World Rabbit Sci. 2006, 14: 231 - 236

EFFECTS OF A DIETARY PROTEIN REDUCTION AND ENZYME SUPPLEMENTATION ON GROWTH PERFORMANCE IN THE FATTENING PERIOD

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Abstract: The effect of a decrease in the dietary crude protein level from 16% to 14%, maintaining the supply of the most limiting amino acids above the recommended levels, and the addition of proteases to the feed during the growing period was investigated. Two diets were formulated to contain 16% or 14% CP and maintaining the same level of total lysine, methionine and threonine (0.77, 0.50 and 0.58%, respectively) by adding synthetic amino acids (14% CP + AA's). To study the effect of protease supplementation, 1 g/kg of commercially available PESCAZYME 5602® was added to the diet containing 16% CP. The three experimental diets were offered *ad libitum* from weaning (35 d of age) to slaughter (63 d) in two growth trials. One hundred and eighty (60 per diet) rabbits were individually housed and 480 rabbits were collectively housed in 120 polyvalent cages (40 cages per treatment) in the growth trials. Forty rabbits (20 per treatment), were assigned to two experimental diets 16% CP and 14% CP + AA's and used to determine total anaerobic bacteria in the ileum and *Clostridium perfringens* in the caecum. There was no mortality either in the individual or collective trial and *Clostridium perfringens* was not detected. However, a reduction of total anaerobic bacteria was detected at ileum (15.9 $\times 10^8 \pm 3$ *vs.* 7.7 $\times 10^8 \pm 0.3$ CFU/g on average for diets 16% CP and 14% CP+AA's, respectively). There was no effect neither for the addition of proteases no for the dietary CP level on growth performances either in the 35 to 49 d period or in the 49 to 63 d period. There was no interaction between type of diet and type of housing. However animals individually caged showed better growth rate and feed conversion rate than animals collectively caged in the whole growing period (55.6 *vs.* 46.1 g/d and 2.19 *vs.* 2.45, respectively). From the results of this study it can be concluded that protein concentration in the diet can be reduced to a level of 14% maintaining the levels of most limiting amino acids above recommended levels and supplying 10g digestible protein per MJ digestible energy without any impairment of growth performance.

Key words: rabbit, dietary protein, protease supplementation, growth performances.

INTRODUCTION

The ban on the use of antibiotics as growth promoters has emphasized the interest in establishing further restrictions to feed formulation aiming to minimize enteric diseases. An excessive supply of protein in the diet could increase mortality in the growing period by leading to a high nitrogen ileal flow (De Blas *et al*., 1981, Gutiérrez *et al.*, 2003; Chamorro *et al*., 2005). Caecal microbiota is able to use protein as source of energy for growth and some pathogenic bacteria, such as *Clostridium perfringens*, are favoured for higher level of ileal flow of nitrogen (Emaldi *et al*., 1979, Chamorro *et al.*, 2005). An interaction with age could also occur, as proteolytic enzyme activity is lower in young than in adult animals (Dojana *et al.*, 1998). Accordingly, the results obtained by García *et al.* (2004 and 2005) showed that dietary addition of proteases reduced the ileal flow and mortality in the

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Received *January 2006* - Accepted *August 2006*.

fattening period. Reducing the dietary level of protein could be a strategy to prevent digestive disorders. However, levels of dietary crude protein (CP) lower than 15% also reduced growth performance (De Blas and Mateos, 1998). Increasing availability of dietary amino acids could permit further reductions of dietary protein maintaining growth performance and reducing nitrogen excretion at the same time (Xiccato *et al.,* 2002). Protein requirements have been established for the whole fattening period. However, dietary requirements could change throughout this period, as they could be higher in the post weaning period, as occurs in other non-ruminant species.

The aim of the present investigation was to study the effect of a decrease in dietary CP level from 16 to 14%, maintaining the supply of the most limiting amino acids, and the addition of proteases to the feed during the growing period of rabbits.

MATERIAL AND METHODS

Diets

A basal diet containing 16% CP and 11.5 g digestible protein (DP) per MJ of digestible energy (DE) was formulated according to the nutrient recommendations of De Blas and Mateos (1998). Another diet was formulated to decrease CP content up to 14% (10.0 g DP/MJ DE) by increasing barley, soybean oil and wheat straw and decreasing alfalfa meal and soybean meal in order to maintain the CP faecal digestibility of diet, according to FEDNA (2003). This diet was supplemented with the most limiting amino acids (lysine, methionine and threonine), maintaining the same supply of total amino acids of diet with 16% CP. To study the effect of protease supplementation, 1 g/kg of commercially available PESCAZYME 5602® (Finnfeeds International Ltd., Marlborough, UK) was added to diet containing 16% CP. This supplement contained 4000 IU/kg of pure subtilisin protease. The ingredient and chemical composition of the three experimental diets are shown in Table 1. Neither feeds nor drinking water were medicated at any time during the trial (only salinomycire was added as coccidiostatic).

Animals and housing

Two growing trials with individually and collectively housed rabbits were performed simultaneously in the Fattening Rabbit Unit at the Poultry and Rabbit Research Centre facilities. In both trials, New Zealand × Californian rabbits weaned at 35 d of age were used. No control of sex was performed. Animals were housed individually in flat-deck cages measuring 35 cm × 46 cm × 30 cm high. Polyvalent cages (38 × 100 × 30 cm) were used to kept 4 rabbits per cage in the collective housing trial. Heating and forced ventilation systems allowed the building temperature to be maintained at 19 ± 4ºC in the two trials. Rabbits were handled according to the principles for the care of animals in experimentation published by the Spanish Royal Decree 223/88 (1988).

Growth trials

One hundred and eighty (60 per diet) weaned rabbits were blocked by litter, assigned at random to the different treatments and individually housed. Growth rate and feed intake was recorded from 35 to 49 days, and from 50 to 63 days of age. Another 480 weaned rabbits were also blocked by litter, assigned at random to experimental diets and collectively housed in 120 polyvalent cages (40 cages per treatment). Growth rate and feed intake was recorded from 35 to 63 days of age. Mortality was recorded daily in both trials.

Microbiological trial

Forty weaned rabbits (20 per diet) weighing 770 ± 14 g were blocked by litter and assigned at random to two experimental diets 16% CP and 14% CP + AA's to determine total anaerobic bacteria in the ileum and *Clostridium perfringens* in the caecum. Following a 10-d adaptation period, the animals (1350 ± 34 g live weight) were slaughtered by cervical dislocation between 19.00 and 21.00 h. The last

Table 1: Ingredient and chemical composition of experimental diets.

	16% CP	16% CP+Pescazyme	14% CP + AA's
Ingredients (% as fed)			
Alfalfa hay (18% CP)	31.0	31.0	29.1
Sunflower meal 30% CP	8.0	8.0	8.0
Soybean meal 44% CP	4.45	4.45	-
Barley grain	21.7	21.7	25
Wheat bran	24.5	24.5	24
Wheat straw	6.3	6.2	8.97
Soybean oil	2.5	2.5	3
Monocalcium phosphate	0.4	0.4	0.4
Sodium chloride	0.5	0.5	0.5
Pescazyme 5602	-	0.1	-
HCl-Lysine	0.15	0.15	0.33
L-Threonine	-	-	0.14
DL, Methionine	-	-	0.05
Premix[1]	0.5	0.5	0.5
Chemical analysis (% as fed)			
Dry matter	92.0	91.9	92.1
Crude protein	16.1	16.5	14.2
NDF	34.2	32.7	34.2
ADF	17.5	17.1	18.1
ADL	4.3	4.45	4.1
Starch	18.6	18.6	19.7
Digestible energy (DE, MJ/kg)[2]	10.2	10.2	9.9
Digestible protein (DP)[2]	11.7	11.7	9.9
DP/DE (g/MJ)	11.5	11.5	10.0
Lysine[2]	0.768	0.768	0.770
Methionine + Cystine[2]	0.540	0.540	0.50
Threonine[2]	0.583	0.583	0.608

[1]Provided by Trouw Nutrition España S·A·(Madrid, Spain). Premix included 0.40% of vitamin and mineral premix and 20 ppm of salinomycin. Mineral and vitamin composition (mg/kg of diet): Mg 290; Na 329; S 275; Co 0·7; Cu 10; Fe 76; Mn 20; Zn 59.2; I 1.25; choline, 250; riboflavin 2; niacin 20; pyridoxine 1; phytylmetaquinone 1; alpha-tocopherol 13; thiamine 1; retinol 2.5; cholecalciferol 0.019

[2] Values estimated according to FEDNA (2003).

20 cm of ileum were taken and ileal contents were removed. Ileum samples from the same treatments were pooled in groups of two rabbits. Ileum and caecum content samples were aseptically taken and transferred into sterile tubes. The sterile tubes were then placed into GENbag anaer 45534 (bioMérieux S.A., Marcy I'Etoile, France) for further determination by keeping the viability of anaerobic microflora. Then, 1 g from each sample was aseptically removed from the sterile tube and transferred to another

sterile tube being diluted from 10^{-1} to 10^{-9} in Buffered Peptona Water BK018HA (Biokar Diagnostic, Zac Dether-Allonne-Beauvais, France) and mixing gently with vortex. From each of the 10^{-8} and 10^{-9} ileum dilution, 0.1 mL was plated onto a Anaerobe Basal Agar CM0972 (Oxoid Ltd., Basingstoke, Hamphsire, UK) supplemented with 5% sterile Defibrinated Horse Blood SR0050 (Oxoid Ltd., Basingstoke, Hamphsire, UK). Regarding *Clostridium perfringens* determinations, from each of the 10^{-1} and 10^{-2} caecum dilution, 0.1 mL was plated onto a Perfringens Agar Base CM0587 (Oxoid Ltd., Basingstoke, Hamphsire, UK) supplemented with Shahidi-Ferguson-Perfringens Selective Supplement SFP SR0093 (Oxoid Ltd., Basingstoke, Hamphsire, UK). All plates were incubated at 37ºC for 3 to 5 days in anaerobic environment placed into GENbag anaer 45534 (bioMérieux S.A., Marcy l'Etoile, France). After incubation colonies were counted and recorded as CFU per g of contents.

Analytical Methods

Chemical analysis of diets was made using the procedure of Van Soest *et al*. (1991) for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignine (ADL), and AOAC (2000) methods for dry matter, crude protein (CP) and starch determinations.

Statistical Analysis

Data were analysed as a completely randomised design with type of diet and type of cage as the main sources of variation by using the General Linear Model (GLM) procedure of SAS (1990). The cage was considered as the experimental unit (n = 40 per treatment) in the collective trial. Initial weight of rabbits was included as linear covariate in the statistical analysis of growth traits. Data in tables are presented as Least square means.

RESULTS AND DISCUSSION

There was no mortality either in the individual or collective trials and *Clostridium perfringens* was not detected. Accordingly, the hypothetic reduction of mortality of young rabbits with a decrease of protein intake could not be tested. However, a reduction of total anaerobic bacteria was detected at ileum in this sense ($15.9\times10^{8} \pm 3$ *vs.* $7.7\times10^{8} \pm 0.3$ CFU/g on average for diets 16% CP and 14% CP + AA's, respectively). According to Emaldi *et al*. (1979), caecal microbiota is able to use protein as a substrate for growth, indicating that main activities were, in decreasing order, ammonia- user, ureolytic, proteolytic and cellulolytic. Besides, a decrease in the dietary level of protein should reduce the nitrogen flow at ileum (Chamorro *et al.*, 2005) and consequently the availability of substrate for bacterial growth.

The effects of dietary CP concentration and protease supplementation on growth performances are shown in Tables 2 and 3. There was no effect of the diet on growth performances both in the 35 to 49 d and in the 49 to 63 d period (Table 2). Accordingly, no effect of diet was observed in the whole fattening period (from 35 to 63 d) either for animals individually or collectively caged (Table 3). No interaction between type of cage and diet were detected. Present recommendations to optimise growth rate and feed efficiency are given in a range from 9.7 to 10.7 g of digestible protein (DP)/MJ of digestible energy (DE). However, these values were obtained from rabbits with a lower growth capacity than present animals (35 *vs.* 55 g/d) (De Blas *et al.*, 1981, Fraga *et al.*, 1983; Fekete and Gippert, 1985), so that an increase of protein requirements with growth potential might be expected. The results obtained in the present experiment (Tables 2 and 3) suggest that a ratio of 10 g DP/MJ DE is enough to maintain growth performances up to 55.6 g/d in individually caged animals. These results could be a consequence of a higher efficiency of the utilization of digestible protein for growth retention in the current breeds and hybrids. According to the review of Fraga (1998), this efficiency increases with growth rate as the relative contribution of maintenance requirements of protein decreases.

Table 2: Effect of dietary crude protein level and supplementation with a protease (Pescazyme) on growth performance of the animals individually housed.

	Diet			SEM[1]	*P-value*
	16% CP	16% CP+Pescazyme	14% CP+AA's		
Initial live weight (35 d), g	789	791	791	13	NS[2]
Intermediate live weight (49 d), g	1495	1494	1499	17	NS
Final weight (63 d), g	2354	2344	2349	39	NS
Period 35-49 d:					
Weight gain, g/d	50.3	50.2	50.6	1.2	NS
Feed intake, g/d	102	104	102	2	NS
Feed conversion rate	2.05	2.10	2.02	0.04	NS
Period 49-63 d:					
Weight gain, g/d	61.4	60.7	60.7	2.5	NS
Feed intake, g/d	138	140	137	3	NS
Feed conversion rate	2.37	2.39	2.35	0.10	NS

[1]SEM = Standard error of means (n =60); [2]NS = non significant ($P > 0.05$)

Growth performance not only depends on an adequate dietary supply of digestible protein but also on amino acids. The results of our study show that the supplementation of the diet with the most limiting amino acids (lysine, methionine and threonine), maintaining the recommended levels (De Blas and Mateos, 1998), permitted a reduction of dietary CP (from 16% to 14%) without negative effects on growth performance throughout the whole fattening period. These results are in agreement with those observed by Xiccato *et al.* (2002). However, a similar reduction in dietary CP level could reduce the growth rate if the supply of other essential amino acids is not taken into account (Maertens *et al.*, 1997). Further investigations on the availability of amino acids in raw materials and the requirements of amino acids are necessary to reduce the dietary protein level without impairment of growth performance.

Dietary supplementation with proteases did not affect any traits studied in any period. Previous results (García *et al.,* 2004 and 2005) showed that the supplementation with protease decreased the mortality in the first 14 d-period after weaning by a reduction of nitrogen flow at ileum. However, this effect could not be tested in our study because of the absence of mortality in all dietary treatments.

Table 3: Effect of dietary crude protein level ,supplementation with protease (Pescazyme) and type of cage on growth performance in the whole growing period (35-63 days).

	Diet			Type of cage		SEM[1]	*P-value*	
	16% CP	16% CP +Pescazyme	14% CP +AA's	Individual	Collective		Diet	Cage
Initial weight (35 d), g	772	773	780	791	759	13	NS[2]	0.003
Final weight (63 d), g	2207	2189	2203	2349	2050	34	NS	0.0001
Weight gain, g/d	51.2	50.6	50.8	55.6	46.1	1.0	NS	0.0001
Feed intake, g/d	117	117	115	121	113	2	NS	0.0001
Feed conversion rate	2.32	2.34	2.30	2.19	2.45	0.04	NS	0.0001

[1]SEM = Standard error of means (n =60); [2]NS = non significant ($P > 0.05$)

The type of housing affected growth performances (Table 3). Animals collectively caged showed lower growth rate (-17.1%) and worse feed conversion rate (+12%) and final weight at 63 d (-12.7%) than animals individually caged. The lower feed intake observed in animals collectively caged (-6.6%) with respect to those individually caged could partially explain the reduction of growth rate. Several authors have reported a lower feed intake in animals collectively caged, although density of animals in the present work (10.5 animals/m^2) is far from those described as critical to limit the voluntary feed intake and to reduce the growth performances and the comfort of the animals (from 15 to 20 animals/m^2) as suggested by Maertens and De Groote (1984) and Aubret and Duperray (1992) and recently reviewed by Trocino and Xiccato (2006). Thus, other aspects of animal behaviour could have contributed to explaining these results. According to the observations of Podberschek *et al.* (1991), the budget time used for feeding is lower in collectively caged animals than in individually caged ones. Furthermore, the animals collectively caged increase the time used for locomotion. This fact could also contribute to explain the reduction of feed efficiency due to the higher maintenance requirements of animals in collective cages.

From the results of this study it can be concluded that the CP concentration in the diet can be reduced to a level of 14% maintaining the levels of most limiting amino acids above requirements and supplying 10 g DP/MJ DE without any impairment of growth performance.

Acknowledgements: This work was supported by a CDTI Project 04-0136.

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