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EFFECTS OF DIFFERENT DIETARY PROTEIN LEVELS IN N.Z.W. RABBITS : BLOOD CONSTITUENTS AND URINARY NITROGEN COMPOUNDS.

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Introduction

The effects of varying dietary protein levels upon protein metabolism have been previously described in rabbits submitted to either protein excess and deprivation (Greppi et al.-1982 ,Corti et al.-1983, Corti et al. -1984a, Corti et al. 1984b, Rosi et al.- 1985, Greppi et al. -1984). In the present study we investigated the effects of feeding rabbits marginally low or high protein diets.

Materials and methods

20 NZW rabbits housed in stainless steel individual metabolic cages were divided into two groups when weighting about 1 kg B.W. and mantained for four weeks on two experimental diets (H and L) whose CP content was respectively 220 and 140 g/kg D.M. The composition of the diets is shown in Tab. 1. After this adaptation period a 20 days nutrients balance trial followed. Half of the rabbits from each dietary group were sacrificed after 12 days . Body weight is shown in Fig. 1. Blood samples were obtained before sacrifice by cardiac puncture. Blood specimens (lithium heparin added) were rapidly centrifugated, plasma samples were stored at -20°C until analysis were performed. Complete urine collections were performed daily, sulphuric acid was added to collection flasks. Urine specimens were stored at -20°C.

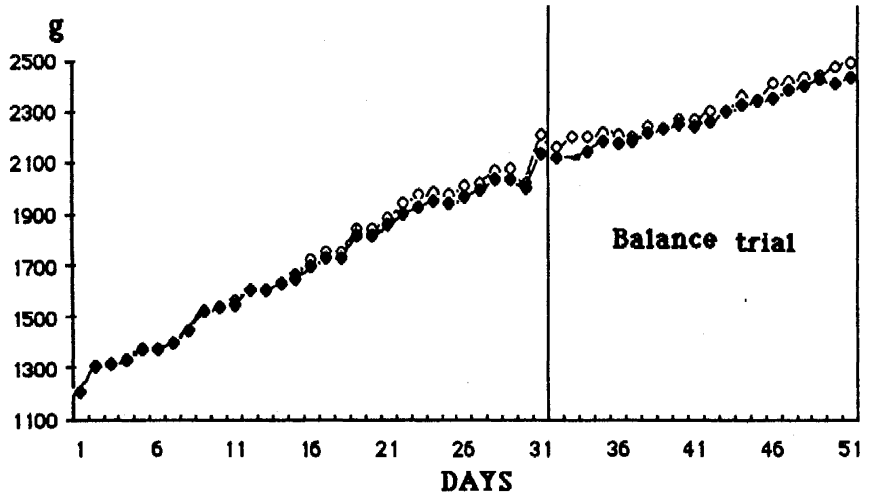
Analytical procedures: commercial kits from Boehringer Biochemia Robin were used for all the determinations except those of alanine and α-NH_2-nitrogen which were analyzed according to the methods of Williamson et al. (1967) and Goodwin (1968) respectively. Plasma creatine and creatinine were analyzed by an enzymatic-colorimetric procedure whereas creatinine in urine by the usual colorimetric procedure. The enzymatic activities were determined at 37°C. All the analysis, except α-NH_2-nitrogen, were performed with the aid of a microcentrifugal analyzer (Multistat III *Instrumentation Laboratory).*

Results and discussion

Blood constituents.

The average values for each treatment are shown in tab.2. The present values agree with those obtained previously for rabbits fed a standard diet (Greppi et al. -1983) and with those reported by other authors (Ghisalberti et al -1981, Chiericato et al. -1985). Out of the examined parameters only plasma urea levels exhibited a highly significant difference between the dietary groups.

Fig 1. Live weight in experimental period



Daily mean values for body weight in male rabbits fed diet containing 220 g CP/kg D.M.(•=diet H) or 140 g CP/kg D.M. (o=diet L).

The absolute difference (3.9 mmol/l) appears as large as the difference (3.8 mmol/l) we previously found between adult rabbits (about 3.4 kg B.W.) fed diets with 81 and 277 g CP/ kg dry matter thus indicating a steeper increase of plasma urea when protein intake increases under the conditions of the present study. This different response, however, cannot be explained with the effect of age since no clear relationship has been observed when the effect of protein intake upon plasma urea has been studied in rabbits of different age (Rosi et al.-1985). Other significant differences ($P<0.05$) have been found for cholesterol, α–NH_2-nitrogen, calcium, phosphorus and γ-GT which were all lower in the plasma of the rabbits given the high soy diet. The difference in plasma cholesterol could reflect the overall difference in lipid and energy metabolism between rabbits fed the experimental diets which led to a significantly higher lipid accumulation in the L group (Greppi et al. -1988). The possibility exists, however, that the source and the level of dietary proteins affect bile acids and cholesterol metabolism through an influence upon bile acids absorption or microbiological modifications (Pacini et al. -1988). The effect of protein intake upon plasma calcium and phosphorus confirms our earlier findings (Corti -unpublished results). The higher plasma levels of calcium and phosphorus when protein intake is low could be a result of differences in the rate of urinary excretion of these minerals. The effect of the diets upon plasma α-NH_2-nitrogen concentration agrees with the close inverse relationship between the sum of the individual amino acids and protein intake which is caused by the accumulation in the plasma of non essential amino acids (Corti et al.- 1984a). Alanine, however, in the present experiment does not differ significantly between groups.

Urinary nitrogen metabolites

The effect of day to day variation and treatments has been tested by a two ways ANOVA procedure. Urea urinary excretion was significantly affected by dietary protein levels but not by day to day variation. Urea daily excretion figures reported by other workers (Motyl et al. 1986) for standard diet fed male rabbits of different breed and body weight (28 mmol/$kg^{0.75}$/day) are intermediate between those observed in the present work. Day to day variation affected creatinine urinary excretion which, on the contrary, was not significantly influenced by the dietary treatment. Amino nitrogen excretion was affected neither by diets nor by day to day variation. During the urine

collection trial the excretion of nitrogen compounds was constant except for urea in the L group which rose significantly as demonstrated by the analysis of regression between days on trial and urinary excretion. It would appear that in the long run group differences tend to decrease. One possible explanation for this difference could be related to the decreasing rate of growth toward the end of the trial with correspondingly lower amino acids requirements. Amino nitrogen excretion increases when protein level in the diet is high (Greppi et al-1986), the present results show that this effect cannot be observed when protein intakes do not present large differences. When individual average creatinine excretion during the whole trial was related to the body weight, the correlation coefficients were : $R = 0.62$ $P < 0.05$ (group H) , $R = 0.21$ n.s. (group L). This observation pairs with the significant decrease of creatinine excretion per kg B.W. through the trial. These findings could indicate that even if creatinine excretion did not differ significantly between groups some kind of effect might have been present as a consequence of different protein intakes.

Summary

The effects of dietary protein level upon blood and urine biochemical constituents were investigated in male rabbits weighting about 2 kg. Rabbits in the experimental groups, 10 subject each, were administred diets with 220 g (H) and 140 (L) g CP/ kg dry matter respectively. Total N, urea, creatinine, α–NH_2-N were analyzed in urine; creatinine, creatine, urea, glucose, total protein, albumin, alanine, α-NH_2-N, cholesterol, Ca, inorganic P, ALT, AST, LDH and γ-GT activity in plasma. Total N and urea urinary concentrations were significantly higher in the high protein diet group. With the exception of urea the other blood constituents which were significantly affected by the dietary treatment exhibited higher plasma levels in the low protein diet fed rabbits. Urinary urea excretion shows two-fold increase when rabbits are given the high protein diet (33 v. 17 mmol/kg $^{0.75}$/day). Creatinine urinary level varies widely from day to day but it does not seem to be influenced by the diet. Amino nitrogen excretion behaves in the same way. When blood constituents were compared, statistically significant differences appeared for urea (8.1 mmol/l in group H v. 4.2 mmol/l in group L), γ -GT, Ca, inorganic P, α-NH_2-N, and cholesterol.

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EFFETS DU NIVEAU PROTEIQUE CHEZ LES LAPINS N.Z.W: METABOLITES PLASMATIQUES ET URINAIRES.

Résumé

Les effets du niveau protéique du régime ont été analysés chez des lapins mâles adultes dont le poids vif était de 2 kg environ.
L'expérimentation porte sur la comparaison de deux régimes qui se distinguent par la teneur en protéines. Vingt lapins mâles adultes (2 groupes de 10) ont été élevés en cage individuelle à métabolisme et alimentés avec des régimes apportant 90 grammes d'aliment à 22 % de protéines pour le groupe A et 14% pour le groupe B. Les urines récoltées tous le jours ont été utilisées pour déterminer la concentration de: azote total, creatine, creatinine, α-NH_2-N et urée. A la fin de l'expérience du bilan, le prélèvement d'échantillons sanguins a permis l'évaluation de la concentration plasmatique de: colesterol,Ca, P inorganique,ALT,AST,LDH et γ-GT.
L'excrétion en urée résulte plus élevée pour les animaux à régime contenant un niveau protéique supérieur. En ce qui concerne les variables hématiques la concentration plasmatique met en évidence des différences hautement significatives pour: urée, γ-GT, Ca, α-NH_2-N et colesterol.

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Tab. (1) Composition of experimental diets (%) and proximate analysis of diets (% of DM)

Diet	H	L
Soja 50%	44	25
Straw	20	20
Solka Flock	7	7
Corn Starch	24	43
Corn oil	2.5	2.5
Dicalcium Phosphate	3	3
Calcium Carbonate	1	1
Mineral Mixture	0.5	0.5
Vitamin Mixture	0.25	0.25
Methionine (p.p.m.)	200	200
Dry matter	90.88	89.86
Crude Protein(Nx6.25)	21.68	13.95
N	3.47	2.23
Crude fat	3.26	3.07
Crude fibre (Weende)	20.19	17.99
N-Free Extractives	47.11	58.83
Energy kJ/kg	19102	18406
Ash	7.76	6.16

Diets were obtained from Laboratorio Piccioni (Gessate-Milano)

Tab. (2) Average values of blood constituents (± s.d.)

		Diet		
Parameter	Unit	High protein	Low protein	Significance Student	Wilcoxon
Glucose	mmol/l	9.11 ± 0.74	9.28 ± 1.49		
Tot. protein	g/l	64.2 ± 4.7	67.4 ± 6.2		
Albumin	g/l	41.7 ± 3.5	41.0 ± 4.7		
Globulin	g/l	23.1 ± 3.6	27.3 ± 6.2		
Creatine	µmol/l	240 ± 61	259 ± 62		
Creatinine	µmol/l	77 ± 24	70 ± 16		
Urea	mmol/l	8.10 ± 1.99	4.23 ± 0.58	**	**
Alanine	µmol/l	435 ± 163	522 ± 170		
α-NH_2-N	mg/l	106 ± 15	122 ± 20	*	
Cholesterol	mmol/l	1.39 ± 0.29	2.34 ± 0.98	*	*
Phosphorus	mmol/l	2.12 ± 0.40	2.46 ± 0.42	*	
Calcium	mmol/l	4.34 ± 0.24	4.52 ± 0.19	*	
AST	U.I. 37°C	35.9 ± 15.3	50.7 ± 37.9		
ALT	U.I. 37°C	41.2 ± 22.9	34.4 ± 16.7		
LDH	U.I. 30°C	321 ± 182	359 ± 199		
γ-GT	U.I. 37°C	12.0 ± 2.4	14.6 ± 2.2	*	

Tab.(3)Average values of urinary nitrogen metabolites daily excretion (± s.d.)

		Diet			
Parameter	Unit	High protein	Low protein	Effects Diets	Days
Urea	g/day	3.62 ±0.65	1.86 ±0.36	**	n.s.
Creatinine	µmol/day	943 ±0.103	860 ±0.091	**	n.s.
α-NH_2-N	mg/day	2.53 ±0.40	2.56 ±0.33	n.s.	n.s.

* P< 0.05** P < 0.01

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Efffects of different dietary protein content in N.Z.W. rabbits: haematological and urinary parameters.

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