EFFECT OF DIETARY PROTEIN DILUTION ON THE PERFORMANCE AND N-EXCRETION OF GROWING RABBITS

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Abstract - By making use of the diet dilution technique 6 diets were produced after progressively blending a high protein diet with a low protein diet, in order to obtain a serie of iso-energetic diets (10.4 MJ DE kg^{-1}) with linearly decreasing protein content between 170.1 (diet 1) and 137.5 g kg^{-1} (diet 6). Lysine, methionine + cystine and threonine were at 1.45 times their assumed requirement in the high protein diet to avoid deficiencies of these amino acids in the experimental diets. A total of 234 weanlings (32 d old) were fed during 6 weeks *ad libitum* one of these 6 diets.
Weekly body weight gain and feed intake data demonstrated an age dependent response to the dietary protein content. A protein level below 157 g kg^{-1} resulted in significant ($P<0.001$) lower growth rate (diet 1: 45.3; diet 6: 36.6 g d^{-1}) and feed intake during the first 3 weeks post weaning. However, during the finishing period weight gain on low protein diets equalled that of the high protein diets (diet 1: 45.7; diet 6: 47.1 g d^{-1}).
Comparative slaughter technique revealed a significant increased N-excretion with increasing dietary protein content. N-excretion was reduced by 39% on the lowest protein diet compared to the rabbits fed the highest protein diet. However, their overall growth rate was 9% lower.
The results suggest that dietary protein and amino acid levels of growing rabbits have to be considered into different age periods, in order to match them better with the requirements. An important reduction of the N-excretion could then be achieved, using phase feeding, without altering the performance.

INTRODUCTION

Animal excreta is no longer exclusively considered to fertilize the land. In several European countries having a high density of animal production, the quantity and composition of excreta produced exceeds the areas of land capable of using it as fertiliser and it can truly be described as a pollutant. Especially nitrogen and phosphorus are considered as the main contaminants and country or EC directives (will) limit the number of manure-producing animals per hectare of land related to limits of total N and P ha^{-1} per year depending of the destination of the zone.
Like for other animals, reduction of the excretion is directly related to the quality and quantity of the feed given. Improvement in the efficiency of nitrogen deposition can be obtained by matching the dietary amino acid composition with the requirements for maintenance and for tissue protein accretion. The availability of commercial synthetic amino acids allows the use of low protein diets by avoiding an excess of each amino acid above the requirement. Excessive amounts of dietary protein are utilized metabolically for energetic purposes with a negative impact on the environment.
Current protein and amino acids (AA) recommendations (LEBAS, 1989) for growing rabbits are based on experiments mainly performed about 20 years ago (see review LEBAS, 1983). However, with increasing use of hybrid strains, average daily growing performance amount actually 40-45g instead of 30-35 g. More recently, efforts have been made to estimate AA requirements based on rabbit whole-body tissue composition (MOUGHAN *et al*, 1988; SCHULTZE *et al*, 1988). Their findings suggest that if a balance of essential AA relative to lysine, based on current AA recommendations is used, several of these AA will be in excess of requirement. However, in their experiments rabbits were fed on a restricted basis resulting in a low growth rate. Further-more, in a lot of experiments, graded supplements of the amino acid under test are added to the basal diet supposed to be deficient only for that amino acid. The changed AA balance between diets, the uncertainty if the response can totally be ascribed to the first-limiting amino acid, especially at high levels of supplementation, and the difficulty in devising a basal diet adequate in all other indispensable AA are considered as weak points of this method (D'MELLO, 1982).
The approach used in the present experiment was to study low dietary protein levels being adequate in lysine, methionine + cystine and threonine. The synthetic analogues of these AA are relatively cheap and widely used in animal feeding. By making use of the diet dilution technique (FISHER and MORRIS, 1970), diets with decreasing concentrations of protein with a near constant amino acid balance are obtained.

The purpose of the present experiment was to study the effect of decreasing dietary protein content on the performances and N-excretion. Effects on carcass composition and N-retention are reported elsewhere (MAERTENS *et al.*, 1996).

MATERIAL AND METHODS

Table 1 : Ingredient and chemical composition of summit and mixture experimental diets[1] 1 and 6 (g/kg^{-1})

	Summit mix	Dilution mix
INGREDIENTS (g kg^{-1})		
Alfalfa meal	250	
Wheat	130	
Wheat middlings	186	
Cassava meal	50	600
Sunflower meal (28%)	120	
Soybean meal	90	
Corn gluten feed (20%)	65	
Flax chaff	25	350
Animal fat	11	25
Molasses (cane)	40	
Min./vit. pre-mix[a]	25	25
Coccidiostat	1	
L-Lysine HCL	3	
DL-Methionine	2.4	
L-Threonine	1.6	
COMPOSITION (g kg^{-1})		
Crude protein	183.1	52.8
ADF	155.6	155.5
Crude fat	38.5	46.5
DE (MJ kg^{-1})[b]	10.4	10.4
Ca[c]	9.1	10.1
P[c]	6.2	2.9

[a]Provided by Trouw NV and contained (g kg^{-1}): Na, 60; Ca, 115; P, 45; I, 0.02; Co, 0.03; Se, 0.012; Cu, 0.3; Mn, 1.3; Zn, 2.3; Fe, 4.0; vitamin E, 0.68; vitamin K_3, 0.012; thiamin, 0.013; choline, 4.50; riboflavin, 0.11; panthothenic acid, 0.27; pyridoxine, 0.013; nicotinic acid, 0.54; vitamin B_{12}, 0.0006; vitamin A, 320.000 IU kg^{-1}; vitamin D_3, 70.000 IU kg^{-1}.
[b]Calculated (MAERTENS *et al*, 1990)
[c]Calculated values, based on the Dutch feedstuffs table.

Table 2 : Protein and amino acid composition of summit and dilution mixture and experimental diets[1] 1 and 6 (g/kg^{-1})

	Summit (S)	Dilution (D)	Diet 1 (90 % S + 10 % D)	Diet 6
Crude protein	183.1	52.8	170.1	137.5
Isoleucine	7.2	1.6	6.6	*5.2*[2]
Leucine	12.7	2.9	11.7	*9.3*
Lysine	10.5	1.6	9.6	7.4
Methionine				
Corn gluten feed (20%) + cystine	8.3	1.0	7.6	*5.7*
Fenylalanine + tyrosine	13.8	3.2	12.7	*10.1*
Threonine	8.4	1.4	7.7	6.0
Tryptophan[3]	2.3	0.4	2.1	1.6
Valine	9.2	2.8	8.6	7.0
Arginine	11.2	3.2	10.5	*8.5*
Histinine	4.6	0.7	4.2	*3.2*

[1]Dietary protein and amino acids are based on the analysis of summit and dilution diet
[2]Amino acids levels in italics are lower than the recommendations of LEBAS (1989)
[3]Calculated values

A high protein diet (Summit mixture) was formulated to contain 10.4 MJ DE kg^{-1} (MAERTENS *et al*, 1990) and lysine, methionine + cystine and threonine levels at 1.45 times their assumed requirement. Dietary lysine requirement was assumed to be 7.3 g kg^{-1} (TABOADA *et al*, 1994). All other essential nutrients were at least above the recommendations of LEBAS (1989) for growing rabbits. An iso-energetic low protein diet (Dilution mixture) consisting of cassava meal and animal fat as energy sources and flax chaff as fibre source was prepared. Summit and dilution mixtures were blended progressively in the ratio 90% summit + 10 % dilution mix (diet 1) to 65% summit + 35% dilution (diet 6) in order to produce a series of 6 diets with linearly decreasing crude protein (CP) content between 170.1 g and 137.5 g kg^{-1} and nearly constant amino acid balance. The ingredient composition of the summit and the dilution mixtures is reported in Table 1. The chemical and amino acid concentrations of the experimental diets (Table 2) were calculated using the analyzed values of the summit and dilution mix. Pelleted diets (Æ 3.2 mm) were fed *ad libitum* throughout the whole experimental period (42 d). Two hundred and thirty four weanlings (32 days old), deriving from the final cross of the Institutes' own lines (MAERTENS, 1992), were blocked by litter and allotted *at random* to the 6 experimental diets (sex was ignored). Only litters invluding at least six young rabbits homogeneous in body weight were used. Rabbits were caged per 3, so that 13 cages and 39 rabbits were assigned to each diet. Rabbits were weighed individually on a weekly

basis. Feed intake was recorded each week per cage. The flat-deck cages, measuring 600 x 430 x 300 *mm high*, were equipped with a nipple drinker and an outside placed feeder. Building heating system and forced ventilation allowed the temperature to be maintained between 18 and 22°C. A cycle of 10 h of light and 14 h of darkness was used throughout the experimental period of 42 d.
The comparative slaughter technique was used to determine N-excretion. Twelve additional weanlings (4 replicates of 3 rabbits) within the same weight range were euthanised to estimate the initial empty body (digesta-free body) composition. At the end of the growing trial, 4 complete blocks were euthanised. After cooling, the content of gut and bladder was removed and the digesta-free body weight was determined. All these rabbits were frozen, ground, freeze-dried and homogenised (per pen). Nitrogen was measured by the Kjeldahl procedure.
Zootechnical data were analyzed as a randomized block design by a GLM procedure, using the SAS®/STAT version 6 (1990) with blocks and type of diet as main sources of variation.

RESULTS AND DISCUSSION

The intended high zootechnical performance level was achieved (Table 3). Daily weight gain (DWG) amounted 45-45.5 g on diets with a CP content of at least 157 g kg^{-1} while feed conversion (FC) was 3.1 between 32 and 74 days of age.

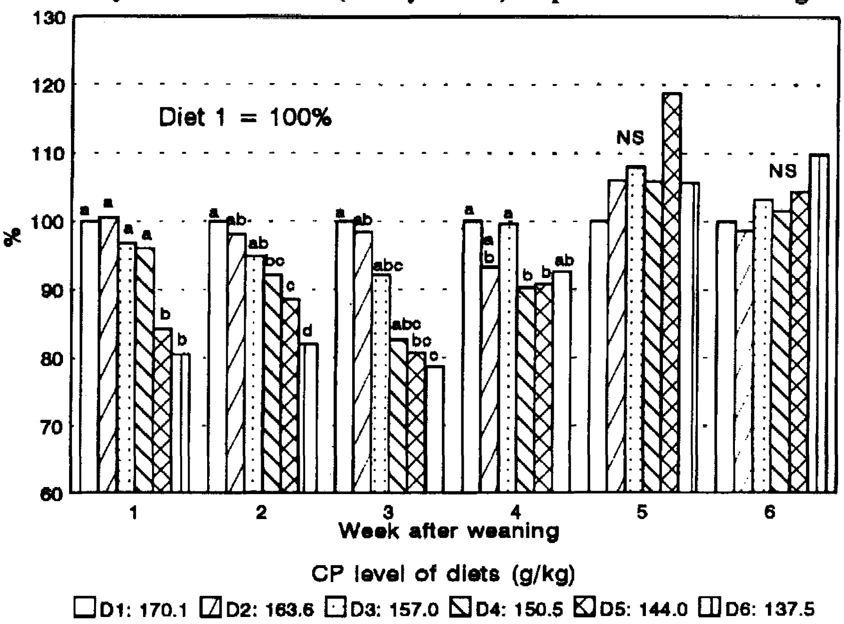
Table 3 : Summary of the performances of rabbits during the 6 week fattening period fed diets differing in CP concentration

Diet - g CP kg[1]	1 - 170.1	2 - 163.6	3 - 157.0	4 - 150.5	5 - 144.0	6 - 137.5	SEM[1]	Stat. sign.
Body weight (g)								
at 32 d	809	809	798	773	802	774	6.7	0.07
at 74 d	2717[a2]	2703[a]	2691[a]	2567[b]	2597[b]	2521[b]	28.3	<0.001
Weight gain ($g\ day^{-1}$)								
0 - 3 weeks	45.3[a]	44.9[a]	43.1[ab]	41.4[b]	38.7[c]	36.6[c]	0.9	<0.001
3 - 6 weeks	45.7	45.1	47.2	44.9	47.1	46.5	0.9	>0.1
0 - 6 weeks	45.5[a]	45.0[a]	45.1[a]	43.2[b]	42.9[b]	41.6[c]	0.6	<0.001
Feed intake ($g\ day^{-1}$)								
0 - 3 weeks	115.6[a]	115.0[a]	111.9[a]	105.4[bc]	100.9[c]	96.1	1.9	<0.001
3 - 6 weeks	167.9[a]	165.5[a]	167.5[a]	156.5[b]	154.7[bc]	149.6[c]	2.5	<0.001
0 - 6 weeks	141.8[a]	140.2[a]	139.7[a]	131.0[b]	127.8[bc]	122.8[c]	1.9	<0.001
Feed conversion								
0 - 3 weeks	2.56	2.57	2.60	2.55	2.62	2.63	0.04	>0.1
3 - 6 weeks	3.69[a]	3.69[a]	3.56[ab]	3.49[b]	3.29[c]	3.22[c]	0.05	<0.001
0 - 6 weeks	3.12[a]	3.12[a]	3.10[ac]	3.03[bc]	2.98[bd]	2.96[d]	0.03	<0.001
Mortality	1/39	1/39	2/39	3/39	1/39	1/39	-	NS

[1]Standard error of means
[2]Treatment means followed by the same letter are not significantly different from each other at P=0.05

Diet composition had a highly significant effect both on DWG, daily feed intake (DFI) and FC as well. Diets 1 to 3 showed high and comparable performance. From a dietary CP concentration of 150.5 g kg^{-1} on (diet 4), decreased ($P< 0.05$) DWG and DFI were observed. Further dilution (diet 5 and 6) depressed the DWG of the total period (6 weeks) with 6 and 9% compared to diet 1, respectively. Because the decrease in DFI was larger than in DWG, rabbits showed a more ($P<0.05$) favourable overall FC especially on diets 5 and 6.
The effect of CP concentration was age dependent (Table 3 & Figure 1). The first 3 weeks after weaning, a decreasing CP concentration had a very pronounced negative effect ($P<0.001$) on DWG. Difference between the highest and the lowest CP concentration amounted to 19%. Although diets 5 and 6 resulted in a significant lower DWG compared to diets 1 to 4, also a decreasing trend was observed from diet 1 to 4.

Figure 1 : Effect of dietary CP level on DWG (weekly results). Expressed as % of the highest protein diet



The weekly zootechnical results demonstrated that protein (amino acid) requirements of rabbits are age dependent during the post weaning period. This agrees with the only report found were the response of supplemented AA was studied in two weekly periods instead of considering the whole fattening period (PARIGI-BINI *et al.*, 1988). Before the age of 7-8 weeks rabbits claim a dietary protein content of at least 157g kg^{-1} under our dietary conditions. Diets with a protein content below this level, resulted in significant decreased DWG and DFI although lysine, threonine and methionine and cystine were above the actual recommendations. Our results are in agreement with earlier studies, which indicated that a dietary protein content below 155 g kg^{-1} results in decreased DWG when the whole fattening period is considered (LEBAS, 1983; OUHAYOUN and CHERIET, 1983; SANTOMA *et al*, 1985; LEBAS and OUHAYOUN, 1987).

The increased DWG (although not significant) during the finishing period (last two weeks) indicates clearly that the protein and AA requirements decrease with increasing age. Our results suggest that if dietary lysine, methionine + cystine and threonine levels are above the requirements, low protein diets can be fed without deteriorating DWG during the finishing period. However, although some compensatory growth occurred on the low protein diets (diet 5 and 6), overall DWG was still 5 (diet 4 and 5) to 9% (diet 6) lower ($P<0.01$). Low protein diets decreased DFI during the total fattening period. It is well known that diets deficient in AA results in a decreased DFI (COLIN, 1974; LEBAS and OUHAYOUN, 1987). When we relate the dietary AA levels (Table 2) with the feed intake data, DFI on diets having AA levels below the recommendations (diets 4, 5 and 6) dropped significantly. This suggest that in early fattening stage, the recommendations of some of these AA are near to the requirements.

Decreased DFI due to the low protein (AA) content was larger (7 to 13%) than the corresponding decreased DWG and results in a more favourable FC. However, this effect was only observed during the finishing period when rabbits on the low protein diets showed a tendency to increased DWG (OUHAYOUN *et al.*, 1979). The significantly decreased FC during the finishing period can further be explained by the difference in live weight at 8 weeks and not by differences in dietary energy content. The comparable DFI and FC on diets 1, 2 and 3 prove indirectly that the diets were iso-energetic as intended, because rabbits adjust their feed intake to the dietary energy content (LEBAS, 1989).

Between protein intake and N excretion, a correlation coefficient of 0.993 was found (Figure 2). Differences in N-excretion were very pronounced when related to diet 1 (Figure 3). A CP dilution of 7.7% (diet 3) reduced N-excretion with 11% without altering performances. A further dilution to a CP content of 137.5 gkg^{-1} resulted in a reduction of 38%, however, DWG worsened with 9%. Although DWG of rabbits was altered when fed diets containing less than 157 g kg^{-1}CP, the decrease in N-excretion (g/rabbit) was much larger than could be expected by the protein dilution.

Further experiments are necessary to match better the CP and AA levels to the real requirements of the rabbits during the different stages of fattening. With phase feeding, performances equal to high protein diets could then be achieved with important reductions of the N-excretion.

Figure 2 : Response of N-excretion (g/rabbit) on protein intake

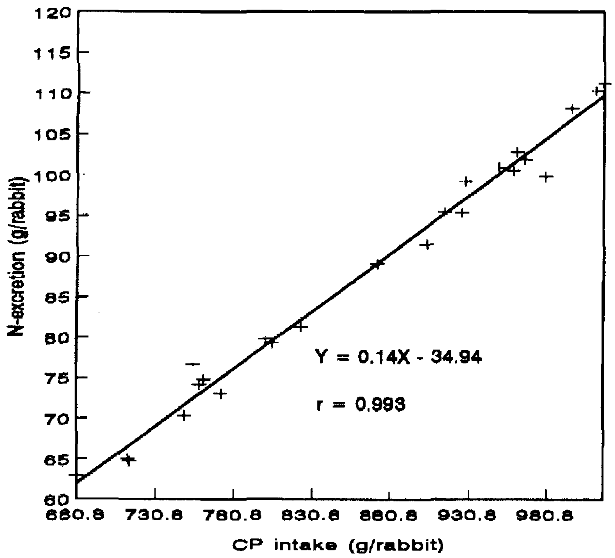
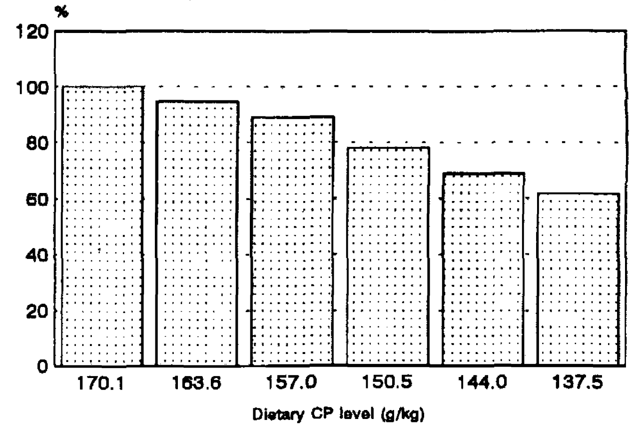


Figure 3 : N-excretion of rabbits between 32 and 74 d of age (% relative of diet 1)



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Effetto della diluizione proteica nella dieta sulle performance e l'eliminazione dell'azoto dei conigli in accrescimento - Mediante l'utilizzo della tecnica della diluizione si sono formulate 6 diete dopo aver progressivamente miscelato una dieta ad alto contenuto proteico con una dieta a basso contenuto proteico, al fine di ottenere una serie di diete isoenergetiche con un contenuto di proteine decrescenti in modo lineare tra 170,1 (dieta 1) e 137,5 g kg^{-1} (dieta 6). La Lisina, la Metionina più Cistina e la Treonina sono state utilizzate ad un livello di 1,45 il fabbisogno tabulare nella dieta iperproteica, per evitare carenze di questi aminoacidi nelle diete sperimentali.
Durante un periodo di 6 settimane ognuna di queste diete è stata somministrata *ad libitum* a 234 conigli allo svezzamento (32 giorni di età). Gli incrementi ponderali ed i consumi alimentari rilevati settimanalmente hanno dimostrato una risposta dipendente dall'età in funzione del contenuto proteico della dieta. Nelle prime 3 settimane post-svezzamento, con un livello proteico inferiore a 157 g kg^{-1}, la velocità di crescita ed il consumo di alimento sono risultati significativamente inferiori (dieta 1: 45,3 g d^{-1} ; dieta 6: 36,6 g d^{-1}). In ogni caso, durante il periodo di finissaggio l'incremento di peso ottenuto con le diete a basso contenuto proteico ha eguagliato quello delle diete ad alto contenuto proteico (dieta 1: 45,7 g d^{-1}; dieta 6: 47,1 g d^{-1}).
La macellazione comparativa ha rilevato un significativo incremento dell'eliminazione di azoto all'aumentare del contenuto proteico della dieta (39% nelle diete ipoproteiche rispetto alle diete iperproteiche); comuque, il tasso medio di crescita è stato più basso del 9%.
I risultati suggeriscono che i contenuti in proteine e aminoacidi nelle diete dei conigli in accrescimento devono essere considerati entro differenti periodi di età, al fine di ottimizzarli in base ai fabbisogni. Un'importante riduzione dell'escrezione azotata può essere realizzata utilizzando l'alimentazione per fasi, senza alterare le performance.