INFLUENCE OF DIETARY PROTEIN REDUCTION AND ENZYME AND/OR AMINO ACID SUPPLEMENTATION ON FATTENING PERFORMANCE OF RABBITS

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ABSTRACT

The effect of dietary protein reduction combined with protease enzyme and/or addition of synthetic limiting amino acids was studied on growth performance of 240 single-housed (n=48/group) and 240 group-housed (3 rabbits/cage, n=48/group) NZW rabbits fed five diets from 35 to 63 d of age. The control diet (C) with 16.7% crude protein (CP) content was supplemented with 0.4% DL-methionine and 0.1% HCl-lysine and contained 0.34% Met and 0.75% Lys. Dietary CP was reduced to 14.2% (three diets) or 13.1% (one diet). The first 14.2% CP diet was supplemented with 1 g/kg protease (Avizyme®1500) but contained 15% less Met and Lys than the control diet (P group). The second 14.2% CP feed was supplemented with more DL-methionine (0.7%) and HCl-lysine (0.3%) than the control to reach the same essential amino acid (AA) dietary levels but without added enzyme (AA group). The third 14.2% CP diet was supplemented with 1 g/kg enzyme plus more AA's (0.7% and 0.3%) than the control (PAA-I group). Compared to the control feed, CP was lowered to 13.1% but the highest level of synthetic Met (0.8%) and Lys (0.4%) plus 1 g/kg enzyme was used in the 13.1% CP diet (PAA-II group). Diets were formulated to have similar DE, EE and CF contents. Growth rates between 35 and 49 d and 49 d body weights were higher (P=0.007) in the C and PAA-I groups (39.0, 1540 and 38.2 g/d, 1528 g), intermediate in PAA-II (37.4 g/d, 1518 g) and lower in the P and AA groups (34.9, 1482 and 34.5 g/d, 1477 g). Between 49 and 63 d, weight gain did not differ but over the whole fattening period it was better (P=0.033) in the PAA-I and C, moderate in PAA-II and poorer in the P and AA groups. Between 35 and 63 d, differences on feed intake and feed conversion among groups were not significant. The 63 d body weight tended to be higher (P=0.063) in the PAA-I, C and PAA-II groups (1995, 1993, 1991 g), intermediate in AA (1933 g) and lower in the P group (1903 g). Health risk was not significantly affected (47.8, 45.3, 52.2, 46.7, 41.6% in order of the C, P, AA, PAA-I, PAA-II groups; P>0.05). In conclusion, reducing the dietary CP content to 14.2% combined with either added protease enzyme or amino acids was not effective because growth rate was significantly decreased. Growth performance was maintained without any increase of health risk even with a 20% lowering of CP from 16.7% to 13.1% if the diet was supplemented with protease plus higher doses of essential amino acids.

Key words: Nitrogen, Protease, Amino acid, Growth.

INTRODUCTION

Rabbit manure is considered an environmental polluter in Europe due to its nitrogen and phosphorus content (Maertens and Luzi, 1996). The EU Directive 91/676/EC stated that member states must identify the vulnerable areas where the load of N of livestock origin cannot exceed 170 kg/ha/year (Xiccato *et al*., 2005). Accordingly, N and P excretion of rabbit farms are intensively studied (Maertens, 1999; Maertens *et al*., 2005; Xiccato *et al*., 2007). The solution to high N and P excretion can be reduction of output and/or the N and P content of rabbit faeces. Dietary N is not totally utilized in the rabbit. That can be improved via nutritional strategies such as dietary protein reduction with or without enzyme and/or amino acid addition (Maertens and Luzi, 1996; Trocino *et al*., 2000; El-Mandy *et al*., 2002; El-Nagmy *et al*., 2002; Zanaty *et al*., 2002; Maertens, 2002; García *et al*., 2005; García-Palomares *et al*., 2006ab) and phase-feeding (Maertens and Luzi, 1997; Xiccato *et al*., 2002).

Suckling rabbits start to consume solid feed at about 17 days of age and generally have access to the high-protein breeding diet of the doe. Before the ban on using antibiotics as growth promoters, enteric diseases due to excess protein in growing feeds could be avoided. Health risk may increase with protein rich, antibiotic free diets. Moreover protein and amino acid requirements vary according to age in association with changes of endogenous digestive enzyme activity and caecal fermentation. Phase-feeding can satisfy the actual nutritional needs of animals but rabbits could susceptible to any change of diet and the farmers prefer to use only one feed during the fattening period.

This work studied the effect of dietary protein reduction from 16.7% to 13.1% combined with protease enzyme and/or supplementation with limiting amino acids on growth performance of rabbits. Analyses of nutrient digestibilities are in progress and not presented here.

MATERIALS AND METHODS

Dietary groups and animals

Rabbits received a diet containing 16.7% crude protein (CP), 0.75% lysine and 0.34% methionine in the Control group (Table 1). Other diets were produced to reduce the CP content by 15% or 20% of control level (i.e. from 16.7 to 14.2 or 13.1%, respectively) by increasing wheat and soybean meal at the expense of alfalfa meal. The first 14.2% CP diet was supplemented with 1 g/kg subtilisin protease enzyme (min. 4000 U/g protease, 400 U/g amylase and 300 U/g xylanase) but contained 15% less Met+Lys than the control diet (P group). The second 14.2% CP feed was supplemented with more synthetic methionine and lysine than the control to reach the same essential amino acid (AA) dietary levels but without added enzyme (AA group). The third 14.2% CP diet was supplemented with enzyme plus more AA than the control diet (PAA-I group). Compared to the control diet, CP was lowered by 20% (from 16.7 to 13.1%) in the 13.1% CP diet that was supplemented with enzyme and highest levels of synthetic AA (PAA-II group). Diets were formulated to have similar DE, EE and CF contents. Each feed was antibiotic free but supplemented with anticoccidial robenidine.

New Zealand White rabbits aged 35 days were weighed, ear-tagged and weaned and assigned to one of the five dietary groups according to body weight and litter. They were transferred from Alsótold rabbit farm to the Gödöllő experimental unit (65 km distance). A growth trial was conducted with 240 rabbits kept individually (48 rabbits/group) and 240 in groups (3 rabbits/cage, 48 rabbits/group) in all-wire cages (61x30x28 cm, i.e. 1830 and 610 cm^2 individual surface) from August to October 2007 in a climate controlled building (20-23°C, 16L:8D) up to 63 days of age. Feed and drinking water were offered *ad libitum*.

Data collection and management

Feed intake and feed conversion were precisely determined with single housed rabbits. Since group rearing is usual and advised in commercial farms because rabbits are highly social (EFSA, 2005), the housing effect on production was also studied. Feed consumption was only estimated for group housing (total intake of group/alive animals) Morbidity corresponded to frequency of enteric disease or severe loss of weight. Health risk was the sum of morbidity and mortality. Growth data of ill and dead rabbits were excluded from the evaluation.

Statistical analyses

The effects of five dietary treatments (Control, P, AA, PAA-I and PAA-II) and two housing systems (one or three rabbits/cage) on growth performance were assessed by two-way ANOVA with interaction (not detailed here). Contrasts were evaluated by Student's *t*-test. Morbidity, mortality and health risk rates were subjected to Chi-square tests with Yates correction. All analyses were performed with Statgraphics 6.0 (1992) statistical software.

Table 1: Ingredients, chemical composition and calculated nutritive value of diets

	Control (17%CP)	P (14%CP+Protease)	AA (14%CP+AA)	PAA-I (14%CP+Protease+AA)	PAA-II (13%CP+Protease+AA)
Crude protein reduction (%)	-	15	15	15	20
Met+Lys reduction (%)	-	15	-	-	-
DL-Methionine addition (%)[1]	0.4	0.35	0.7	0.7	0.8
HCl-Lysine addition (%)[2]	0.1	0.04	0.3	0.3	0.4
Protease inclusion (g/kg)[3]	-	1.00	-	1.00	1.00
Ingredients (%):					
Alfalfa meal	53.7	49.2	46.2	45.6	34.4
Oats	10.0	10.0	10.0	10.0	10.0
Barley	8.0	8.0	8.0	8.0	10.0
Extr. sunflower meal	8.0	-	-	-	-
Wheat grain	5.0	10.0	10.0	10.0	10.0
Dried apple pulp	5.0	5.5	5.5	5.0	9.0
Full fat soybean meal	3.5	4.5	4.5	5.0	5.5
Wheat bran	1.5	3.0	4.0	4.0	4.0
FaserMix wheat straw pellet	-	4.5	6.0	6.5	11.0
KNP-843-Ro-AS premix[4]	4.0	4.0	4.0	4.0	4.0
Chemical composition (%):					
Dry matter	89.73	89.66	89.67	89.67	89.72
Digestible energy (MJ/kg)	9.68	9.65	9.65	9.70	9.70
Crude protein	16.71	14.25	14.25	14.25	13.10
Ether extract	3.65	3.75	3.69	3.76	3.70
Crude fibre	16.82	16.83	16.82	16.82	16.76
NDF	30.60	31.80	32.19	32.27	32.82
ADF	21.92	21.85	21.77	21.74	21.51
ADL (Lignin)	4.70	4.48	4.42	4.38	4.24
Lysine	0.75	0.63	0.75	0.75	0.75
Methionine	0.34	0.28	0.35	0.35	0.35
Met+Cys	0.58	0.50	0.56	0.56	0.54
Threonine	0.64	0.56	0.55	0.55	0.50
Calcium	1.30	1.23	1.20	1.19	1.04
Phosphorus	0.61	0.55	0.55	0.55	0.54
Vitamin A (NE/kg)	10000	10000	10000	10000	10000
Vitamin D_3 (NE/kg)	1000	1000	1000	1000	1000
Vitamin E (mg/kg)	60	60	60	60	60

[1]Synthetic methionine (Biometin), [2]Synthetic lysine (Biolizin), [3]Avizyme®1500 (Danisco A/S, Copenhagen, Denmark): a blend of min. 4000 U/g protease (subtilisin, from *B. Subtilis*), 400 U/g amylase and 300 U/g xylanase (developed for corn/sorghum based poultry diets), [4]Supplemental medication: 50 mg/kg robenidine

RESULTS AND DISCUSSION

The 15% reduction of dietary CP content (from 16.7% to 14.2%) combined with added protease enzyme (P group) reduced growth performance (Table 2). The 35-63 d feed conversion tended to worsen and weight gain was significantly reduced thus rabbits had lower 49 and 63 d live weights than control animals. Similar results were found when lowered CP was combined with added synthetic amino acids (AA group). When however the 15% CP reduction was combined with enzyme plus AA addition (PA-I group) results were identical to the control group. Lowering the CP content by 20% (from 16.7 to 13.1%) combined with enzyme plus extra AA additions (PA-II group) seemed to lower mean feed intake but compared to P group, improve the 35-63 d feed conversion (P=0.157). Body weight at 63 d of age was higher than for P rabbits and similar to that of control and PA-I animals.

Health risk was not significantly affected (P=0.201) but it was 10% higher in the AA group than in the PAA-II group (Table 2). In addition to omission of in-feed antibiotics, the reason for unusual high health risk could be that the rabbits were exposed to higher stress (i.e. weaning and transfer from a more hygienic farm to a unit having poorer conditions). Rabbits housed in threes gained 7.7% less between 35 and 63 d of age and had 5% lower 63 d body weight than those caged singly. Health risk was 9.6% higher with group housing (P=0.049).

Table 2: Effect of dietary protein reduction and supplemental protease enzyme (Avizyme) and/or synthetic amino acids (Biometin and Biolizin) on growth performance of weaned rabbits

	day	Group						Housing (rabbits/cage)			SEM
		Control n=92	P n=95	AA n=90	PA-I n=92	PA-II n=89	Prob.	One n=229	Three n=229	Prob.	
Body weight (g)	35	998	998	1000	997	1006	0.930	999	1001	0.702	3
	49	1540[b]	1482[a]	1477[a]	1528[b]	1518[ab]	0.007	1562[a]	1457[b]	0.001	3
	63	1993[b]	1903[a]	1933[ab]	1995[b]	1991[b]	0.063	2013[a]	1913[b]	0.001	13
Weight gain (g/d)	35-49	39.0[b]	34.9[a]	34.5[a]	38.2[b]	37.4[ab]	0.007	40.5[a]	33.1[b]	0.001	0.5
	49-63	35.1	34.0	33.1	36.1	35.4	0.497	33.8	35.7	0.078	0.6
	35-63	37.3[b]	34.4[a]	34.3[a]	37.6[b]	36.6[ab]	0.033	37.5[a]	34.6[b]	0.001	0.4
Feed intake (g/d)	35-49	102	96	100	102	98	0.498	99	121	-	1
	49-63	124	123	127	130	115	0.249	124	136	-	2
	35-63	113	113	114	117	107	0.371	113	128	-	2
Feed conversion	35-49	2.44	2.56	2.52	2.48	2.47	0.688	2.49	-	-	0.03
	49-63	3.95	3.93	4.12	3.79	3.69	0.735	3.90	-	-	0.10
	35-63	3.05	3.22	3.05	3.03	2.93	0.157	3.05	-	-	0.03
Health risk (%)	35-63	47.8	45.3	52.2	46.7	41.6	0.201	41.9	51.5	0.049	-

Values in the same row with unlike superscripts differ

CP content below 15% caused slower growth after weaning but that was compensated for during finishing (Maertens and Luzi, 1996). Moreover weight gain was not decreased at 13.8% CP in the second period of fattening (Maertens, 2002). Xiccato *et al*. (2002) reported that CP in the first period may be lowered to 14% with adequate AA supply and, because of lower needs with advanced age, even 13% CP may enough during the finishing period. García-Palomares *et al*. (2006b) compared growth rates of rabbits fed a 16% CP diet, a 16% CP diet supplemented with 1 g/kg protease (Pescazyme), or a 14% CP diet with added AA's. Growth was not affected by feeding and they concluded that CP can be reduced to 14%. Added enzyme complex in 16 or 18% CP diets tended to improve growth rates and nutrient digestibility in rabbits aged 4 weeks (El-Mandy *et al*., 2002). El-Nagmy *et al*. (2002) studied the effect of diets containing 14, 16 or 18% CP combined with 0.6 or 0.7% Met+Cys in 28-day old rabbits. Growth rate was reduced only with a 14% CP diet with 0.6% AA's. Our results agree with above findings.

García-Palomares *et al*. (2006b) found lower growth rate and feed intake and worse feed conversion in collectively than in individually caged rabbits. They explained it by behaviour, i.e. more time spent for locomotion and less for feeding with group housing (Podberscek *et al*., 1991), since the individual surfaces (950 and 1610 cm^2, respectively) were above the critical 625 cm^2 area per animal (EFSA 2005). In this study the 610 cm^2 for group housing was less than that. However, growth rate with 550 or 825 cm^2 surface area per rabbit kept in threes or in pairs did not differ (Eiben *et al*., 2002).

CONCLUSIONS

Reducing the dietary CP content to 14.2% combined with either added protease enzyme or amino acids, was not effective because growth rate was significantly decreased and, in the latter case, health risk was higher. Growth performance was maintained without any increase of health risk even with a 20% lowering of CP from 16.7 to 13.1% if the diet was supplemented with protease plus higher levels of essential amino acids.

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REFERENCES

EFSA (European Food and Safety Authority) 2005. Scientific Report (EFSA-Q-2004-023) "The impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits" of the Scientific Panel on Animal Health and Welfare (AHAW), annex to *EFSA Journal (2005), 267, 1-31.*

Eiben Cs., Szendrő Zs., Radnai I., Bíró-Németh E. 2002. Effect of weaning age, cage size and stocking density on the performance of fattening rabbits. *In: Proc. 3rd Sci. Conf. Rabbit Prod. in Hot Climates, 2002 October, Hurghada, Egypt, 409-414*.

El-Mandy M.R., Salem F.A., El-Homeland Y.M. 2002. Effect of Kemzyme in rabbit rations differing in their protein levels on digestion, blood constituents and carcass traits. *In: Proc. 3rd Sci. Conf. Rabbit Prod. in Hot Climates, 2002 October, Hurghada, Egypt, 495-505*.

El-Nagmy K.Y., Ibrahim M.R.M., Abdel-el Samee M.O. 2002. Effect of dietary protein and total sulphur amino acid levels on performance, nutrient digestibility and carcass traits of growing crossbred rabbits. *In: Proc. 3rd Sci. Conf. Rabbit Prod. in Hot Climates, 2002 October, Hurghada, Egypt, 469-480*.

García A.I., García J., Corrent E., Chamorro S., García-Rebollar P., De Blas J.C., Carabaño R. 2005. Effect of rabbit age, type of protein and feed enzyme addition on the apparent dry matter and crude protein digestibility of rabbit feed. *In: Proc. 11émes Journées de la Recherche Cunicole, 2005 November, Paris, France, 197-200.*

García-Palomares J., Carabaño R., García-Rebollar P., De Blas J.C., García-Ruiz A.I. 2006a. Effects of a dietary protein reduction during weaning on the performance of does and suckling rabbits. *World Rabbit Sci., 14 (1), 23-26.*

García-Palomares J., Carabaño R., García-Rebollar P., De Blas J.C., Corujo A., García-Ruiz A.I. 2006b. Effects of a dietary protein reduction and enzyme supplementation on growth performance in the fattening period. *World Rabbit Sci., 14, 231-236.*

Maertens L., Luzi F. 1996. Effect of dietary protein dilution on the performance and N-excretion of growing rabbits. *In: Proc. 6th World Rabbit Congress, 1996 July, Toulouse, France, Vol 1, 237-242.*

Maertens L. 2002. The diet dilution technique as alternative method to study protein (amino-acids) requirements of growing rabbits. *In: Proc. Joint Scientific Meeting WG1 and WG4. COST Action 848 and ECVAM, October 2002, JRC Ispra, Varese, Italy, pp 27.*

Maertens L., Luzi F. 1997. Reduction of N-excretion of growing rabbits using phase feeding. *In: Proc. 10. Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere, 1997 May, Celle, Germany, 136-142*.

Maertens L. 1999. Towards reduced feeding costs, dietary safety and minimal mineral excretion in rabbits: a review and opinion article. *World Rabbit Sci., 7, 65-74.*

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.*

Podberscek A.L., Blackshaw J.K., Beattie A.W. 1991. The behaviour of group penned and individually caged laboratory rabbits. *Appl. Anim. Behav. Sci., 28 (4), 353-363.*

Statgraphics® 1992. Reference Manual, Version 6.0, *Manugistics Inc., Rockville, MD, USA*

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

Xiccato G., Trocino A., Sartori A., Queaque P.I. 2002. Reducing dietary protein in phase feeding programs for growing rabbits. *In: Proc. Joint Scientific Meeting WG1 and WG4. COST Action 848 and ECVAM, October 2002, JRC Ispra, Varese, Italy, 26.*

Xiccato G., Schiavon S., Gallo L., Bailoni L., Bittante G. 2005. Nitrogen excretion in dairy cow, beef and veal cattle, pig, and rabbit farms in Northern Italy. *Ital. J. Anim. Sci. Vol. 4 (Suppl. 3), 103-111.*

Xiccato G., Trocino A., Fragkiadakis M., Majolini D. 2007. A survey on rabbit farms in Veneto Region: technical data and nitrogen excretion estimate. *In: Proc. 12th French Rabbit Days, 2007 November, Le Mans, France, 167-169.*

Zanaty G.A. 2002. The use of biogen as a natural growth promoter for growing New Zealand White rabbits. *In: Proc. 3rd Sci. Conf. Rabbit Prod. in Hot Climates, 2002 October, Hurghada, Egypt, 507-523*.