

Effect of protein source and enzyme supplementation on ileal protein digestibility and fattening performance in rabbits

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Abstract

An experiment was conducted to determine the effects of source of protein (soybean vs sunflower meal) and enzyme supplementation (no enzyme vs protease addition vs protease + xylanase addition) on digestion and growth traits of rabbits. Treatments were arranged factorially in six isonutritive diets. Two hundred and forty rabbits weaned at 25 or 35 days were used to measure dry matter and crude protein apparent ileal digestibility of diets at 35 or 45 days of age, respectively. Another one hundred and eighty animals weaned at 35 days were fed *ad libitum* in individual cages during four weeks to determine fattening performance. Source of protein did not affect digestion efficiency or feed conversion rate in the whole fattening period, but animals fed soybean meal-based diets showed higher feed intake, grew faster and had higher mortality than those fed sunflower meal-based diets. Addition of enzymes increased ileal apparent digestibility, especially in youngest animals when supplements contained xylanase besides protease activity. Enzyme supplementation did not affect any of the growth traits studied, but decreased fattening mortality in sunflower meal-based diets.

Additional key words: digestion, exogenous enzymes, growth traits, soybean meal, sunflower meal.

Resumen

Efecto de la fuente de proteína y de la suplementación con enzimas sobre la digestibilidad ileal de la proteína y los rendimientos productivos de conejos en cebo

Se realizó un experimento para determinar los efectos de la fuente de proteína (harina de soja vs harina de girasol) y de la suplementación enzimática (no suplementación vs adición de proteasas vs adición de proteasas + xilanasas) sobre parámetros digestivos y de crecimiento en conejos en cebo. Los tratamientos se estructuraron factorialmente en seis piensos isonutritivos. Se usaron doscientos cuarenta conejos destetados a los 25 ó 35 días de edad para medir la digestibilidad ileal aparente de la materia seca y de la proteína bruta de los piensos a los 35 ó 45 días de edad, respectivamente. Un grupo adicional de 180 animales destetados a los 35 días de edad fue alimentado *ad libitum* en jaulas individuales durante 4 semanas para determinar rendimientos productivos en el periodo de cebo. La fuente de proteína no afectó a la eficacia digestiva o a la conversión del alimento en el conjunto del periodo de cebo, pero los animales alimentados con piensos basados en soja crecieron más rápido y presentaron una mayor mortalidad que los que recibían dietas basadas en harina de girasol. La adición de enzimas incrementó la digestibilidad ileal aparente, cuando los suplementos contenían actividad xilanásica además de proteásica, especialmente en los animales más jóvenes. La suplementación con enzimas no afectó a ninguno de los parámetros de crecimiento estudiados, pero redujo la mortalidad en cebo en piensos basados en harina de girasol.

Palabras clave adicionales: digestión, enzimas exógenas, harina de girasol, harina de soja, parámetros de crecimiento.

Introduction

Intensive rearing systems are increasingly used at present in commercial rabbit farms. They are associated

to weaning at early ages (around 35 days) to reduce parturition interval and to increment numerical productivity of rabbit does. As a consequence, gut pathologies in young rabbits are frequent because of the incomplete development of the digestive physiology at these ages (Lebas *et al.*, 1971; Dojana *et al.*, 1998; Gutiérrez *et al.*, 2002a).

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The supply of balanced diets might help to control postweaning mortality by limiting pathogen microbiota populations. In this way, previous work showed that a decrease of the flow of nutrients (as starch or lactose) towards the fermentative area reduced diarrhoea incidence (Gutiérrez *et al.*, 2002a,b). Other studies also indicated that an increase of the nitrogen flow reaching the terminal ileum incremented the populations of *Clostridia* spp. (Haffar *et al.*, 1988), total anaerobic bacteria (García-Palomares *et al.*, 2006) and those of *Helicobacter* spp., *Campylobacter* spp. and highly pathogenic *Clostridium perfringens* (Chamorro *et al.*, 2005). A positive relationship between the level of crude protein in the diet and the intestinal proliferation of *C. perfringens* has also been described in other non-ruminant species, as broiler chickens (Drew *et al.*, 2004) and dogs (Zentek *et al.*, 2004). These results might contribute to explain the increment in the incidence of enteric disorders and mortality reported in fattening rabbits when dietary crude protein content increased (De Blas *et al.*, 1981; Maertens and De Groote, 1988; Chamorro *et al.*, 2005), or crude protein ileal digestibility decreased (Gutiérrez *et al.*, 2003).

The aim of this trial was to determine the effect of the dietary supplementation with two types of exogenous enzymes in rabbit feeds based either on soybean (*Glycine max* L.) or sunflower meal (*Helianthus annuus* L.) on its ileal digestion efficiency, and to try to relate the resulting daily ileal flows of dry matter and protein to fattening rabbit performance, including mortality.

Material and Methods

Diets

Six experimental diets were factorially (2×3) arranged with two protein sources (sunflower meal, T1 to T3, or soybean meal, T4 to T6) supplemented or not with Pescazyme 5602[®] or Porzyme 8300[®], supplied by Danisco, Marlborough, UK. The Pescazyme 5602[®] supplement contained 4,240 U g⁻¹ of protease subtilisin (EC. 3.4.21.62). The Porzyme 8300[®] supplement contained 2,827 U g⁻¹ of protease subtilisin (EC. 3.4.21.62) and 5,426 U g⁻¹ of endo-1,4 beta-xylanase (EC 3.2.1.8). All the experimental diets were formulated to be iso-nutritive and to meet or exceed the nutrient requirements of growing rabbits (De Blas and Mateos, 1998). The enzymes were added to the feed at different doses (1 g kg⁻¹ of Pescazyme 5602[®] and 1.5 g kg⁻¹ of Porzyme

8300[®]) in order to provide the same protease activity but different xylanase activity. The ingredient composition and chemical analysis of the diets is shown in Table 1. All the feeds included 5 g kg⁻¹ of lucerne hay fibre mordanted with ytterbium according to the procedure described by Uden *et al.* (1980). Ytterbium was used as indigestible marker. The marked fibre was produced from dehydrated alfalfa washed with detergent in an automatic washing machine. Diets were pelleted and animals were given *ad libitum* access to feed and water during all the experiment.

Animals and housing

All the experiments were carried out with New Zealand x Californian rabbits from the H(AV) x R line

Table 1. Ingredient and chemical composition of experimental diets (g kg⁻¹)

	Source of protein	
	Sunflower meal	Soybean meal
Barley	260	220
Wheat bran	220	240
Lucerne hay	195	195
Wheat straw	46.7	82.7
Sunflower hull	19.5	56.1
Sunflower meal 36	220	—
Soybean meal 46	—	170
Soybean oil	20	20
DL Methionine	0.10	0.70
L-Lysine	3.00	—
L-Threonine	0.70	0.45
Sodium chloride	5.00	5.00
Vitamin/mineral premix ¹	5.00	5.00
Fibre + Yb ²	5.00	5.00
<i>Determined nutrient composition, g kg⁻¹ DM</i>		
Dry matter	892	887
Crude protein	194	202
Neutral detergent fibre	347	345
Acid detergent fibre	188	192
Acid detergent lignin	42.3	48.4

¹ Provided by Trouw Nutrition España S.A. (Madrid, Spain): Mineral and vitamin composition (mg kg⁻¹ of feed): Mg, 290 (MgO); NaCl, 329; S, 275; Co, 0.7 (CoCO₃.1H₂O); Cu, 10 (CuSO₄.5H₂O); Fe, 76 (FeSO₄.1H₂O); Mn, 20 (MnO); Zn, 59.2 (ZnO); I, 1.25 (KI); choline chloride, 250; riboflavin, 2; niacin, 20; pyridoxine, 1; menadione sodium bisulfite, 1; α-tocopherol, 20; thiamine, 1; retinol, 8,375 IU kg⁻¹ of feed; and cholecalciferol, 750 IU kg⁻¹ of feed. ² Lucerne hay fibre marked with Ytterbium.

selected at the Politechnical University of Valencia, Spain. Animals were housed individually in flat-deck wired cages measuring 350 × 460 × 290 mm. A cycle of 12-h of light and 12-h of dark was used throughout the experiment. The light was switched on at 07:30. Heating and forced ventilation systems allowed the building temperature to be maintained between 15 and 24°C. Rabbits were handled according to the principles for the care of animals in experimentation published in the Spanish Royal Decree 1201/2005 (BOE, 2005).

Digestibility trial

A total of two hundred and forty rabbits, weaned at 25 or 35 days of age and weighing respectively 441 ± 25 or 777 ± 31 g, were randomly blocked by litter without regard to their sex and allocated to each experimental diet (20 animals per weaning age and diet). Following a 10-days feed adaptation period (at 35 and 45 days of age, respectively), animals were slaughtered by cervical dislocation between 19:00 and 22:00 to minimize the influence of caecotrophy. The last 20 cm of ileum were excised and ileal contents were removed, frozen, and freeze-dried. Samples were then ground and because of the small quantity of sample, pooled in groups of two rabbits of the same treatment to measure dry matter (DM), crude protein (CP) and ytterbium. Ileal digestibility of DM and CP were determined by the dilution technique using ytterbium as a marker and according to the following equations:

$$\text{Apparent ileal DM digestibility (\%)} = [1 - (\text{dietary ytterbium concentration} / \text{ileal ytterbium concentration})] \times 100.$$

$$\text{Apparent ileal CP digestibility (\%)} = [1 - (\text{dietary ytterbium concentration} \times \text{ileal CP concentration} / \text{ileal ytterbium concentration} \times \text{dietary CP concentration})] \times 100.$$

Growth trial

One hundred and eighty rabbits (30 per diet) weaned at 35 days of age and weighing 825 ± 71 g were blocked by litter without regard to their sex and assigned at random to the different treatments. Animals fed the experimental diets during four consecutive weeks. Feed intake, weight gain and mortality of the rabbits was determined from 35 to 49 and from 49 to 63 days of age.

Analytical methods

All chemical analyses were conducted in duplicate. Procedures of the AOAC (2000) were used to determine DM (930.15), and CP (954.01) of diets and ileal content. Neutral-detergent fibre, acid-detergent fibre and acid-detergent lignin were determined according to the sequential method of Van Soest *et al.* (1991). Ytterbium content of diets and ileal digesta were analysed by atomic absorption spectrometry (Smith Hieftje 22, Thermo Jarrel Ash, MA, USA) using pre-dosed samples of faeces to prepare common matrix standards. Previously, samples were ashed (600°C) and then digested by boiling with a solution of 1.5 M HNO₃ and 0.05 M KCl.

Statistical analysis

Data were analysed as a completely randomised block (litter) design with type of diet as main effect, by using the GLM procedure of the SAS software (SAS Institute, 1991). Mortality was considered as a continuous variable because of the large number of repetitions per treatment (n = 30). Weaning weight was used as a linear covariate in all the traits studied. Orthogonal contrasts were made to test for the effect of source of protein, enzyme supplementation, type of enzyme and their interactions. The effect of age and of the interactions of age with type of diet was also analyzed in digestibility traits. A covariance analysis (GLM procedure, SAS Institute, 1991) was made to predict fattening mortality, using daily ileal flows of DM and CP as linear covariates, by pooling data from this and previous experiments. Regression procedures (SAS Institute, 1991) were used to relate daily live weight gain to ileal digestible CP consumption.

Results

Digestibility trial

The apparent ileal digestibilities (AID) of the six experimental diets are shown in Table 2. Average digestibilities of DM and CP were higher when measured in the younger (35 days) than in the older (45 days) animals (47.2 vs 44.5% for DM and 72.6 vs 70.7% for CP, P = 0.013 and P = 0.003, respectively). Source of protein had no influence, but supplementation with

Table 2. Effect of protein source and enzyme supplementation on apparent dry matter and protein ileal digestibility (%) of diets at two ages

	Source of protein						Significance of contrast ^{1,2}				
	Sunflower meal			Soybean meal							
	Control	Pesczyme	Porzyme	Control	Pesczyme	Porzyme	1	2	3	4	5
Treatments											
	T1	T2	T3	T4	T5	T6					
<i>35 days</i> ³											
DM	40.6	46.9	56.6	42.9	45.1	50.9	NS	< 0.001	< 0.001	NS	NS
CP	70.4	72.9	75.9	69.8	72.0	74.7	NS	< 0.001	0.07	NS	NS
<i>45 days</i> ³											
DM	39.5	46.3	48.8	39.0	43.7	49.6	NS	< 0.001	0.09	NS	NS
CP	67.7	71.5	73.0	67.9	71.7	72.6	NS	< 0.001	NS	NS	NS

¹ SEM: Standard error of means (n = 10): 1.78 for DM and 1.06 for CP apparent ileal digestibility, NS: non significant (P > 0.15).

² Contrasts: 1, source of protein (T1+T2+T3 vs T4+T5+T6); 2, enzyme supplementation (T1+T4 vs T2+ T3+T5+T6); 3, type of enzyme (T2+T5 vs T3+T6); 4, interaction source of protein x enzyme supplementation (T2+T3-T1 vs T6+T5-T4); 5, interaction source of protein x type of enzyme (T3-T2 vs T6-T5). ³ Age has a significant effect both on DM (P = 0.013) and CP (P = 0.003) apparent ileal digestibility. An interaction (P = 0.15) between age and type of enzyme on DM apparent ileal digestibility diet was found. Other interactions between age and type of diet were non significant (P > 0.15).

enzymes increased significantly (P < 0.001) the AID of DM and CP (by 19.7 and 6.0% as average, respectively). The effect of enzyme supplementation on AID of DM and CP tended to be more evident in the youngest animals when it contained xylanase besides protease activity. No significant effect of any of the other interactions studied was observed on these traits.

Growth trial

The effect of treatments on performance in the two-weeks after weaning (35-49 days) and in the whole fattening period (35-63 days) is shown in Table 3. Animals fed diets based on soybean meal showed a higher average daily gain (51.0 vs 49.1 g, P = 0.005) and feed intake (138 vs 132 g, P = 0.001), but a similar feed conversion ratio, and had a higher mortality (11.1 vs 4.44%; P = 0.09) than those fed on sunflower meal-based diets in the period from 35 to 63 days of age. Growth rate and the effect of source of protein were higher in the first two-weeks after weaning than in the whole fattening period, where a higher (by 2.5%, P = 0.05) feed efficiency for soybean meal-based diets was also observed. Enzyme supplementation did not affect significantly any of the traits at any of the periods studied. However, an interaction source of protein

x enzyme supplementation was observed on the mortality incidence in the whole fattening period (P = 0.06), as the addition of both types of enzymes reduced the mortality with respect to the control diet in sunflower-based diets, whereas no significant response was detected in soybean-based diets.

Discussion

The higher values of AID of DM and CP observed in the younger animals (35 vs 45 days) were not expected, as several studies (Lebas *et al.*, 1971; Dojana *et al.*, 1998; Gutiérrez *et al.*, 2002a) have shown that the activities of pepsin and pancreatic enzymes increased with age in weaning rabbits. These results might be partially explained by a relatively lower ileal flow of endogenous nitrogen at 35 than at 45 days of age. Endogenous losses constitute a significant proportion of the apparent ileal flow in rabbits, and are highly dependent on feed intake (García *et al.*, 2004), which in turn increases sharply after weaning.

The source of protein did not affect AID of the CP of the experimental diets. This result agrees with previous work that reported a similar faecal (Maertens and De Groote, 1984; Fekete and Gippert, 1986; Villamide *et al.*, 1991; Llorente *et al.*, 2005, 2006) and ileal

Table 3. Effect of protein source and enzyme supplementation on growth performance and mortality at different periods

	Source of protein						SEM	Significance of contrasts				
	Sunflower meal			Soybean meal				1	2	3	4	5
	Control	Pesczyme	Porzyme	Control	Pesczyme	Porzyme						
	T1	T2	T3	T4	T5	T6						
<i>Period 35-49 days</i>												
Daily gain, g	56.9	54.8	59.7	60.6	61.8	61.1	1.43	<0.001	NS	NS	NS	NS
Daily feed intake, g	117	112	118	122	122	123	2.54	0.002	NS	NS	NS	NS
Feed conversion rate, g g ⁻¹	2.08	2.10	1.99	2.02	1.99	2.02	0.03	0.05	NS	NS	0.06	NS
Mortality, %	3.33	0.00	3.33	3.33	6.67	10.00	3.76	NS	NS	NS	NS	NS
<i>Period 35-63 days</i>												
Daily gain, g	48.3	48.3	50.7	51.0	51.1	50.8	0.77	0.005	NS	NS	NS	NS
Daily feed intake, g	130	130	135	139	138	138	2.24	<0.001	NS	NS	NS	NS
Feed conversion rate, g g ⁻¹	2.69	2.70	2.66	2.72	2.70	2.71	0.02	NS	NS	NS	NS	NS
Mortality, %	10.00	0.00	3.33	6.67	16.67	10.00	4.75	0.09	NS	NS	0.06	NS

SEM: standard error of means (n = 30), NS: non significant (P > 0.15). Contrasts: 1, source of protein (T1+T2+T3 vs T4+T5+T6); 2, enzyme supplementation (T1+T4 vs T2+ T3+T5+T6); 3, type of enzyme (T2+T5 vs T3+T6); 4, interaction source of protein x enzyme supplementation (T2+T3-T1 vs T6+T5-T4); 5, interaction source of protein x type of enzyme (T3-T2 vs T6-T5).

digestibility of CP (Gutiérrez *et al.*, 2003; García *et al.*, 2005; Llorente *et al.*, 2005, 2006) in partially dehulled sunflower meal (36% CP) than in soybean meal. However, animals fed sunflower-based diets showed a lower daily ileal CP flow (5.86 vs 6.41 g d⁻¹ in the soybean-based diets), determined from the average AID of CP at 35 and 45 days and the average CP consumption from 35 to 49 days of age. This effect might be explained because of both a lower feed intake and CP concentration in the sunflower-based diets. Ileal flows of DM and CP also decreased in parallel to the improvement of AID observed with enzyme supplementation. The highest effect on DM and CP AID values in the youngest animals was obtained with the enzyme complex containing xylanase besides of proteolytic activity. Fibre digestion is limited in rabbits because of the short fermentation time in this species (De Blas *et al.*, 1999), and develops with age (Marounek *et al.*, 1995), which would explain the increase of DM digestion efficiency observed in the present experiment with the use of exogenous fibrolytic enzymes in early-weaned (25 days) rabbits. The additional improvement of CP digestibility with xylanase addition might be related to a higher accessibility of proteolytic enzymes to dietary protein when cell wall polysaccharides are partially predigested (Bedford, 1995; Villamide and Fraga, 1998).

Both the substitution of soybean with sunflower meal and the enzyme supplementation of sunflower meal-based diets reduced significantly fattening mortality. This effect might be related to the parallel decrease observed in the flow of CP to the terminal ileum. Figure 1 shows the significant linear relationship (+5.1 ± 2.0% of fattening mortality per each 1 g d⁻¹ increment of ileal CP flow; P = 0.03) obtained between these variables in this study, together to our previous works using a similar methodology. When DM flow was used

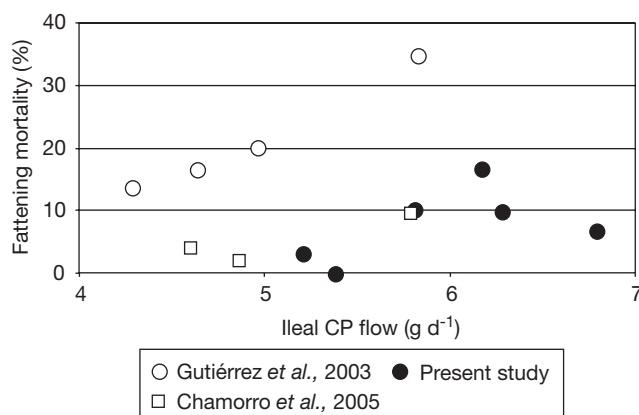


Figure 1. The effect of the average ileal CP flow in the two-weeks after weaning period on the average mortality in the whole fattening period according to several experiments.

instead of CP flow as the independent variable in the model, the relationship was less significant ($P > 0.15$). These results could be explained by the proliferation of harmful bacteria as *Clostridia* spp. and *Escherichia coli* with an increase in the ileal flow of protein in rabbits (Haffar *et al.*, 1988; Cortez *et al.*, 1992). Recently, Chamorro *et al.* (2005) have also found a positive correlation between the ileal CP flow and the frequency of detection at the terminal ileum of *C. perfringens*. A toxin produced by this bacterium has been described as the more probable factor causing epizootic rabbit enteropathy, a pathology responsible of high mortality rates in commercial farms that affects mainly to young animals aged between 3 and 10 weeks (Pérez de Rozas *et al.*, 2005).

None of the exogenous enzymes studied reduced fattening mortality when supplemented soybean-based diets. The presence in soybean meal of antigenic substances has been related to a higher diarrhoea incidence in several non ruminant species, including rabbits (Scheele and Bolder, 1987). This anti-nutritive activity could be not altered by the exogenous enzymes used in this trial, and might also contribute to explain the higher mortality observed with soybean than with low-antigenic sources of protein as sunflower meal in this and in previous studies (Gutiérrez *et al.*, 2003).

Live weight gain was very high in the two first-weeks after weaning period (59.1 g d^{-1} , as average). Figure 2 indicates a linear ($P = 0.02$) and a trend ($P = 0.15$) for a quadratic effect of ileal digestible CP consumption (idCPc, calculated as the difference between CP intake and ileal total CP flow, determined as previously described), on the average daily live weight gain in this period (ADG35-49). The regression equation obtained was:

$$\begin{aligned} \text{ADG35-49} = & -283 (\pm 184) + 43.0 (\pm 24.6) \text{idCPc} - \\ & - 1.34 (\pm 0.82) \text{idCPc}^2; \\ n = & 6; R^2 = 0.90; P = 0.03. \end{aligned}$$

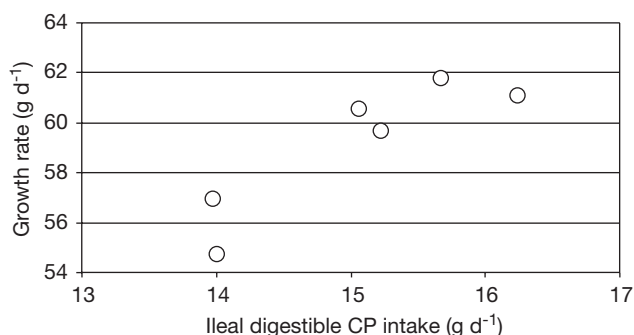


Figure 2. The effect of ileal digestible CP intake on live weight gain during the two-weeks after weaning period.

From this equation it can be derived that maximal growth rate in this period in highly growing rabbits might be reached for a value of idCPc of 16.0 g d^{-1} . However, more information is necessary to assess this recommendation, as this optimal value is close to the upper extreme value studied. The effect of diets on weight gain during the whole fattening period was smaller, which suggests a decrease of CP requirements with age.

The results from this study indicate that sunflower meal should be preferred to soybean meal in starter diets for rabbits in order to minimize digestive disorders. They also suggest that enzyme supplementation of sunflower diets might further reduce fattening mortality through a decrease of the amounts of nutrients reaching the fermentative area.

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