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The protein requirement of adult marmosets: nitrogen balances and net protein utilization of milk proteins, soy protein, and amino acid mixtures*)

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Summary: Nitrogen balance studies were conducted in adult male marmosets *(Callithrix jacchus)* using purified and semipurified diets with protein levels between 0 % and 7 %. Daily nitrogen loss in a state of prolonged protein free nutrition was 131 ± 16 mg/kg body weight$^{0.75}$. Zero nitrogen balance resulted from mean daily intake of 261 mg nitrogen/kg$^{0.75}$ when high quality protein sources were used. Very low protein intake or the lack of arginine and histidine in an amino acid mixture induced coprophagy. It is concluded that the protein requirement of adult marmosets is very similar to the protein requirement of adult humans (based on metabolic body weight). About 6–7 % high quality protein, based on dry matter, suffice to avoid a negative nitrogen balance in all individuals.

Zusammenfassung: An erwachsenen männlichen Weißbüscheläffchen *(Callithrix jacchus)* erfolgten Stickstoffbilanzmessungen bei Verfütterung von „halbsynthetischen" und „synthetischen" Diäten mit Proteingehalten von null bis sieben Prozent. Der tägliche Stickstoffverlust bei längerer proteinfreier Ernährung („Abnutzungsquote") betrug 131 ± 16 mg/kg Körpergewicht^{0,75}. Eine N-Bilanz = ± 0 wurde mit der Zufuhr von 261 mg N/kg^{0,75} in Form hochwertiger Proteine erreicht. Sehr geringe Proteinaufnahme oder das Fehlen von Arginin und Histidin in einer Aminosäurenmischung führte zu Koprophagie. Es wird gefolgert, daß der Proteinbedarf erwachsener Krallenaffen dem des erwachsenen Menschen (auf der Basis des metabolischen Körpergewichts) sehr ähnlich ist. Etwa 6–7 % Protein hoher Qualität, auf Trockensubstanz bezogen, genügen zur Vermeidung einer negativen Stickstoffbilanz.

Schlüsselwörter: Stickstoffbilanz, Proteinbedarf, Krallenaffen, Callitrichidae

Key words: nitrogen balance, protein requirement, marmoset, *Callitrichidae*

Introduction

The minimum protein requirement of monogastric omnivorous mammals is still subject to debate. We have, for the first time, conducted nitrogen balance trials with marmosets *(Callithrix jacchus)* fed diets varying in sources and concentrations of protein.

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C. jacchus is one of the few New World primate species which are kept in significant numbers as laboratory animals. Field observations suggest a mixed diet of plant material such as buds, young leaves, sprouts, fruits, tree sap and animal prey like insects, reptiles, eggs and nestling birds. In captivity, *C. jacchus* thrives and reproduces on mixed diets simulating the diet regimen assumed to prevail in their natural tropical forest biotope.

Dietary recommendations for marmosets include a high intake of protein, usually well above 20 % of dry matter (see 5, 7), suggesting an especially high protein requirement. Ausman et al. (1) reported a low efficiency of protein utilization for another small New World primate, the squirrel monkey *(Saimiri sciureus)*, compared to Old World macaques. In adult cebus monkeys (2), members of the same subfamily as *Saimiri*, nitrogen balance for maintenance required at least 340 mg N per kg metabolic weight ($W^{0.75}$), a rather high value compared to the requirements of pigs, dogs and humans. We have maintained tamarins *(Saguinus fuscicollis)* over months on a diet with 6 % protein of high quality (6). These results indicate a rather "normal" protein requirement for *Callitrichidae*, the family to which marmosets and tamarins belong.

The following results are chosen from our balance experiments to arrive at estimates of the protein requirements of adult marmosets and to allow for interspecific comparisons. In addition, estimates of net protein utilization were derived from these data.

Materials and methods

Adult male *Callithrix jacchus*, between two and four years of age and weighing 350–450 g, were housed individually in metabolic cages at room temperatures of 24 ± 1 °C and a relative humidity of 55–60 %. Artificial light was provided from 0600 to 1800 hrs. The cages allowed for separation of urine from spilled food and feces. Food and water was offered ad libitum.

The semipurified diets contained constant levels of a vitamin mixture and a mineral and trace element mixture, 7 % soybean oil, 5 % coconut fat, and 5 % cellulose powder. Cornstarch, saccharose and occasionally glucose were used in rather constant proportions. The tested protein sources and/or amino acid mixtures were added at the expense of the carbohydrate mixture. Gross energy of the diets varied between 4.6 and 4.8 kcal/g of dry matter. All diets were pelleted (5 mm). More details on diets, protein sources, and amino acid mixtures are given in (8, 9).

Diet 3/3 contained 1.6 % of the essential amino acid mixture plus 0.5 % glycine, plus 0.8 % diammoniumcitrate. Diet 3/7 contained 1.6 % of the essential amino acid mixture plus 4.0 % glycin, plus 0.8 % diammoniumcitrate. The essential amino acid mixture was made up of arg 11.99, his 3.75, ile 13.29, leu 15.75, lys 9.82, met 5.64, phe 10.84, thr 7.37, try 2.60, and val 15.18 (sum total deviates from 100 because of his and lys hydrochloride). Kjeldahl-N was 0.80 % in diet 3/3 and 1.135 % in diet 3/7.

Test proteins were a soybean protein concentrate ("Danpro A"), a whey protein concentrate ("Globulal 70 N"), and acid casein ("Edible"). All protein sources were supplied by Meggle Corporation, Wasserburg, Bavaria. Collection periods for balance trials lasted five days. They followed a nine-day pre-period which allowed for adjustment to the respective test diet. Feces were lyophilized, the samples of urine and rinsing water were frozen. Nitrogen in feed, feces and urine was determined by a modified Kjeldahl method. Additionally, phosphorus in feed, feces, and urine was determined in most of the balance trials as well as urinary creatinine. Endogenous nitrogen in feces and urine was determined in collection periods after

Table 1. Total*) nitrogen loss on a protein free diet (mg N/animal/day).

		Previous diet 18 % protein			Previous diet 6 % protein		
Day	Animal	Bo	Di	Hu	Da	Ha	Vo
1– 4		119	131	141	81	81	86
5– 7		89	118	90	74	72	72
8–10		73	88	73	65	69	62
11–13		66	82	64	58	63	60
14–16		66	76	74	57	66	65
17–19		63	80	61	68	60	57
20–22		65	73	55	62	59	55

*) including calculated surface loss

nine days on a nitrogen free diet or a diet containing 1 % protein. Nitrogen losses via body surface were calculated as 20 mg N per kg body weight$^{0.75}$. Net protein utilization was calculated as follows:

$$NPU = \frac{\text{nitrogen retention + endogenous fecal N + endogenous urine N} \times 100}{\text{nitrogen intake}}$$

Results

Endogenous nitrogen

Nitrogen losses were determined over a period of 22 days on a protein-free diet. Three of the marmosets had previously received an 18 % protein diet, three others the same semipurified diet containing only 6 % protein. Nitrogen losses for each animal are listed in Table 1. There are marked differences in nitrogen excretion due to previous protein intake. While a plateau is reached after seven days in the animals previously on low protein, 10 days are needed to reach minimum excretion in the other three marmosets. During days 11 to 22 (Table 2), the mean total nitrogen loss is 65 ± 7 mg per day (47 % urine, 38 % feces, 15 % surface). Calculated via individual metabolic body weights, total nitrogen loss amounts to 131 ± 16 mg N per kg$^{0.75}$ per day. Feed intake was somewhat reduced

Table 2. Nitrogen losses day 11–22, mg N/animal/day.

	Previous protein 18 %			Previous protein 6 %			
Animal	Bo	Di	Hu	Da	Ha	Vo	$\bar{x} \pm s$
feces	28	28	20	26	24	19	24 ± 4
urine	26	41	33	25	29	30	31 ± 6
f + u	54	69	53	51	53	49	56 ± 7
			Total loss*) mg N/body weight$^{0.75}$				
	125	159	120	130	139	116	131 ± 16

*) including calculated surface loss

Table 3. Nitrogen balances (mg N/animal/day) on low protein intake.

		% dietary protein		
		1	3	6
Globulin,	Bo	–36	–10	+ 6
Globulin,	Vo	–68	–11	+ 8
Casein,	Bo	–34	–17	+ 2
Casein,	Vo	–55	–32	+18
Soy-Conc.,	Bo	–37	–10	+11
Soy-Conc.,	Vo	–48	–31	± 0
	$\bar{x}$	–46	–19	+ 8

throughout the depletion period in all six animals. Body weights dropped 9 % to 13 % during the 22 days on the protein-free diet.

Nitrogen balances from trials with three different protein sources are shown in Table 3. Each source has been fed at protein levels (N × 6.25) of 1 %, 3 %, and 6 % incorporated in the same purified diet. While diets with 3 % or less protein yielded negative balances without exception ($\bar{x} = -46$ mg N/day and -19 mg N/day for 1 % and 3 % protein, respectively), all balances based on the 6 % protein were positive ($\bar{x} + 8$ mg N).

Similar results were obtained from a different set of balances listed in Table 4. Here, all diets with 5 % protein (protein equivalent) or less resulted in negative balances. Again, levels of 6 % and above did lead to positive balances. Marginal supply of essential amino acids (diet 3/3) with low intake of "unspecific nitrogen" produced distinct negative balances. These were considerably improved by the addition of glycine (diet 3/7).

Net protein utilization was calculated for those balance periods in which protein intake led to slightly positive balances (Table 5). Mean values for the protein sources soy concentrate, oat flakes, and milk proteins are 58 %, 58 %, and 53 %, respectively.

Table 4. Individual N-balances (mg N/day) on amino acid diets and protein diets.

		Animal						
Diet	% protein (n × 6.25)	Ha	Ve	Is	Bo	Di	Da	Vo
Amino acids								
3/3	3			−12	−33	−18	−23	
3/7	7			+ 1	− 4	+21	− 9	
5/5	5			− 8	−11	−15	− 8	
7/7	7			+ 5	+ 8	+ 8	+ 5	
Protein								
6 soy	6	+ 8	− 4	+19				
6 soy + ess. AS	6	+ 8	+19	+ 5				
7 oats	7	+19		+12	+12	+31	+27	+1

Table 5. Net protein utilization (%) measured under conditions of marginal protein intake.

Protein source	Bodo	Dino	Hubertus	Django	Habakuk	Volker
6 % soy				54	57	46
6 % soy	61	54	85	64	64	
7 % oats	51	64	–	51	50	47
6 % whey	47					69
6 % soy	48					50
6 % casein	47					68

From four balance trials with amino acid diets, linear regressions of daily nitrogen balance on daily nitrogen intake were calculated, both expressed as mg N/kg bodyweight$^{0.75}$. Zero N-balance resulted from intakes of 238, 268, 261, and 275 mg nitrogen.

In trial phases with very low or zero protein intake, coprophagy occurred almost regularly. No coprophagy was seen in animals on diets with 6 % protein or 6 % of an amino acid mixture simulating egg protein. But omitting histidine and arginine from the amino acid diet did lead to coprophagy. In severe cases, the amount of feces eaten was estimated to be close to 30 % of total output. Some of the coprophagists licked urine from the preputium (10).

Discussion

Nitrogen losses during day 11 to 22 on the protein free diet allow for an estimate of the minimum protein requirement for maintainance in the adult state. As can be seen from Table 1, there is after day 10 only a negligible difference in nitrogen loss between animals which had consumed the high and low protein diets previously. Calculated on the basis of metabolic body weight, these losses amounted to 131 ± 16 mg nitrogen per kg$^{0.75}$ (Table 2). The "absolute nitrogen minimum" in adult humans has been determined by several workers (3, 4, 11, 18). The range of 13 measurements was 111–149 with a mean of 136 mg, all values expressed as daily nitrogen loss per kg body weight$^{0.75}$. We therefore conclude that even the small New World primates exhibit similarity of nitrogen turnover among omnivorous mammals.

Since all three protein sources yielded similar balance data (Table 3) and, in a different set of trials, similar NPU values (Table 5), a net protein utilization of slightly above 50 % can be assumed for protein sources of high quality. Accordingly, the minimum protein requirement would be at least twice the absolute N-minimum. To be on the safe side, the standard deviation (2×) should be added. The daily protein requirement then would be $2 \times (131 + 32) \times 6.25 = 2.4$ g/kg$^{0.75}$.

From the regressions of N-balance on N-intake, derived from another set of data (amino acid diets), one arrives at a very similar result. The mean of the four regressions for zero N-balance is 261 mg N-intake/day/kg$^{0.75}$.

Under our conditions, 2.4 g of protein/kg$^{0.75}$ would correspond to a dietary protein concentration of about 7 %. Actually, all protein concentrations of 6 % and above did lead to positive nitrogen balances (Tables 3 and 4), while 5 % resulted in negative balance in all four animals tested. Such a minimum protein intake, enough to avoid negative nitrogen balance in most individuals, may not suffice to provide optimum amino acid supply under the varying conditions of normal life.

The amino acid diets (Table 4) yielded results very similar to the protein diets. Of special interest, however, is a comparison of diet 3/3 and diet 3/7. The improvement of 3/7 over 3/3 is due solely to the addition of glycine. These results suggest that an amino acid pattern simulating whole-egg protein is deficient in "unspecific nitrogen", when in short supply in the adult state. The improvement of soy protein by a small addition of the essential amino acid mix is very likely due to methionine or methionine plus threonine. The omission of arginine and histidine from the "simulated egg mix" eventually led to rejection of the diet in most cases or to coprophagy. No taste differences could be detected by humans. We conclude that arginine and histidine may well be essential for the adult marmoset.

Positive nitrogen balances are often observed in adult individuals. They may be due to previous losses or to cumulative experimental errors. In our case, comparisons with the phosphorus balances of the same individuals and the results of urinary creatinine yielded estimates of a 1–7 % error range, in most cases well below 5 % overestimation of true nitrogen retention.

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