# NEW CONCEPTS AND OBJECTIVES FOR PROTEIN-AMINO ACID NUTRITION IN RABBITS

**Carabaño R.[1]*, Villamide MJ.[1], García J.[1], Nicodemus N.[1], Llorente A.[1], Chamorro S.[1], Menoyo D.[1], García-Rebollar P.[1], García-Ruiz A.I.[2], de Blas J.C.[1]**

[1]Dept. Producción Animal, Universidad Politécnica de Madrid, E.T.S. Ingenieros Agrónomos, 28040, Madrid, Spain
[2]Nutreco Poultry and Rabbit Research Centre, Casarrubios del Monte, 45950, Toledo, Spain
*Corresponding author: rosa.carabano@upm.es

## ABSTRACT

In the European context, the new legislation to avoid mineral contamination and the ban of antibiotics as growth promoters has led to the definition of new objectives with respect to nitrogen supply. The present study summarizes the state of nitrogen nutrition in rabbits and reviews the role of protein and amino acids on rabbit health and the new nitrogen value of protein sources based on the 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 the ileal nitrogen flow has 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 are summarized. Furthermore, the use of this different unit for amino acid digestibility is discussed.

**Key words**: Amino acids, Ileal digestibility, Microbial growth, Intestinal health, Nutrition, Rabbit.

## INTRODUCTION: A HISTORICAL PERSPECTIVE

Knowledge on 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 paucity of knowledge relative to that reported in ruminant (cattle or sheep) or non ruminant species (pigs or poultry). Also, rabbits are relatively less important with respect to other livestock species, and combined with a slow rate of industrialization, might have contributed to this fact, despite the historical wide contribution of rabbit to the protein supply of the human diet.

From 1940's to early 1970's, the rabbit was considered a laboratory animal and consequently, research was focused to investigate 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, 1964; Yoshida *et al*., 1968, 1971, 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, 1973) has been determined by several authors. From these studies, it was concluded that caecal microbiota are able to use non-protein compounds (as urea) and that the caecotrophy contributes to improve N digestion and retention. However, these studies also affirm that this extra N can not 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, 1975a, 1975b) and those of Davison and Spreadbury (1975) and Spreadbury (1978) led to confirm the essentiality of these amino acids and the high requirements of arginine, in contrast to that observed in poultry. Furthermore, 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).

**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 |
| Energy (MJ/kg) | 10.5 | 10.5 | 10.5 | 11.0 | 10.5 | 11.1 |
| CP (%) | 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 | 0.68 | 0.70 |
| Digestible (%) | | | | | 0.47 | 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 respect to optimal dietary levels of protein for growth, in the early 1980's, our research at UP Madrid was focused to set 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 days) (de Blas *et al.*, 1981, 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 with digestible energy. Therefore, 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 the growth rate and the 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, 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 among the amino acid profile of milk and muscle protein (Moughan *et al.*, 1988; Partridge, 1989) may compromise 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, 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 breeds, 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 consider a new unit for amino acid supply, addressing the need to determine not only "crude" but also "digestible" (at faecal level) amino acid requirements. This unit brings into 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 moment 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 7$^{th}$ World Rabbit Congress (Carabaño *et al.*, 2000) suggested that faecal balance is not correct to characterize the absorption of amino acids at ileal level, and consequently to meet the amino acid requirements. Consequently, recent studies have 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.

Therefore, until the 21$^{th}$ century, the dietary supply of protein and amino acids had the primary and traditional objective to meet rabbit requirements for production. As crude or total units have been widely used in practical diet formulation, an excess of protein is typical in commercial diets. Furthermore, the tendency in the last decade to increase the dietary fibre level and reduce the 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%). However, in the European context, the new legislation to avoid mineral contamination and the ban of antibiotics as growth promoters has led to define new objectives with respect to the 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. According to Maertens *et al.* (2005), 2/3 of N excretion is explained by nitrogen ingestion and has been recently reviewed by Xiccato *et al.* (2006). Therefore, the reduction of dietary protein level could be an effective strategy to satisfy new laws. Furthermore, this reduction may also help to control the growth of pathogenic bacteria that promote higher mortality in the post-weaning period. The experience in pigs and poultry has confirmed the utility of this strategy, but also has brought up the important role of some essential or semi-essential amino acids on gut health.

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

## THE ROLE OF NITROGEN IN MICROBIAL GROWTH

The 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 contains a great 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 DM) while nitrogen is the second in importance (about 15% of total DM) (Table 2).

**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) |
|---|---|---|
| DM | 150 | 60 |
| CP | 24 | 9 |
| Starch | 22 | 0-2 |
| NDF | 52 | 42 |

This latter 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 NDF components) and the high content of endogenous substances in the nitrogen residues (about 65%, see the following sections), both components may equally contribute to resident intestinal microbiota maintenance.

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 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 the 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. Other nitrogen source for microbial growth is the urea recycled into the caecum through the blood.

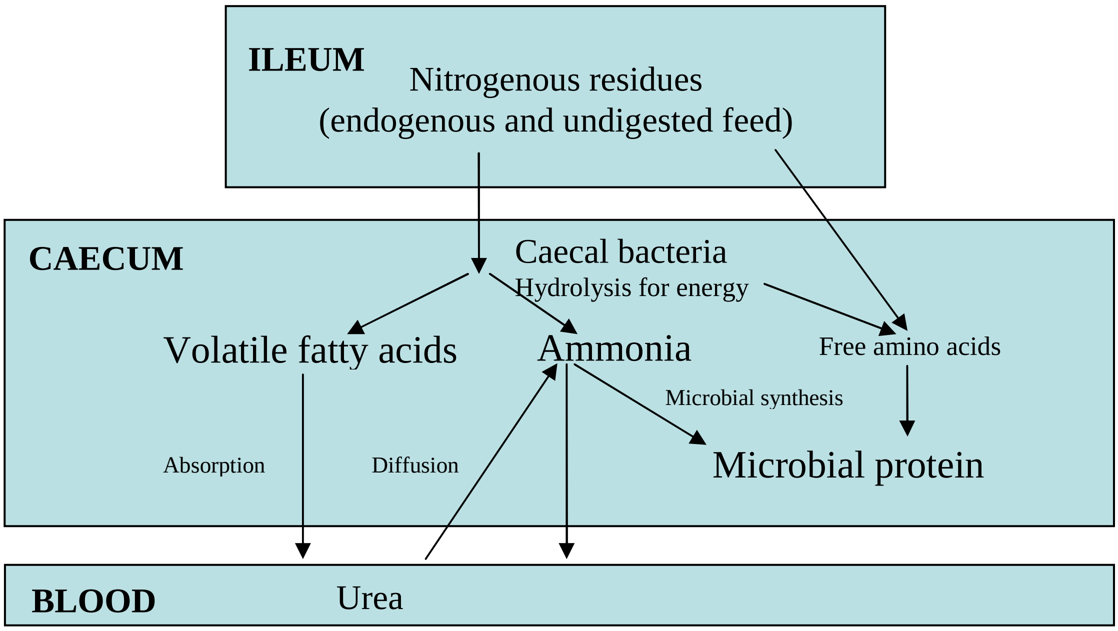


**Figure 1**: Caecal nitrogen metabolism

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) 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 NITROGEN ILEAL 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 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 diets containing excessive protein concentration.

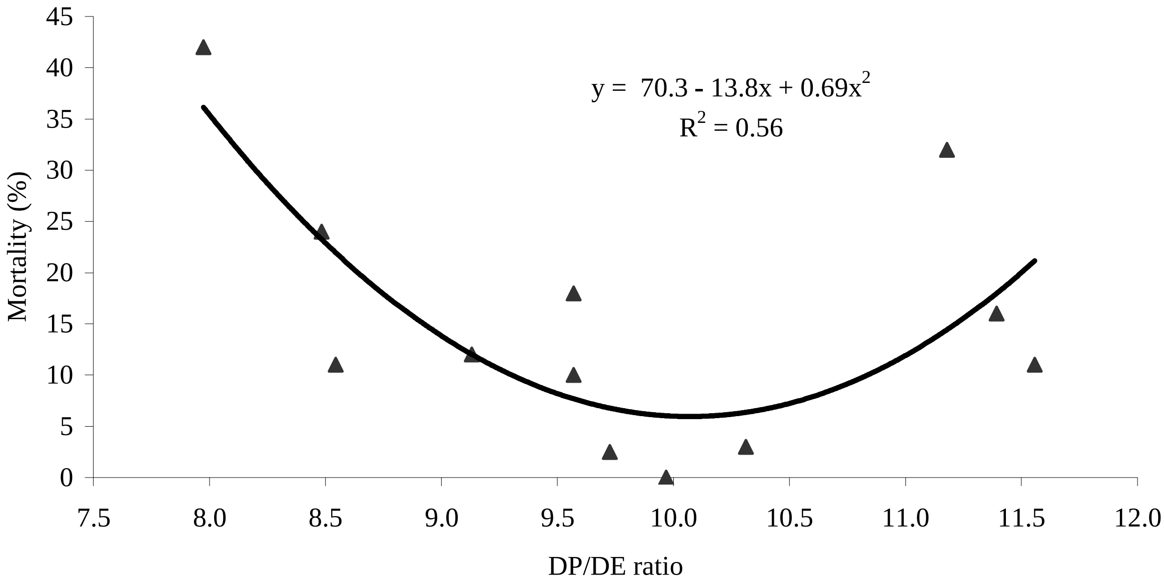


**Figure 2**: Relationship between mortality (%) and the DP/DE ratio in the growing period (from de Blas *et al.*, 1981)

This hypothesis has now been confirmed with the development of ileal digestibility techniques in the post weaning period (Blas *et al.*, 2000; Carabaño and Merino, 2005). 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 pathogens bacteria (*Clostridium perfringens*) and mortality (10 points) due to Epizootic Rabbit Enteropathy (Table 3).

**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 |

Further reductions of protein (from 16 to 14% CP; Chamorro *et al.*, 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%) of the dietary CP level on mortality (5 points) and morbidity due to different pathologies including ERE (Gidenne and García, 2006).

Another way to reduce the nitrogen flow is by including in the diet highly ileal digestible sources. 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%). Sunflower meal or soybean protein concentrated (ethanol treated) showed 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).

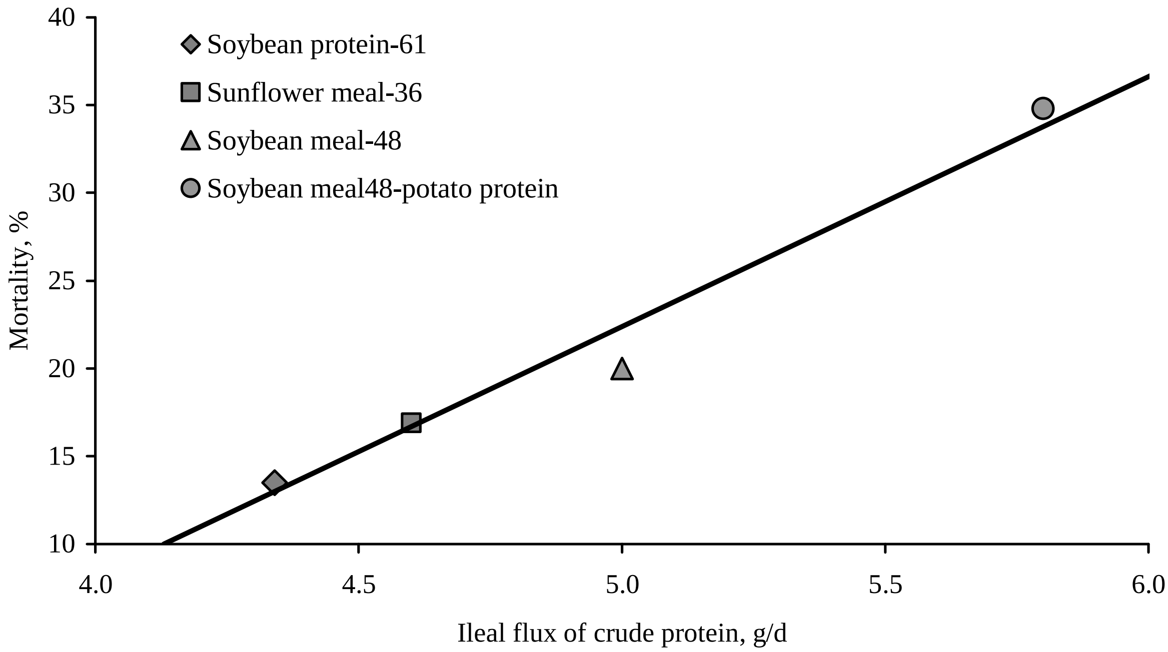


**Figure 3**: Effect of ileal flux of crude protein on mortality from 25 to 60 days of age (Gutiérrez *et al.*, 2003)

The dietary addition of proteases could also help to reduce nitrogen flow but mainly in the post weaning 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 the 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 the sunflower diets. The presence of anti-nutrive factors or allergenic compounds in soybean based diets might exert an additional effect on the mortality. The source of protein may also affect the mucosal integrity of 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 and without 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 it 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 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.

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, 2007b). However, this contribution is variable and is mainly influenced by DM intake, 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 their consequences on the mortality is 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 contribute 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 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, the soluble fibre may also protect intestinal mucose 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.

Above mentioned 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. However, more studies are needed to track ileal nitrogen origin and other characteristics.

**Table 4:** Effect of level and type of dietary CP on intestinal health and ileal CP flow

| Diets | Ileal flow | *C. perfringens* | Other bacteria | Mortality | Authors |
|---|---|---|---|---|---|
| ***CP level:*** | | | | | |
| 18 vs. 16 % CP | ▼ 16% | ▼ | | ▼ 10 points | Chamorro *et al.*(2007) |
| 16 vs. 14 % CP | ▼ 10% | ▼ | | ▼ 5 points | Chamorro *et al.* (unpublished data) |
| 16 vs. 14% CP | ND[1] | ND | ▼ total anaerobic bacteria | No mortality | García-Palomares *et al.* (2006a) |
| 18 vs. 14% CP | ND | ND | ND | ▼ 5 points | Gidenne and García (2006) |
| ***CP type:*** | | | | | |
| Soybean proteins vs. sunflower vs. potato protein | ▼ 35% | ND | ND | ▼ 20 points | Gutierrez *et al.* (2003) |
| Soybean vs. sunflower meal | No effect | ND | ND | ▼ 7 points | García-Ruiz *et al.* (2006) |
| Sunflower meal + proteases | ▼ 15% | ND | ND | ▼ 7 points | García-Ruiz *et al.* (2006) |
| Alfalfa vs. soluble fibre + soybean isolated | No effect | No effect | No effect | No effect | Chamorro *et al.* (2007) |

[1]ND= not determined.

## PRACTICAL CONSEQUENCES OF A REDUCTION OF DIETARY PROTEIN SUPPLY

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

Protein levels around 14% fed to rabbits from weaning (at 35 days 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 days 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 needs for intestinal growth (Lebas and Laplace, 1972; Trocino *et al.*, 2000), and maintenance of the intestinal mucosa functionality. From

21 to 42 days of age, there is an exponential growth of the enzymatic and immunological mechanisms that allows the nutrient assimilation and the protection against pathogens (Lebas and Laplace, 1972; Knight and Crane, 1994; Dasso *et al.*, 2000; Lanning *et al.*, 2000; Campín *et al.*, 2003).

Due to the relatively slower daily gains after weaning, the higher weight of gut maintenance on total requirements can increase significantly 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, 2007c; Baylos *et al.*, 2008) indicate that dietary supplementation with glutamine reduced the mortality caused by ERE, modified ileal microbiota (with a decrease of 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 of 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 to growth performance or 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).

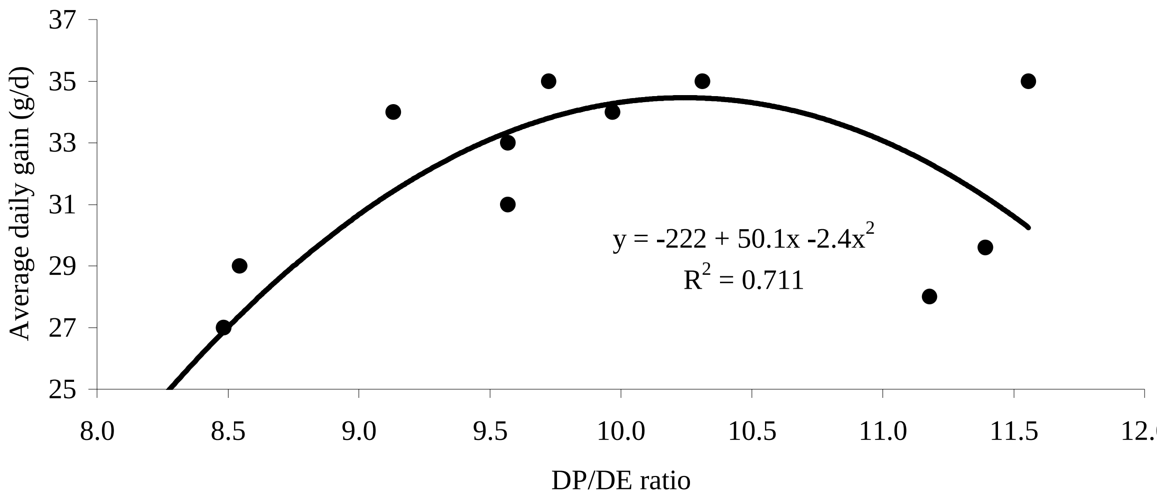


**Figure 4**: Relationship between average daily gain (g/d) and the DP/DE ratio in the growing period (from de Blas *et al.*, 1981)

For lactating does, a reduction of protein level (from 18 to 16%) with a DP/DE ratio of 11.5 g/MJ in the late lactation (21 d 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 21st d of lactation could be decreased to 11.5 g DP/MJ DE, taking into account the decrease of 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 contribute to reduce the N-excretion as nitrogen intake during the 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, some conditions are necessary to obtain adequate performance. A better knowledge of amino acid requirements and improving the characterization of protein and AA 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 the excess of 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, ileum is the last segment where the amino acids are absorbed, so in other non ruminant species, the ileal digestibility is considered the most precise unit to estimate the real availability of the amino acids for animal protein synthesis. However, up to now faecal digestibility has been the unit used for feedstuff evaluation in rabbits.

Ileal and faecal digesta contains important 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 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.

**Table 5**: Amino acid composition of endogenous flow at ileal and faecal level

| | 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 |

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. Therefore, for a more reliable analysis of digestible protein and amino acids of feedstuffs, a correction for endogenous losses must be performed. When this correction is done, a new unit arose and is referred 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 the 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.

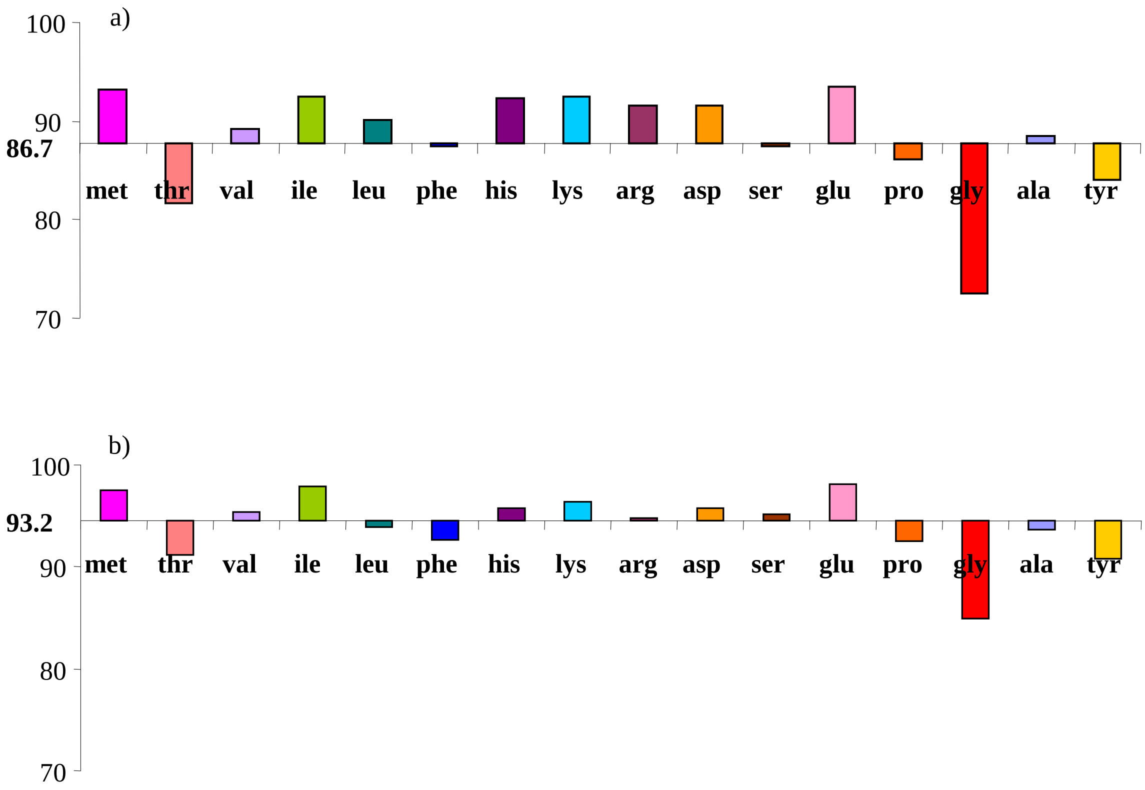


**Figure 5**: Apparent (a) and true (b) ileal amino acid digestibility (%) of soybean meal with respect to apparent (86.7%) and true (93.2%) crude protein digestibility

García *et al.* (2004, 2005) conducted several studies 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.

**Table 6**: Digestibility of the most important sources of protein in rabbit diets

| | 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)[1] | 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[1] | 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[1] | 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[1] | 69.1 | 55.0 | 69.6 | 50.3 | 59.1 | 59.4 | 74.4 | 56.2 | 74.2 | 71.7 | 84.2 | 75.2 |

From Llorente *et al.* (2005, 2006, 2007a); [1]From García *et al.* (2005).

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 shows intermediate values (average of 75.6%), indicating an important 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.

There are important differences among feedstuffs both in protein and amino acid digestibility, being mainly related to 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 the standard ileal digestibility as determined in pigs, due to the higher importance of endogenous protein in rabbits. The 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 sense, 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 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) 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.

## CONCLUSIONS

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

## ACKNOWLEDGEMENTS

Financial support was provided by the Spanish Comisión Interministerial de Ciencia y Tecnología (Projects AGL 2002-05, AGL 2005-03203 and PETRI 95-0689-OP), the Ministery of Industry (Project CDTI, 04-0136) and feed companies NUTRECO, NANTA and COREN.

## REFERENCES

Adams C.E. 1983. Reproductive performance of rabbits on a low protein diet. *Lab. Anim., 17, 340-345*.

Adamson I., Fisher H. 1971. The amino acid requirements of the growing rabbit: Qualitative needs. *Nutr. Rep. Int., 4, 59-64*.

Adamson I., Fisher H. 1973. Amino acid requirement of the growing rabbit: An estimate of quantitative needs. *J. Nutr., 103, 1306-1310*.

Blas E., Falcao L., Gidenne T., PinheiroV., García A.I., Carabaño R. 2000. Measurement of ileal digestibility in rabbits: An inter-laboratory study to compare two markers and to frequencies of digesta collections. *World Rabbit Sci., 8, 131-138.*

Baylos M., Menoyo D., Chamorro S., Sainz A., Nicodemus N., de Blas C., Carabaño R. 2008. Effect of dietary level and source of glutamine on intestinal health in the postweaning period. *In Proc. 9th World Rabbit Congress, 2008 June, Verona, Italy, 529-534.*

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

Cano J.L., Blas E., Soler M.D., Moya V.J., Guillén M.I. 2004. Immune response induced by feed in growing rabbits. *In Proc. 8th World Rabbit Congress, 2004 September, Puebla, Mexico, 765-770.*

Carabaño R., Merino J.M. 2003. Efecto de la cecotrofia sobre la composición química de la digesta y sobre la digestibilidad ileal. *ITEA, 24, 657-659.*

Carabaño R., de Blas C., García A.I. 2000. Recent advances in nitrogen nutrition in rabbits. *World Rabbit Sci., 8, 14-28.*

Carabaño R., García J., de Blas J.C. 2001. Effect of fibre source on ileal apparent digestibility of non-starch polysaccharides in rabbits. *Anim. Sci., 72, 343-350.*

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., de Blas J.C. 2008. New trends in rabbit feeding: Influence of nutrition on intestinal health. *Spanish J. Agri. Res., 6 (Sp. Iss.), 15-25.*

Chamorro S., Gomez Conde M.S., Perez De Rozas A.M., Badiola I., Carabaño R., de Blas J.C. 2007a. 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.*

Chamorro S., Carabaño R., Badiola I., García J., de Blas J.C. 2007b. Effet d'une supplémentation alimentaire en glutamine et en arginine sur la croissance, quelques paramètres de la flore iléale et caecale et la santé intestinale chez le lapereau, sevré précocement et atteint par le syndrome de l'EEL. *In Proc. 12èmes Journées Recherche Cunicole, 2007 November, Le Mans, France, 89-92.*

Chamorro S., Carabaño R., Grant G., García J., de Blas J.C. 2007c. Efecto de la adición de glutamina y arginina sobre la mortalidad y barrera intestinal de gazapos. *In Proc. II Congreso Ibérico Cunicultura, 2007 June, Vila-Real, Portugal, 91-94.*

Cheeke P.R. 1971. Arginine, lysine and methionine needs of the growing rabbits. *Nutr. Rep. Int., 3, 123-128*.

Colin M. 1974. Supplémentation en lysine d'un régime a base de tourteau de sésame chez le lapin. Effets sur les performances de croissance et le bilan azoté estimé par deux méthodes. *Ann. Zootech., 23, 119-132*.

Colin M. 1975a. Effets sur la croissance du lapin de la supplémentation en l-lysine et en dl-méthionine de régimes végétaux simplifiés. *Ann. Zootech., 24, 465-474*.

Colin M. 1975b. Effet de la teneur en arginine du régime sur la croissance et le bilan azoté chez le lapin: relation avec le taux de lysine *Ann. Zootech., 24, 629-638*.

Dasso J.F., Obiakor H., Bach H., Anderson A.O., Mage R.G. 2000. A morphological and immunohistological study of the human and rabbit appendix for comparison with the avian bursa. *Dev Comp Immunol., 24, 797-814.*

Davidson J., Spreadbury D. 1975. Nutrition of the New Zealand White rabbit. *Proc. Nutr. Soc., 34, 75-83*.

de Blas J.C., Mateos G.G. 1998. Feed formulation. *In: de Blas J.C., Wiseman J.(Eds). The Nutrition of the Rabbit. CABI Publishing. CAB International, Wallingford, Oxon, UK, 241-253*.

de Blas J.C., Pérez E., Fraga M.J., Rodriguez J.M., Gálvez J.F. 1981. Effect of diet on feed intake and growth of rabbits from weaning to slaughter at different ages and weights. *J. Anim. Sci., 52, 1225-1232*.

de Blas J.C., Fraga MJ., Rodriguez J.M. 1985. Units for feed evaluation and requirements for commercially grown rabbits. *J. Anim. Sci., 60, 1021-1028*.

de Blas J.C., Taboada E., Nicodemus N., Campos R., Piquer J., Mendez J. 1998. Performance response of lactating and growing rabbits to dietary threonine content. *Anim Feed Sci. Technol., 70, 151-160*.

Dojana N., Costache M., Dinischiotu A. 1998. The activity of some digestive enzymes in domestic rabbits before and after weaning. *Anim. Sci., 66, 501-507.*

Emaldi O., Crociani F., Matteuzzi D. 1979. Bacteria in the caecal content, soft and hard faeces of rabbit. *J. Appl. Bacteriol., 46, 169-172.*

Feugier A., Smit M.N., Fortun-Lamothe L., Gidenne T. 2006. Fibre and protein requirements of early weaned rabbits and the interaction with weaning age: effects on digestive health and growth performance. *Anim Sci., 82, 493-500.*

Forsythe S.J., Parker D.S. 1985. Ammonia nitrogen turnover in the rabbit caecum and exchange with plasma urea. *Br. J. Nutr., 54, 285-292*.

Fraga M.J. 1998. Protein digestion. *In: de Blas J.C., Wiseman J. (Eds). The Nutrition of the Rabbit. CABI Publishing. CAB International, Wallingford, Oxon, UK, 39-54.*

Fraga M.J., Pérez de Ayala P., Carabaño R., 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. *J. Anim. Sci., 69, 1566-1574*.

Fraga M.J., de Blas J.C., Pérez E., Rodríguez J.M., Pérez C.J., Gálvez J.F. 1983. Effect of diet on chemical composition of rabbits slaughtered at fixed body weights. *J. Anim. Sci., 56, 1097-1104.*

Gaman E., Fisher H. 1970. The essentiality of arginine, lisyne and methionine for growing rabbits. *Rep. Int., 1, 57-64*.

García A.I., de Blas J.C, Carabaño R. 2004. Effect of diet (casein-based or protein-free) and caecotrophy on ileal endogenous nitrogen and amino acid flow in rabbits. *Anim. Sci., 79, 231-240.*
García A.I., de Blas J.C, Carabaño R. 2005. Comparison of different methods for nitrogen and amino acid evaluation in rabbit diets. *Anim. Sci., 80, 169-178.*
García J., Pérez-Alba L., Alvarez C., Rocha R., Ramos M., de Blas J.C. 1995. Prediction of nutritive value of lucerne hay in diets for growing rabbits. *Anim. Feed Sc. Technol., 54, 33-44.*
García J., Nicodemus N., Carabaño R, de Blas J.C. 2002. Effect of inclusion of defatted grape seed meal in the diet on digestion and performance of growing rabbits. *J. Anim. Sci., 80, 162-170.*
García-Palomares J., Carabaño R., García-Rebollar P., de Blas J.C., Corujo A., García-Ruiz A.I. 2006a. Effects of a dietary protein reduction and enzyme supplementation on growth performance in the fattening period. *World Rabbit Sci., 14, 231-236.*
García-Palomares J., Carabaño R., García-Rebollar P., de Blas J.C., García A.I. 2006b. Effects of dietary protein reduction during weaning on the performance of does and suckling rabbits. *World Rabbit Sci., 14, 23-26.*
García-Ruiz A.I., García-Palomares J., García-Rebollar P., Chamorro S., Carabaño R., de Blas J.C. 2006. Effect of protein source and enzyme supplementation on ileal protein digestibility and fattening performance in rabbits. *Span. J. Agric. Res., 4, 297-303.*
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. *Br. J. Nutr., 67, 133-147.*
Gidenne T., García J. 2006 Nutritional strategies improving the digestive health of the weaned rabbit. *In: Maertens L., Coudert P. (Eds.) Recent advances in rabbit sciences. Ilvo, Merelbeke, Belgium, 211-227.*
Gómez-Conde M.S., García J., Chamorro S., Eiras P., García Rebollar P.G., Perez De Rozas A., Badiola I., de Blas C., Carabaño R. 2007. Neutral detergent-soluble fiber improves gut barrier function in 25 d old weaned rabbits. *J. Anim. Sci., 85, 3313-3321.*
Gutiérrez I., García P., Carabaño R., de Blas C. 2000. Effect of supplementation with animal plasma and antibiotics on jejunal morphology of early-weaned rabbits. *World Rabbit Sci., 8, 263-267.*
Gutiérrez I., Espinosa A., García J., Carabaño R., de Blas J.C. 2003. Effect of source of protein on digestion and growth performance of early-weaned rabbits. *Anim. Research, 52, 461-472.*
Haffar A., Laval A. Guillou J.P. 1988. Entérotoxémie à Clostridium spiroforme chez des lapins adultes. *Le Point Vétérinaire, 20, 99-102.*
Hill R.R.H. 1986. Digestion of mucin polysaccharides in vitro by bacteria isolated from the rabbit cecum. *Curr. Microbiol., 14, 117-120.*
Hoover W.H., Heitmann R.N. 1975. Cecal nitrogen metabolism and amino acid absorption in the rabbit. *J. Nutr., 105, 245-252.*
INRA 1984. L'alimentation des Animaux Monogastriques: Porc, Lapin, Volailles. *Institute de la Recherche Agronomique, Paris, France.*
INRA 2002. Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage. *In: Sauvant D., Perez J.M., Tran G. (Eds). INRA Editions, Paris, France.*
Knight K.L., Crane M.A. 1994. Generating the antibody repertoire in rabbit. *Adv. Immunol., 56, 179-218.*
Lanning D., Sethupathi P., Rhee K.J., Zhai S.K., Knight K.L. 2000. Intestinal microflora and diversification of the rabbit antibody repertoire. *J. Immunol., 165, 2012-2019.*
Lebas F. 1988. Rabbits. *Liv. Prod. Sci., 19, 289-298.*
Lebas F., 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'age. *Ann Zootech., 21, 337-347.*
Lebas F., Cousin M.C., Sardi G. 1973. Effet de la teneur en protéines de rations a base de soja ou de sésame sur la croissance du lapin. *Ann. Zootech., 22, 83-92.*
Le Floc'h N., Séve B. 2000. Le devenir des protéines et des acides aminés dans l'intestin du porc: de la digestion à l'apparition dans la veine porte. *Prod. Anim., 13, 303-314.*
Llorente A., García A.I., Nicodemus N., Villamide M.J., 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 Proc. XI Jornadas sobre Producción Animal ITEA, 2005 May, Zaragoza, Spain, 26, 497-499.*
Llorente A., García A.I., Nicodemus N., Villamide M.J., Carabaño R. 2006. Digestibilidad ileal aparente y verdadera de aminoácidos de harinas de girasol, productos de soja y guisante en conejos. *In Proc. XXXI Symposium de Cunicultura de Asescu, 2006 May, Lorca, Spain, 117-124.*
Llorente A., Villamide M.J, García A.I., Carabaño R. 2007a. Digestibilidad de la proteína de los aminoácidos de cereales y sus subproductos en conejos. *In Proc. II Congreso Ibérico de Cunicultura, 2007 June, Vila-Real, Portugal, 87-90.*
Llorente A., Villamide M.J, García A.I., Chamorro S., Carabaño R. 2007b. Prédiction de la digestibilité iléale azotée par méthodes in vitro. *In Proc. 12$^{èmes}$ Journées Recherche Cunicole, 2007 November, Le Mans, France, 93-96.*
Maertens L., de Groote G. 1988. The effect of the dietary protein-energy ratio and lysine content on the breeding results of does. *Archiv Fur Geflugelkunde, 52, 89-95.*
Maertens L., Štruklec M. 2006. Technical note: preliminary results with a tannin extract on the performance and mortality of growing rabbits in an enteropathy infected environment. *World Rabbit Sci. 14, 189-192.*
Maertens L, Luzi F, DeGroote G. 1997. Effect of dietary protein and amino acids on the performance, carcass composition and N-excretion of growing rabbits. *Ann. Zootech., 46, 255-268.*
Maertens L., Cavani C., Petracci M. 2005. Nitrogen and phosphorus excretion on commercial rabbit farms: calculations based on the input-output balance. *World Rabbit Sci., 13, 3-16.*
McWard G.W, Nicholson L.B., Poulton B.R. 1967. Arginine requirement of the young rabbit. *J. Nutr., 92, 118-120.*
Merino J.M., Carabaño R. 1992. Effect of type of fibre on ileal and fecal digestibilities. *J. Appl. Rabbit Res., 15, 931-937.*

Motta W., Fraga M. J., Carabaño R. 1996. Inclusion of grape pomace, in substitution for alfalfa hay, in diets for growing rabbits. *Anim. Sci., 63, 167-174*.

Moughan P.J., Schultze W.E., Smith W.C. 1988. Amino acid requirements of growing meat rabbit. 1. The amino acid composition of rabbit whole-body tissue-a theoretical estimate of ideal amino acid balance. *Anim. Prod., 55, 153-162*.

NRC 1977. Nutrient requirements of rabbits. *National Academic of Science, Washington DC, USA*.

Parigi Bini R., Xiccato G., Cinetto M. 1990. 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., Cinetto M. 1991. Utilizzazione e ripartizione dell'energia e della proteina digeribile in coniglie non gravide durante la prima lattazione. *Zootec. Nutr. Anim., 17, 107-120*.

Parigi Bini R., Xiccato G., Cinetto M., Dalle Zotte A. 1992. Energy and protein utilization and partition in rabbit does concurrently pregnant and lactating. *Anim. Prod., 55, 153-162*.

Partridge G.G. 1989. Nutrition of farmed rabbits. *Proc. Nutr. Soc*., *48, 93-101*.

Partridge G.G., Allan S.J. 1982. The effects of different intakes of crude protein on nitrogen utilization in the pregnant lactating rabbit. *Anim. Prod., 35, 145-155*.

Partridge G.G., Lobley G.E., Fordyce R.A. 1986. Energy and nitrogen metabolism of rabbits during pregnancy, lactation, and concurrent pregnancy and lactation. *Br. J. Nutr., 56, 199-207*.

Penney R.L., Folk Jr. G.E., Galask R.P., Petzold C.R. 1986. The microflora of the alimentary tract of rabbit in relation to pH, diet and cold. *J. Appl. Rabbit Res., 9, 152-156*.

Proto V. 1976. Fisiologia della nutrizione del coniglio con particolare riguardo alla ciecotrofia. *Rivista di Coniglicoltura 13(7), 15-33*.

Ramos M.A., Carabaño R., Boisen S. 1992. An in vitro method for estimating digestibility in rabbits. *J. Appl. Rabbit Res., 15, 938-946*.

Reeds P.J., Burrin D.G., Stoll B., Jahoor F. 2000. Intestinal glutamate metabolism. *J. Nutr., 130, 978S-982S*.

Rerat A. 1978. Digestion and absorption of carbohydrates and nitrogenous matters in the hindgut of the omnivorous nonruminant animal. *J. Anim. Sci., 46, 1808-1837*.

Sanchez W.K., Cheeke P.R., Patton N.M. 1985. Effect of dietary crude protein level on the reproductive performance and growth of new zealand white rabbits. *J. Anim Sci., 60, 1029-1039*.

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

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. *Brit. J. Nutr., 39, 601-603*.

Taboada E., Méndez J., Mateos G.G., de Blas J.C. 1994. The response of highly productive rabbits to dietary lysine content. *Liv. Prod. Sci., 40, 329-337*.

Taboada E., Méndez J., de Blas J.C. 1996. The response of highly productive rabbits to dietary sulphur amino acid content for reproduction and growth. *Reprod. Nutr. Develop., 36, 191-203*.

Thacker E.J., Brandt C.S. 1955. Coprophagy in the rabbit. *J. Nutr., 55, 375-385*.

Trocino A., Xiccato G., Sartori A., Queaque P.I. 2000. Feeding plans at different protein levels: effects on growth performance, meat quality and nitrogen excretion in rabbits. *In Proc. 7th World Rabbit Congress, A. Blasco (Ed.), Valencia Univ. publ., Spain, Vol. C, 467-474.*

Vente-Spreeuwenberg M.A.M., Verdonk J.M.A.J., Bakker G.C.M., Beynen A.C., Verstegen M.W.A. 2004. Effect of dietary protein source on fed intake and intestinal morphology in newly weaned piglets. *Livest. Prod. Sci., 86, 169-177*.

Villamide M.J., Maertens L., de Blas C., Pérez J.M. 1998. Feed formulation. *In: de Blas J.C., Wiseman J. (Eds). The Nutrition of the Rabbit. CABI Publishing. CAB International, Wallingford, Oxon, UK, 89-101.*

Xiccato G. 1996. Nutrition of lactating does. *In Proc. 6th World Rabbit Congress, F. Lebas (Ed.), AFC Publ., Toulouse, France, Vol. 1, 29-47*.

Xiccato G., Parigi Bini R., Cinetto M., Dalle Zotte A. 1992. The influence of feeding and protein levels on energy and protein utilisation by rabbit does. *J. Appl. Rabbit Res., 15, 965-972*.

Xiccato G., Trocino A., De Boever J.L., Maertens L., Carabaño R., Pascual J.J., Pérez J.M., Gidenne T., Falcao-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). *Anim. Feed Sci. Technol., 104, 153-168.*

Xiccato G., Trocino A., Nicodemus N. 2006. Nutrition of the young and growing rabbit: a comparative approach with the doe. *In: Maertens L., Coudert P. (Eds.) Recent advances in rabbit sciences. Ilvo, Merelbeke, Belgium, 239-246.*

Yoshida T., Kandatsu M. 1960. Studies on cecum digestion. V. Nitrogen in the water-soluble fraction of cecal contents, hard feces, and soft feces of the rabbit. *Jap. J. Zootech. Sci., 31, 181-187*.

Yoshida T., Kandatsu M. 1964. Studies on cecum digestion. VI. Free amino acids in the cecal contents, hard feces, and soft feces of the rabbit. *Jap. J. Zootech. Sci., 35, 64-69*.

Yoshida T., Pleasants J.R., Reddy B.S., Wostmann B.S. 1968. Efficiency of digestion in germfree and conventional rabbits. *Brit. J. Nutr., 22, 723-737*.

Yoshida T., Pleasants J.R., Reddy B.S., Wostmann B.S. 1971. Amino acid composition of cecal contents and feces in germfree and conventional Rabbits. *J. Nutr., 101, 1423-1430.*

Yoshida T., Pleasants J.R., Reddy B.S., Wostmann B.S. 1972. The pH values and nitrogen fractionations of cecal contents and feces of germfree and conventional rabbits. *Jap. J. Zoot. Sci., 43, 284-289*.