabaño *et al.*, 2008). In addition, the presence of tannins and other phenolic compounds in the diet increases the nitrogen flow towards the caecum (Merino and Carabaño, 1992). However, tannins can protect the intestinal mucosa against oxidative damage and pathogens, and inhibit microbial activity in caecum (Fraga *et al.*, 1991; Motta *et al.*, 1996; García *et al.*, 2002). Maertens and Štruklec (2006) reported a reduction of mortality (due to ERE) in rabbits fed on diets supplemented with tannins. Also, the inclusion of soluble fibre might increase the endogenous nitrogen as suggested in recent works (Chamorro *et al.*, 2007a). Moreover, soluble fibre may also protect intestinal mucosa and reduce the presence of *C. perfringens* and hence mortality (Gomez-Conde *et al.*, 2007). Indeed, in-depth studies are necessary to verify the role of endogenous nitrogen on intestinal health.

The aforementioned results are summarised in Table 4. According to these results, feeding strategies that minimize the ileal nitrogen flow can help to reduce the incidence of digestive disorders. Nonetheless, more studies are needed to track ileal nitrogen origin and other characteristics.

PRACTICAL CONSEQUENCES OF A REDUCTION OF DIETARY PROTEIN SUPPLY

A reduction of protein supply in the diet might be an effective feeding strategy to reduce the nitrogen load passed on to the environment or intestinal disorders. Current commercial levels of dietary protein for fattener and reproductive does range from 16 to 18% CP. These levels exceed the recommendations in several circumstances, as final phases of growth or lactation (Xiccato *et al.*, 2006).

Table 4: Effect of level and type of dietary crude protein (CP) on intestinal health and ileal CP flow.

	Ileal flow (%)	*C. perfringens*	Other bacteria	Mortality (points of %)	Authors
CP level:					
18 *vs* 16 %	▼ 16	▼		▼ 10	(1)
16 *vs* 14 %	▼ 10	▼		▼ 5	(2)
16 *vs* 14%	ND[1]	ND	▼ TAB[2]	-[3]	(3)
18 *vs* 14%	ND	ND	ND	▼ 5	(4)
CP type					
Soybean proteins *vs*. sunflower *vs*. potato protein	▼ 35	ND	ND	▼ 20	(5)
Soybean *vs*. sunflower meal	NE[4]	ND	ND	▼ 7	(6)
Sunflower meal + proteases *vs*. without proteases	▼ 15%	ND	ND	▼ 7	(6)
Alfalfa *vs*. soluble fibre + soybean isolated	NE	NE	NE	NE	(1)

[1]ND= Not determined; [2]TAB=Total anaerobic bacteria; [3]No mortality occurred in this trial; [4]NE=No effect.
Authors; (1) Chamorro *et al.* (2007a), (2) Chamorro *et al.* (unpublished data), (3) García Palomares *et al.* (2006a), (4) Gidenne and García (2006), (5) Gutierrez *et al.* (2003), (6) García-Ruiz *et al.* (2006).

Protein levels around 14% fed to rabbits from weaning (at 35 d old) to slaughter (2 to 2.7 kg) did not impair growth performance (up to 55 g/d), if DP/DE is around 9.5 and the amino acid supply is correct (de Blas *et al*., 1981; Trocino *et al*., 2000; García-Palomares *et al*., 2006a). With this level of protein it is possible to reduce up to 38% of N-excretion in the fattening period (Maertens *et al*., 1997), and also reduce the mortality (see Table 4). However, this level may not be enough to meet the growth requirements in postweaning diets fed to very young animals (21 to 35 d old) (Maertens *et al*., 1997; Feugier *et al*., 2006). Protein and amino acid requirements are relatively high in young rabbits, not only for tissue accretion but also because of the high need for intestinal growth (Lebas and Laplace, 1972; Trocino *et al*., 2000), and maintenance of the intestinal mucosa functionality. From 21 to 42 d of age, there is an exponential growth of the enzymatic and immunological mechanisms that allows the nutrient assimilation and protection against pathogens (Lebas and Laplace, 1972; Knight and Crane, 1994; Dasso *et al*., 2000; Lanning *et al*., 2000; Campín *et al*., 2003). These results would support the use of higher dietary protein levels (around 16-18%) in the postweaning period (from 21 to 35 d) in early- weaned rabbits. After that, protein content in the diet might be reduced to 14-16%, depending on the knowledge about essential amino acid supply.

Due to the relatively slower daily gains after weaning, the higher weight of gut maintenance on total requirements can significantly increase the relative needs for certain essential and non-essential amino acids with respect to advanced stages of growth. In addition, the defence mechanisms of the intestinal barrier can have specific needs for amino acids. Thus, threonine is a major component of mucin proteins, whereas glutamate is the main amino acid used by enterocytes as an energy source which plays an essential role in the repairing mechanisms of mucosa tissue (Le Floc'h and Séve, 2000; Reeds *et al*., 2000). Recent studies in rabbits (Chamorro *et al*., 2007b and 2007c; Baylos *et al*., 2008) indicate that dietary supplementation with glutamine reduced the mortality caused by ERE, modified ileal microbiota (with a decrease in the frequency of detection of several pathogens such as *C. perfringens* and *Helicobacter* spp), and diminished the presence of *Eimeria* spp in the jejunum. Therefore, a reduction in the protein level, even when the supply of most limiting amino acids for growth is maintained (lysine, sulphur and threonine), may reduce the supply of other essential or non-essential amino acids that could also affect growth performance or

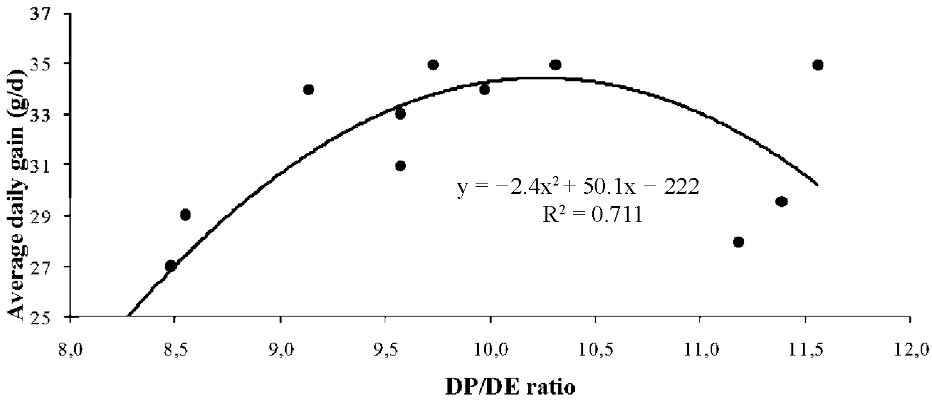

Figure 4: Relationship between average daily gain (g/d) and the DP/DE ratio in the growing period (from de Blas *et al.*, 1981).

mortality. Accordingly, very low levels of protein (12%) have been related with low growth performance and incremental increases in the mortality rate (de Blas *et al.*, 1981) (Figures 2 and 4).

For lactating does, a reduction in protein level (from 18 to 16%) with a DP/DE ratio of 11.5 g/MJ in the late lactation (21 to 35 d) did not affect performance of rabbit does and their litters (García-Palomares *et al.*, 2006b). The protein supply to lactating does after the 21 d of lactation could be decreased to 11.5 g DP/MJ DE, taking into account the decrease in milk yield, which corresponds to the lowest value recommended by Xiccato (1996) and de Blas and Mateos (1998) for highly productive does. As Xiccato *et al.* (2006) suggested, this might also help to reduce the N-excretion as nitrogen intake during lactation, accounting for one-third of the total nitrogen amount.

In practical situations, the proposed dietary CP reduction is not easy to accomplish. As mentioned above, certain conditions are necessary to obtain adequate performance. Better knowledge of amino acid requirements and improving the characterization of protein and amino acid value of raw materials and diets are necessary to minimize the risk of CP reduction. Furthermore, the use of synthetic amino acids is necessary to avoid excess protein.

NITROGEN AND AMINO ACID EVALUATION

The first step to formulate balanced diets with a reduced CP content is the characterisation of the protein value of the feedstuff in terms of nitrogen and amino acid availability. Furthermore, the ileum is the last segment where the amino acids are absorbed, so in other non ruminant species, ileal digestibility is considered the most precise unit to estimate the real availability of the amino acids for animal protein synthesis. Faecal digestibility has however been the unit used up to now for feedstuff evaluation in rabbits.

Ileal and faecal digesta contains large amounts of protein of endogenous origin: 3.8 and 2.5 g/100 g DMI, at ileal and faecal level, respectively (García *et al.*, 2004; Llorente *et al.*, 2006) originating from digestive secretions, epithelial cells and mucins or micro-organisms. This endogenous protein represents about 64% of the total nitrogen flow both at ileal and faecal level. The relative importance of endogenous protein varies with the DM intake, but also varies with the type of diet and the CP origin. Thus, for a diet based on peas or soybean hulls with the same intake and similar chemical composition, the endogenous

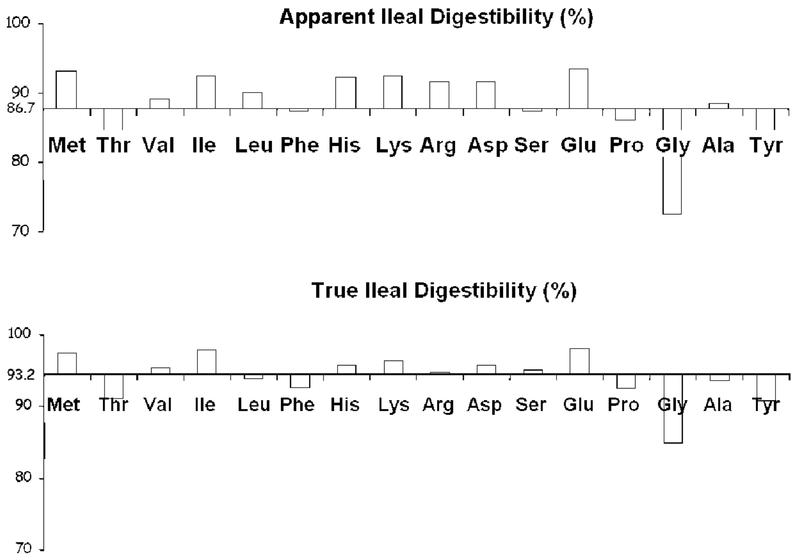

Figure 5: Apparent and true ileal amino acid digestibility of soybean meal with respect to its crude protein digestibility.

protein at the ileal level represents 65 and 55%, respectively. The amino acid composition of endogenous protein at ileal and faecal level is shown in Table 5. The endogenous protein at ileum or faeces contains high concentrations of some essential (Thr, Val, Leu, Ile and Lys) and non-essential (Gln, Gly and Asp) amino acids. For a more reliable analysis of digestible protein and amino acids of feedstuffs, a correction for endogenous losses must therefore be performed. When this correction is done, a new unit arose and is referred to as "standardised" or "true" (TID) instead of "apparent" (AID) ileal digestibility in non-ruminant species (Carabaño *et al.*, 2000).

The variation of each amino acid with respect to CP ileal digestibility for soybean meal is shown in Figure 5. In particular, some amino acids (glycine and threonine) are considerably less digestible than protein, whereas others, such as methionine or isoleucine, are more digestible (from -14 to +6 points for AID and from 8 to 5 points 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, which further leads to a decrease in the variation of amino acid digestibility with respect to protein digestibility.

Recent work in collaboration with feed companies aimed to evaluate the main protein sources used in rabbit diets. Ileal (apparent and true) and faecal (apparent) digestibility of CP and of limiting amino acids of these raw materials are shown in Table 6. True ileal digestibility of CP is relatively high (average of 80.8%), while the apparent values (average of 65.7%) are 15 points lower due to the great importance of endogenous losses. Apparent faecal digestibility (AFD) of CP gives intermediate values (an average of 75.6%), indicating a significant disappearance of protein in the large intestine, although at a different rate for each amino acid. Threonine seems to disappear to a larger extent (from 4 to 46 points) than methionine, while showing higher ileal than faecal values (0.2 to 5.2 points) for some feedstuffs. The important microbial activity in the caecum leads to wide changes in the amino acid composition of digesta and, consequently, the faecal amino acid balance leads to an unclear interpretation (García *et al.*, 2005). Using fistulated does, values for CP faecal digestibility agree with average dietary values (73.2%, n = 164, Xiccato *et al.*, 2003) as determined in growing rabbits.

Table 5: Amino acid composition of endogenous flow at ileal and faecal level (g per 16 g nitrogen).

	García *et al.* (2004)	Llorente *et al.* (2006)	
	Ileum	Ileum	Faeces
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
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

There are important differences between feedstuffs both in protein and amino acid digestibility, mainly connected with their CP content (r = 0.91, 0.81 and 0.61 for AFD, AID and TID, respectively), and with the type of protein (concentrates *vs.* forages or fibrous by-products). Apparent ileal digestibility of CP and threonine for the different feedstuffs determined in rabbits are lower than those obtained in pigs (INRA, 2002), whereas lysine and methionine AID values are similar. However, both CP and limiting amino acids TID values are higher for almost all feedstuffs than standard ileal digestibility as determined in pigs, due to the higher importance of endogenous protein in rabbits. TID evaluation is time consuming and expensive because of the use of semi-purified diets supplied to cannulated animals and the amino acid analysis. Therefore, we attempted to predict them from easier and less costly methods. In this respect, encouraging results were obtained using an *in vitro* method (Llorente *et al.*, 2007b) as developed for pigs and adapted to rabbits (Ramos *et al.*, 1992). Although the *in vitro* CP digestibility was higher than the corresponding *in vivo* values (22.5, 11.9 and 5.8 points as averages for AID, AFD and TID, respectively), the precision of their estimation was high (i.e., the coefficient of variation was lower than 5.5% for TID) even when the *in vitro* CP digestibility was used as a predictor.

Once the amino acids of the raw materials are evaluated for ileal digestibility, the other objective that must be approached in the near future is their specific requirements. As previously mentioned, amino acid requirements, both for growing and doe rabbits, have been expressed on the basis of total or AFD (see Table 1). There are no recommendations of apparent or true ileal digestible amino acids, but the values should be different. In an attempt to study the practical effect of formulating with these units, the AID and TID threonine requirements were estimated from the raw material composition of experimental diets used to obtain threonine requirements (de Blas *et al.*, 1998). The "estimated" AID and TID threonine requirements should be 0.37 and 0.51%, respectively. When ileal values were used instead of total ones

Table 6: Digestibility (%) of the most important sources of protein in rabbit diets (García *et al.*, 2005; Llorente *et al.*, 2005, 2006, 2007a).

	Apparent Faecal Digestibility				Apparent Ileal Digestibility				True Ileal Digestibility			
	CP	Lys	Met	Thr	CP	Lys	Met	Thr	CP	Lys	Met	Thr
Soybean meal (47% CP)	95.9	96	97	91.9	86.8	92.5	93.2	81.7	93.2	96.4	97.4	91.1
Full-fat soybean	91.4	94.4	94.1	88.0	82.3	90.3	91.0	76.4	90.1	95	96	87.6
Soybean hulls	65.3	75.2	72.7	66.9	31.3	62.2	60.1	20.7	52.9	72.9	71.7	49.2
Sunflower meal (28% CP)	82.9	86.4	91.4	79.8	75.6	88.4	91.6	74.3	87	98	95.3	89.8
Sunflower meal (34% CP)	85.2	79.6	92.1	77.1	80.7	84.5	93.8	73.8	86.1	91.5	96.7	74.4
Sunflower meal (36% CP)	84.9	85.4	92	80.7	79.8	85.3	92.7	76.5	88.1	93.1	95.5	88.4
Peas	81.7	88.6	83.5	72.7	75.5	90	87.5	63.4	87.9	97.1	97.2	83.8
Wheat	75.9	75.7	86.4	56.4	67	74.6	88.7	44	89.1	94.2	99.3	84.3
Maize	65.8	63.5	78.4	42.1	49.4	63.2	81.8	15.8	78.2	92.2	94.0	62.0
Barley	68.3	59.7	73.5	53.3	61.9	61.6	80.3	45.7	79.6	79.2	89.8	72.5
Wheat shorts	68.1	74.2	81.2	51.3	65.8	78.0	86.8	44.1	84.4	93.5	96.6	77.2
Wheat bran	56.1	48.0	60.2	52.6	52.9	47.2	69.0	43.9	69.8	65.2	79.1	74.4
Gluten feed	75.7	82.6	85.3	65.9	65.5	78.1	85.0	49.8	78.1	88.5	92.2	71.5
Alfalfa hay	68.3	76.2	75.5	60.7	54.3	71.8	76.9	43.5	73.4	85.8	87.3	69.3
Alfalfa hay	69.1	55.0	69.6	50.3	59.1	59.4	74.4	56.2	74.2	71.7	84.2	75.2

(Table 6) in a practical formula, the price decreased (3.3 and 2.8%, for AID and TID, respectively), favouring the inclusion of concentrates and cereal by-products instead of forages.

Over the last few years, many advances have been achieved in the nitrogen nutrition of rabbits; there are however many opportunities to expand knowledge on amino acid metabolism further in order to meet specific requirements for improving health and welfare of rabbits.

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