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PROTEIN METABOLISM IN ADULT RABBITS FED DIETS WITH DIFFERENT PROTEIN LEVELS: NITROGEN BALANCE

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INTRODUCTION

The determination of nutrient balance is used extensively in nutrition research. It is obviously of great significance to know how much of a given nutrient in a feed is retained in body tissue.

The importance of dietary protein in nutrition of growing rabbits is well recognized. However informations about the protein requirements for maintenance are still limited. In the well nourished adult that mantains the same weight over a long period of time also body composition must be constant. If so, the intake of various nutrients must approximately pair the amount that are lost from the body. Thus when adult animals are considered, N balance near zero or slightly positive should indicate protein adequacy. Unfortunately N balance trials may be affected by inaccuracies. However they furnish useful informations if care is taken in animal adaptation prior to balance, if trials are long enough and the collection of excreta is accurate (Colin and Lebas 1976, Asplund 1979).

The present study attempts to determine the effects of different Crude Protein intakes upon nitrogen balance in adult rabbit.

MATERIALS AND METHODS

Twenty adult male NZW rabbits of the same age and live weigh were used. They were housed in individual stainless steel cages.

Animals were gradually adapted to consume 110 g/d of a standard diet (STD), over a 15 days period. During this adaptation period water was provided ad libitum, food intake and live weight were recorded.

At the end of this period the animals were weight-sorted in four groups of five. They continued on the STD diet for 8 days, which represented the '0' period of the balance trial. Afterthat one group continued on the STD diet (18.3 % CP), the other groups were transferred to three experimental diets: a high protein diet (HP) with 27.7% CP, a moderate low protein diet (MLP) with 8.1% CP and a low protein diet (LP) with 4.8% CP. The composition of the diet and proximate analysis are reported in Table 1.

The experiment was divided in six consecutive collection periods of eight days. In the course of the experiment, 110 g of diet were supplied daily to each rabbit. Water was provided ad libitum.

The food intake was recorded daily and live weight was recorded every two days. Complete excreta collections were performed.

48 h urine and daily faeces from each animal were collected. The urine was collected into flasks with HCl and toluene, measured and aliquots were stored at -20°C. Daily faeces were dryed in air at 40°C for 24 hours. Dryed faeces from eight consecutive collections were weighted, pooled and milled before sampling for analyses.

Total nitrogen was estimated by Kjeldahl procedure. Nitrogen retention was calculated by the differences between N intake and N losses with faeces and urine.

RESULTS AND DISCUSSION

Food intake, body weight and body weight gain are shown in Table 2 which reports average values for each period. In the LP group a significant depression of food intake occurred in the course of the whole experimental period, whereas a slight decrease of food intake was observed in the 2nd and 3rd period in the case of animals fed the HP diet. Since the diets were isoenergetic and isofibrous the food intake depression we observed in the LP group could be ascribed to the effect of the low protein dietary content. It is well known that animals fed low protein reduce their voluntary food

consumption. This response has been interpreted as a protective mechanism (Peret 1972). The possibility exists that poor palatability could have contributed to the differences in food intakes. This effect could be related to the presence of starch in the LP diet. The starch percentage, however, was not very large.

When the whole experimental period was considered, the rabbits in the HP, MLP and STD groups displayed almost identical live weight gains. This indicates that adult rabbits can tolerate variations in the protein content of the diet over a wide range. The animals fed the LP diets exibited live weight losses starting from the 2^{nd} period of the trial. When compared to the '0' period the urinary N excretion during the experimental period (2^{nd}-5^{th} periods) showed the following changes: - 80% in the LP group, - 60% in the MLP group, + 65% in the HP group. The corrisponding changes in faecal N losses were: - 45%, - 35%, + 25% in the LP, MLP and HP group respectively.

From these data one could argue that amino acid and protein catabolism as reflected by urinary N excretion, is greatly affected by varying dietary protein levels, wheares digestion and absorption are affected to a minor extent. However the contribution of endogenous losses should not be neglected. Though direct estimations of endogenous N losses are not available, one could suppose that endogenous contribution is much more important in the case of faecal losses rather than in the case of urinary losses. Furthermore the effects of different protein intakes upon digestibility is complicated by recycling of N escaped from digestion in the small intestine by mean of caecotrophy.

The apparent digestibility was very low in the LP group (20%), whereas in the MLP group it attained a 52% level (2^{nd} - 5^{th} periods). The apparent digestibility did not appear to be affected by feeding the HP diet. Rabbits given this diet attained apparent digestibility levels similar to those observed in the STD group. The present data indirectly show taht endogenous N may become the major contributor of faecal N losses when the dietary N influx is greatly reduced.

In spite of the large variability which affected N balance data, the present results show that animals on the MLP diet, which provided minus than 8 g/d of Crude Protein, were able to mantain a positive N balance and to gain

weight. In addition, when the effects of an excess of dietary protein on N balance was investigated, it appeared that the N retention in the course of the whole trial was similar in the HP and in the STD group. The average N retention was 0.449 and 0.475 g/d in the HP and in the STD groups respectively (2^{nd}-5^{th} periods).

Even though, in the case of rabbits given the HP diet, the protein intake went beyond the capability to utilize the surplus N, the high N influx did not exceed the capability to catabolize this large intake. Thus animals were able to prevent toxicity.

The ineffectiveness of high protein diets toward N retention is not surprising since adult animal's cells, unlike those of the young, have limited capability to modify the protein synthesis rates.

According to Møllegard (1955) this last mechanism is the main factor of protein utilization. It has been shown that the maximun N retention is more closely related to age than to dietary levels (Millward et al. 1974), or the energy intake (Ørskov et al. 1983).

The ratio of N retained to N intake was, in percent, 10.98 $\pm$ 2.68 and 17.78 $\pm$ 5.43 in the HP and in the STD groups respectively (2^{nd} - 5^{th} periods). Within the explored range, the relationship between N intake and N retention did not appear to be linear. The regression could be expressed by the following equation: $Y = -0.545 + 0.632X - 0.094X^2$ where Y is N retained and X the N intake during the period from the 2^{nd} to the 5^{th} balance trial ($r = 0.96$, $p<0.001$).

CONCLUSION

The present study provides evidence that the adult rabbit is able to mantain live weight over a wide protein intake range. N balance data show that N equilibrium, as calculated by interpolation, might be attained with a N intake of 1.02 g/d. It would appear that usual raccomandations tend to overestimate protein need of adult rabbits.

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TABLE 1 - COMPOSITION OF EXPERIMENTAL DIETS (%)
AND PROXIMATE ANALYSIS OF DIETS (% OF DM)

	LP	MLP	STD	HP
CORN	18,7	23,5	18,5	5,0
HAY	30,0	30,1	15,0	-
CORN STARCH	29,0	7,0	-	-
MALT	5,0	5,6	-	-
BEAN	5,0	5,0	2,0	-
ORGE BARLEY	-	12,0	15,0	11,0
WHEAT BRAN	-	-	18,0	-
ALFA-ALFA	-	-	15,0	30,0
SOJA	-	-	12,0	30,0
GROATS	-	-	-	12,0
YEAST TORULA	-	-	2,0	2,0
SOLRA FLOCK	3,5	1,5	-	-
HERRING MEAL	-	-	-	3,8
CORN OIL	2,0	1,5	-	1,6
ANIMAL FAT	3,0	2,6	-	1,6
DICALCIUM PHOSPHATE	3,0	1,0	1,5	1,5
LIMESTONE	-	-	1,0	1,0
VITAMIN AND MINERAL MIXTURE	0,8	0,8	0,5	0,5
DRY MATTER	92,0	91,0	90,0	91,0
CRUSE PROTEIN	4,75	8,13	18,3	27,7
CRUDE FAT	6,7	6,8	3,4	6,8
CRUDE FIBRE	15,2	15,0	13,9	12,5
N-FREE EXTRACTS	64,85	60,47	54,5	42,9
ASH	8,5	9,6	9,9	10,1
ENERGY KCAL	3387	3300	3358	3360

DIETS WERE OBTAINED FROM LAB. Dott. PICCIONI s.n.c.

TABLE 2

DIET	PERIOD 0 $\bar{x}$	SD	1 $\bar{x}$	SD	2 $\bar{x}$	SD	3 $\bar{x}$	SD	4 $\bar{x}$	SD	5 $\bar{x}$	SD
FEED INTAKE g/die (MEAN ± SD)												
LP	108.5	1.6	100.1	10.2	87.7	11.3	79.4	19.3	76.1	18.7	69.1	14.9
MLP	109.3	0.6	102.6	15.2	107.8	2.1	108.6	1.0	107.9	1.3	106.6	1.5
STD	104.1	7.6	107.1	2.1	105.1	6.5	104.7	6.3	100.6	13.6	108.1	1.7
HP	108.6	2.0	108.2	5.0	99.7	11.7	98.9	7.9	105.9	1.9	103.8	2.8
LIVE WEIGHT g (MEAN ± SD)												
LP	3426	264	3467	279	3446	283	3408	295	3372	300	3326	302
MLP	3421	181	3447	226	3468	204	3536	190	3561	171	3570	179
STD	3420	155	3469	159	3519	155	3528	158	3547	168	3556	183
HP	3427	180	3457	174	3481	206	3534	212	3555	202	3580	231
DAILY GAIN g/die (MEAN ± SD)												
LP	3.1	4.2	5.1	3.4	-2.6	4.8	-4.8	4.5	-4.5	-8.0	-5.8	7.5
MLP	2.5	5.2	3.2	2.8	2.6	7.6	8.5	4.3	3.1	3.2	1.1	1.9
STD	2.5	4.3	6.1	3.0	6.2	2.1	1.1	6.4	2.3	1.7	1.1	1.6
HP	3.3	6.0	3.8	5.3	3.0	7.4	6.6	6.5	2.5	8.3	3.3	5.9
DIETARY N INTAKE g/die (MEAN ± SD)												
LP	2.855	0.037	0.698	0.063	0.615	0.069	0.564	0.104	0.530	0.121	0.489	0.093
MLP	2.877	0.013	1.214	0.161	1.275	0.021	1.285	0.010	1.277	0.013	1.262	0.016
STD	2.737	0.178	2.818	0.050	2.764	0.151	2.758	0.147	2.648	0.320	2.846	0.041
HP	2.858	0.047	4.143	0.179	3.966	0.379	3.987	0.283	4.266	0.069	4.182	0.102

TABLE 3

DIET	PERIOD 0 $\bar{x}$	SD	1 $\bar{x}$	SD	2 $\bar{x}$	SD	3 $\bar{x}$	SD	4 $\bar{x}$	SD	5 $\bar{x}$	SD
FECAL N LOSS g/die (MEAN ± SD)												
LP	0.804	0.130	0.532	0.108	0.424	0.058	0.354	0.105	0.402	0.129	0.432	0.182
MPL	0.892	0.058	0.459	0.112	0.623	0.086	0.699	0.222	0.682	0.183	0.602	0.161
STD	0.830	0.187	0.799	0.093	0.759	0.040	0.733	0.066	0.672	0.179	0.708	0.122
HP	0.894	0.058	0.982	0.058	0.933	0.178	0.961	0.117	1.181	0.296	1.125	0.122
URINARY N LOSS g/die (MEAN ± SD)												
LP	1.602	0.153	0.497	0.034	0.372	0.031	0.468	0.339	0.374	0.084	0.269	0.064
MLP	1.358	0.297	0.590	0.139	0.503	0.155	0.471	0.168	0.548	0.125	0.550	0.258
STD	1.530	0.426	1.337	0.203	1.640	0.088	1.592	0.104	1.589	0.206	1.421	0.186
HP	1.587	0.172	2.126	0.467	2.633	0.272	2.435	0.595	2.716	0.281	2.621	0.255
APPARENT N RETENTION g/die (MEAN ± SD)												
LP	0.448	0.177	-0.331	0.064	-0.181	0.059	-0.258	0.448	-0.246	0.150	-0.212	0.117
MLP	0.627	0.264	0.165	0.309	0.148	0.083	0.115	0.123	0.047	0.119	0.110	0.211
STD	0.374	0.422	0.682	0.211	0.363	0.200	0.433	0.095	0.387	0.467	0.717	0.244
HP	0.377	0.211	1.035	0.522	0.400	0.300	0.591	0.678	0.369	0.244	0.436	0.283
APPARENT N DIGESTIBILITY % (MEAN ± SD)												
LP	71.79	4.17	22.07	7.48	27.41	8.48	24.17	9.81	25.44	12.	20.92	17.28
MLP	68.93	4.50	61.62	8.68	51.10	6.25	45.53	15.38	46.64	14.69	52.28	11.23
STD	69.87	4.46	71.42	3.20	72.22	1.60	73.20	1.70	74.12	5.54	76.00	4.04
HP	68.72	1.74	76.16	0.67	75.50	3.59	75.78	2.95	72.20	6.73	73.08	2.52

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SUMMARY

Six consecutives N balance trials, each of 8 days were performed in order to evaluate the relationship between N intake and N retention in adult rabbits. Four diet were employed, they were isoenergetic and isofribous. The Crude Protein content (% of dry matter) was: 4.8 (Low Protein diet), 8.1 (Moderate Low Protein diet), 18.3 (STD diet) and 27.7 (High Protein diet). The animals were housed in metabolic individual stainless steel cages, 110 g of diet were provided daily to each rabbit, water was provided ad libitum. Each diet was administered to five animals. The initial average live weight was about 3.4 Kg. N analyses was performed on the 48 h urine from each animal, whereas eight days pooled faeces were used. N retained was 0.445, 0.475, 0.117 g/d in the HP, STD and MLP group respectively.

In the LP group the N balance was negative (-0.245 g/d). These data were obtained from four consecutive balance trials, the first period after the change of the diet was excluded from calculation of the average N retention. The relationship between N intake and N retention (Y) could be expressed by the following equation: $Y = -0.545 + 0.632\ X - 0.094\ X^2$ ($r = 0.96$). From this equation a zero N balance is attained when N intake is 1.017 g/d.

RIASSUNTO

E' stata condotta una prova di bilancio azotato su 20 conigli adulti NZW. La prova fu suddivisa in sei successivi periodi di bilancio della durata di 8 giorni ciascuno. Gli animali (del peso medio iniziale di circa 3,4 Kg) furono alloggiati in gabbie singole da metabolismo di acciaio inossidabile. Furono impiegate quattro diete isocaloriche ed isofibrose del tenore proteico di: 4.8% (dieta a basso tenore proteico), 8,1 (dieta a relativamente basso

tenore proteico), 18,3 (dieta standard) e 27,7 (dieta ad alto tenore proteico).A ciascun animale vennero offerti 110 g/die di alimento mentre l'acqua di bevanda venne offerta ad libitum. L'azoto trattenuto risultò pari a 0,455, 0,475 e 0,117 g/die rispettivamente nei gruppi alimentati con le diete con l'8,1, il 18,3 e il 27,7% di proteina greggia. Il gruppo alimentato con la dieta a basso tenore proteico, invece, presentò un bilancio azotato negativo (-0,245 g/die). Questi valori medi furono calcolati sulla base dei risultati ottenuti in quattro successivi periodi di bilancio, non si tenne conto dei dati relativi al primo periodo dopo il cambio della dieta. La relazione tra la quantità giornaliera di N trattenuto e l'apporto azotato risultò esprimibile dalla seguente equazione: $Y = - 0.545 + 0.632 X - 0.094 X^2$ ($r = 0.96$). In base a questa equazione un apporto azotato giornaliero di soli 1,017 grammi dovrebbe essere sufficiente a mantenere un bilancio azotato uguale a zero.

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