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EFFECT IN RATS OF PARTIAL REPLACEMENT OF COW'S MILK PROTEIN BY SUPPLEMENTARY NITROGEN

SUMMARY—A number of experiments studied growth of weanling rats and urinary nitrogen excretion in young adult rats when cow's milk protein was partially replaced by varying levels of supplementary nitrogen. A mixture of nonessential L-amino acids (NEAA) or a mixture of diammonium citrate and glycine (DAC-Gly) was used as the source of nitrogen. Substitution of the 15% milk protein diet to the extent of 10% slightly reduced growth; significant growth reduction occurred with substitutions of 20% and greater. Fortification of the diets containing 10.5% milk protein and the supplementary nitrogen sources with sulfur amino acids did not restore growth to the maximum rate obtained with the 15% milk protein diet. Additional supplementation with tryptophan further improved growth slightly but not to the maximum rate. Several other essential amino acids, alone or in combination, had no apparent effect. Based on urinary nitrogen excretion, comparable results were obtained with young adult rats by substituting nitrogen for milk protein in the diets. The reduced performance following substitution of milk protein with supplementary nitrogen may be due partly to decreased utilization of sulfur amino acids and possibly to decreased utilization of all essential amino acids.

INTRODUCTION

WHEN ESSENTIAL amino acids form the sole dietary source of protein for rats, a lower growth rate is observed than when the same level of dietary N is supplied by a well-balanced amino acid mixture containing approximately equal amounts of essential and nonessential amino acids (Rose et al., 1948; Stucki et al., 1962; Young et al., 1968).

Animal proteins of high biological value may contain some or all of the essential amino acids, expressed per unit of total protein nitrogen, in excess of required amounts for the rat. Bender (1965) suggested that egg protein contains a surplus of 10–20% of all essential amino acids. In this case, essential amino acids are catabolized to meet calorie needs or are used for synthesis of nonessential amino acids and other N-containing compounds. Therefore, isonitrogenous replacement of the excess essential amino acids with supplementary nitrogen (from nonessential amino acids or other utilizable N sources) should not decrease dietary N utilization and animal performance.

For man, studies in this laboratory (Scrimshaw et al., 1966; Huang et al., 1966) and in others (Snyderman et al., 1962, Kofranyi et al., 1964) suggested that high quality protein N can be partially replaced with supplementary nitrogen without reducing nitrogen retention.

In the present series of experiments, weanling rats were fed dried skim milk as the source of dietary protein. A mixture of nonessential L-amino acids (NEAA), patterned as in cow's milk, or a mixture of diammonium citrate and glycine (DAC-Gly) was used as supplementary nitrogen to replace part of the protein nitrogen. The influence of the protein nitrogen replacement on growth rate was studied. Some experiments evaluated the effects of adding several essential amino acids to the diets containing the supplementary N sources.

The influence of age was also evaluated because available data indicate that for both man (Rose, 1957; Holt et al., 1965) and rats (Rama Rao et al., 1964; Smith et al., 1967) the requirements for essential amino acids per g total nitrogen differ in young and adults.

EXPERIMENTAL & RESULTS

Methods

A preliminary experiment (Table 1) confirmed general experience that maximum growth rates are achieved with a diet containing 15% protein from unsupplemented skim milk. This concentration of dietary protein was used, therefore, as a control level in the growth studies. The net protein utilization (NPU) of skim milk protein by rat assay (Bender et al., 1957) was 71 ± 2 compared with 63 ± 2 for unsupplemented casein as a standard.

Male weanling rats (Charles River Laboratories, North Wilmington, Mass., CD strain) were fed the experimental diets ad libitum for 14 days. The basal diets contained: dried skim milk (Dried Nonfat Skim Milk, Carnation Company, Van Nuys, California), 28.1 to 50.5% (10–18% protein); corn oil, 10%; salt mixture, 5% (Rogers et al., 1965); vitamin mixture, 0.5% (Rogers et al., 1965); choline chloride, 0.2%; and dextrin:sucrose (2:1 weight ratio) to make a total of 100%. In all diets lactose was partially substituted for the dextrin:sucrose mixture to achieve the lactose level present in the control diet (15% skim milk protein). Weekly weight gain and daily food intake were recorded.

The essential amino acid content of the basal diet, calculated from the data of Orr et al. (1957), and the essential amino acid requirements for rat growth as reported by Rama Rao et al. (1964) are given in Table 2. The diet appears to meet all requirements although it is borderline for the sulfur amino acids, methionine and cystine. In some experiments methionine and cystine (all L-amino acids purchased from General Biochemicals, Inc., Chagrin Falls, Ohio) were added in a ratio by weight of 3 to 1 in order to reach a calculated sulfur amino acid content of 0.65% in the diet.

Experiments 1 and 2

Experiment 1 was designed to determine the extent to which a mixture of NEAA could isonitrogenously replace a 15% milk protein diet without affecting growth in weanling rats. Body weight gains are summarized in Table 3. A highly significant ($P < 0.01$) decrease in growth, PER, and food efficiency was observed only for

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Table 1—Effect of dietary level of skim milk protein on the 14-day growth rate of weanling rats.

Group No.	Dietary skim milk protein %	Food intake g/day	Weight gain[1] g/14 days	FE[2] %	PER[3]
1	10	8.8	45.5 ± 3.3	36.9	3.69
2	12	8.7	56.4 ± 1.5	46.2	3.86
3	15	9.9	76.4 ± 4.0	54.8	3.67
4	18	9.6	77.9 ± 2.1	57.8	3.22

[1]Beginning weight of rats was 45 ± 2 g. Weight gain is mean ± standard error for 8 rats per group.
[2]Food efficiency (FE) is (g gain per g food intake) × 100.
[3]Protein efficiency ratio (PER) is g gain per g protein consumed.

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Table 2—Calculated % of essential amino acids in experimental diets compared with requirements for young growing rats.

Amino acid	Experimental diets					Amino acids required[2]
		% of milk protein N replaced				
	Basal[1]	10	15	20	30	
	(% of Diet)					
Isoleucine	0.96	0.86	0.82	0.77	0.67	0.55
Leucine	1.47	1.32	1.25	1.18	1.03	0.70
Lysine	1.17	1.05	0.99	0.94	0.82	0.90
S-amino acids (Methionine + cystine)	0.50	0.45	0.43	0.40	0.35	0.50
Aromatic amino acids (Phenylalanine + tyrosine)	1.49	1.34	1.27	1.19	1.04	0.72
Threonine	0.69	0.62	0.59	0.55	0.48	0.50
Tryptophan	0.20	0.19	0.18	0.17	0.15	0.11
Valine	1.03	0.93	0.88	0.83	0.72	0.55
Arginine	0.55	0.50	0.47	0.44	0.39	—
Histidine	0.39	0.35	0.33	0.31	0.27	0.25

[1] Calculated values from data of Orr et al. (1957).
[2] From Rama Rao et al. (1964).

the 30% substitution in this experiment.

Therefore, a second experiment was conducted with larger groups of rats to evaluate the effect of 20% and 30% replacements of milk protein N by two sources of supplementary nitrogen. The results are summarized in the lower half of Table 3. Significant ($P < 0.01$) decreases in growth rate, FE, and PER occurred for both sources of nitrogen at the two substitution levels.

Experiment 3

Because the sulfur amino acids, methionine and cystine, are limiting in cow's milk protein for weanling rats, a third experiment was conducted to determine the influence of DAC-Gly substitution of milk protein N in diets supplemented with sulfur amino acids. Table 4 summarizes these results. Comparison of groups 5 and 9 reveals that inclusion of DAC-Gly in the diet resulted in a lower weight gain ($P < 0.05$) than when the diet contained 10.5% milk protein alone. This effect appeared to be related to the reduced food intake, particularly because efficiency of food conversion was similar for both groups.

Growth rates were similar for diets containing 10.5% milk protein, with or without added DAC-Gly, supplemented with 0.15–0.28% sulfur amino acids (compare group 6 vs. 10, 7 vs. 11). The highest level of S-amino acid supplementation reduced body weight gain significantly when the diet contained DAC-Gly (group 8) but not when the only other dietary N was milk protein (groups 4 and 12). These findings suggest that the DAC-Gly mixture affected utilization of sulfur amino acids at the high and possibly at the low dietary sulfur amino acid levels.

Although addition of 0.15–0.28% S-amino acids to 10.5% milk protein diets containing DAC-Gly allowed weight gains which were similar to the 10.5% milk protein diets without the added nitrogen, growth rate remained lower than that on the 15% milk protein diet which supplied the same level of total N and was supplemented with sulfur amino acids.

Experiments 4 and 5

A study was conducted with addition of various essential amino acids to diets containing 30% of total dietary N from the NEAA mixture. The added amino acids achieved the concentrations calculated to be present in the 15% milk protein diet. Arginine, lysine, or threonine addition to the diets (Expt. 4, Table 5) failed to improve weight gain significantly.

The effects of adding tryptophan, tryptophan and threonine, or a combination of several essential amino acids, were also studied (Expt. 5, Table 5). Tryptophan significantly ($P < 0.05$) improved the growth rate, mainly as a consequence of higher food intake (group 4). Addition of tryptophan and threonine together or in combination with arginine and lysine did not result in a higher growth rate than that achieved with tryptophan alone.

Experiment 6

DAC-Gly replaced from 10–50% of skim milk protein N while concentrations of 0.65% sulfur amino acids and 0.21% tryptophan were maintained as in the control diet. The results, summarized in Table 6, show that a 10% replacement of milk protein produced a small but nonsignificant ($P > 0.05$) decrease in growth rate of weanling rats. A significant reduction ($P < 0.01$) in growth rate did occur when 20% of the protein was replaced by DAC-Gly. Proportionately greater reductions were observed with still higher rates of replacement by DAC-Gly despite the unchanged amounts of sulfur amino acids and tryptophan in the diet.

Experiment 7

In young adult rats isonitrogenous replacement of milk protein with the DAC-Gly mixture was studied by monitoring changes in urinary nitrogen excretion. The basal diet contained 4% milk protein, while the remaining

Table 3—Fourteen-day growth of weanling rats during isonitrogenous replacement of skim milk by nonessential amino acids or glycine and diammonium citrate.

	Level of milk protein in diet %	Nitrogen source	% total dietary N from added N source	Food intake g/day	Weight gain g/14 days	FE %	PER
Expt. 1[1]	15.00	—	0	9.4	73.8 ± 4.0	56.1	3.74
	13.50	NEAA[2]	10	9.3	69.1 ± 2.2	53.1	3.54
	12.75	NEAA	15	9.4	66.8 ± 1.9	50.8	3.38
	12.00	NEAA	20	10.0	67.0 ± 2.3	47.9	3.19
	10.50	NEAA	30	9.3	56.0 ± 2.4	43.0	2.87
Expt. 2[3]	15.0	—	0	9.8	68.0 ± 2.4	49.6	3.30
	12.0	NEAA	20	9.3	53.4 ± 2.2	41.0	2.73
	12.0	DAC-Gly[4]	20	9.9	56.8 ± 2.1	41.0	2.73
	10.5	NEAA	30	8.9	48.3 ± 2.0	37.8	2.58
	10.5	DAC-Gly	30	9.5	49.5 ± 2.5	37.2	2.48

[1] Beginning weight of rats was 36 ± 2 g. Weight gain is mean ± standard error for 8 rats per group.
[2] Amino acid (g) per 100 g mixture: L-alanine, 6.5; L-aspartic, 13.72; L-glutamic, 44.03; glycine, 3.72; L-proline, 20.90; L-serine, 11.09.
[3] Beginning weight of rats was 46 ± 3 g. Weight gain is mean ± standard error for 14 rats per group.
[4] Diammonium citrate and glycine mixture. Each compound supplied equal amounts of N per unit weight of mixture.

Table 4–Fourteen-day growth of weanling rats on diets containing skim milk protein supplemented by S-amino acids, with and without added glycine and diammonium citrate (Experiment 3).

Group No.	Level of milk protein in diet %	% total dietary N from added DAC-Glycine	Methionine & cystine added[1]	% dietary methionine & cystine[2]	Food intake g/day	Weight gain[3] g/14 days	FE %	PER
1	15.0	–	–	0.50	10.3	73.3 ± 4.5	50.8	3.49
2	15.0	–	0.15	0.65	10.6	79.8 ± 3.2	53.8	3.55
3	15.0	–	0.28	0.77	10.6	81.1 ± 4.1	54.6	3.58
4	15.0	–	0.43	0.92	10.7	81.3 ± 3.1	54.3	3.52
5	10.5	30	–	0.35	8.9	49.0 ± 4.1	39.3	2.62
6	10.5	30	0.15	0.50	9.8	62.7 ± 5.1	45.7	3.02
7	10.5	30	0.28	0.65	11.1	73.3 ± 3.2	47.2	3.09
8	10.5	30	0.43	0.77	10.7	64.6 ± 2.1	43.1	2.79
9	10.5	–	–	0.35	11.2	61.0 ± 4.2	38.9	3.71
10	10.5	–	0.15	0.50	10.9	67.1 ± 3.5	44.0	4.13
11	10.5	–	0.28	0.65	11.3	73.7 ± 4.8	46.6	4.32
12	10.5	–	0.43	0.77	12.2	78.9 ± 5.1	46.2	4.23

[1]Methionine and cystine were added in a weight ratio of 3:1.
[2]Calculated values.
[3]Beginning weight of rats was 45 ± 3 g. Weight gain is mean ± standard error for 7 rats per group.

dietary components were similar to those in previous experiments. Lactose was maintained at the basal (4% milk protein) level in all diets.

Except for sulfur amino acids, the basal diet contained concentrations of essential amino acids in excess of those suggested by Smith et al. (1967) for maintenance in young adult rats. These authors suggested 0.29% for the total sulfur amino acids, whereas the present 4% milk protein diet supplied 0.13%. However, with a 30% DAC-Gly substitution, isoleucine and threonine also were probably deficient.

The rats, housed individually in metabolic cages, were given 10 g of the basal diet daily for an initial 10-day adaptation period followed by a 10-day control interval (Period 1). For 10 days (Period 2) after the control phase, group 1 was maintained on the basal diet, while groups 2, 3 and 4 were given diets in which the DAC-Gly mixture replaced 10, 15 and 20% of the milk protein, respectively. During the final 10 days (days 31–40, Period 3) all groups were returned to the basal 4% milk protein diet.

On day 11 of the experiment, daily urine collections began. Two 5-day pooled samples of urine were prepared for each period and analyzed for nitrogen by the Kjeldahl method.

Table 5–Fourteen-day growth of weanling rats on diets containing skim milk protein supplemented with sulfur amino acids, NEAA, and various essential amino acids.

	Group No.	Level of milk protein in diet[1] %	% total dietary N from added NEAA mixture[2]	Essential amino acid added %		Food intake g/day	Weight gain[3] g/14 days	FE %	PER
Experiment 4									
	1	15.0	–	–	–	12.1	85.6 ± 3.0	50.5	3.34
	2	10.5	30	–	–	12.1	68.4 ± 2.3	40.4	2.64
	3	10.5	30	L-Arg.	0.12	12.3	71.0 ± 3.1	41.2	2.67
	4	10.5	30	L-Lys.	0.13	11.9	69.0 ± 2.2	41.1	2.68
	5	10.5	30	L-Threo.	0.10	12.3	75.4 ± 2.7	43.8	2.84
	6	10.5	–	–	–	12.2	70.4 ± 3.1	41.2	3.81
	7	10.5	–	L-Arg.	0.12	13.4	74.9 ± 3.3	39.9	3.66
	8	10.5	–	L-Lys.	0.13	13.6	70.2 ± 2.5	36.9	3.37
	9	10.5	–	L-Threo.	0.10	12.2	71.8 ± 2.3	42.0	3.86
Experiment 5									
	1	15.0	–	–	–	11.7	80.7 ± 2.7	49.2	3.25
	2	10.5	30	–	–	11.3	65.4 ± 2.9	41.3	2.70
	3	10.5	30	L-Threo.	0.10	11.1	63.0 ± 3.3	40.5	2.63
	4	10.5	30	L-Try.	0.06	12.5	74.0 ± 4.8	42.3	2.75
	5	10.5	30	L-Threo.	0.10	11.5	71.0 ± 4.2	44.1	2.85
				+ L-Try.	0.06				
	6	10.5	30	L-Threo.	0.10	11.1	68.3 ± 4.2	44.0	2.80
				+ L-Try.	0.06				
				+ L-Arg.	0.12				
				+ L-Lys.	0.13				

[1]15% milk protein diets were supplemented with 0.15% methionine and cystine (3:1) and the 10.5% milk protein diets with 0.3%.
[2]Composition of the NEAA mixture is given in Table 3.
[3]Beginning weight of rats was 45 ± 3 g. Weight gain is mean ± standard error for 8 rats per group in Experiment 4 and 7 rats per group in Experiment 5.

Table 6—Fourteen-day growth of weanling rats fed diets with different levels of DAC-Glycine replacement of skim milk protein supplemented with sulfur amino acids and tryptophan (Experiment 6).

Group No.	Level of milk protein in diet %	Amino acid added %	% total dietary N from added DAC-Glycine	Food intake g/day	Weight gain[1] g/14 days	FE %	PER
1	15.0	Met-Cys 0.15	0	11.2	84.8 ± 2.4	54.1	3.57
2	13.5	Met-Cys 0.20 + Try 0.02	10	11.1	81.2 ± 2.5	52.3	3.43
3	12.0	Met-Cys 0.25 + Try 0.04	20	10.3	68.4 ± 4.4	47.4	3.10
4	10.5	Met-Cys 0.30 + Try 0.06	30	9.7	61.0 ± 2.3	44.9	2.92
5	7.5	Met-Cys 0.40 + Try 0.10	50	8.3	35.0 ± 2.8	30.1	1.94

[1] Beginning weight of rats was 55 ± 3 g. Weight gain is mean ± standard error for 7 rats per group.

Rats were weighed at the beginning of the study (mean weight 200 g) and at the end of each dietary period. They showed little change in weight during the entire 40-day span.

Table 7 shows that 15 or 20% substitution of milk protein with DAC-Gly resulted in a significant increase ($P < 0.01$) in levels of urinary nitrogen excretion during the first five days of Period 2 (days 21–25). During the last five days of this period, urinary nitrogen excretion decreased for groups 2 and 3 (10 and 15% substitution) but was still high for group 4 (20% substitution).

When the rats were returned to the basal diet (Period 3) a sharp reduction in urinary N output occurred in groups 2–4, indicating improved retention of dietary nitrogen on the basal diet as compared with partial replacement of skim milk protein N by supplementary nitrogen.

Urinary nitrogen excretion during the last five days of Period 2 was significantly greater ($P < 0.01$) for rats fed the diet containing 20% of N from DAC-Gly compared with other groups. However, the level of urinary nitrogen excretion was not significantly higher ($P > 0.05$) for rats fed diets with the 10 and 15% substitution than for rats given the basal diet throughout (group 1).

In contrast to these results, the reduced urinary N excretion following return to the basal diet was highly significant ($P < 0.01$) for all groups given DAC-Gly. The control group (group 1), maintained throughout the experiment on the basal diet, did not show a significant change in urinary N excretion ($P > 0.1$) between Periods 2 and 3.

Experiment 8

Because the maintenance requirement of young adult rats for S-amino acids (methionine and cystine) may be higher than the amount supplied by the basal diet (Smith et al., 1967), the effect of partial replacement of milk protein nitrogen by DAC-Gly was studied while a constant dietary level of S-amino acids and tryptophan was maintained. In this study, the basal diet and 20% and 30% levels of substitution were utilized. The experimental design was similar to that of Experiment 7, except that six rats were used per dietary group.

Table 8 shows values of urinary nitrogen excretion for the last five days (15–20) of Period 1 during feeding of the basal 4% protein diet, for days 25–30 of Period 2, and for the first five days (days 31–35) of Period 3. The results show that even with the concentration of S-amino acids and tryptophan maintained at the level present in the basal diet, urinary nitrogen excretion increased for both levels of substitution with DAC-Gly. However, maintenance of the dietary S-amino acid and tryptophan levels appeared to result in a smaller urinary nitrogen increase following 20% replacement than was observed in the earlier experiment without supplemental dietary S-amino acids (Table 7).

DISCUSSION

GROWTH depression resulted from isonitrogenous substitution of cow's milk protein by supplementary nitrogen, either a mixture of nonessential L-amino acids, patterned as in cow's milk, or DAC-Gly.

Various investigators have examined the interrelationship between glycine and methionine. Glycine is known to alleviate the growth depressing effects of high levels of dietary methionine (Hardin et al., 1951; Roth et al., 1950; Benevenga et al., 1967; Wertz et al., 1968) and methionine protects against excess glycine (Wertz et al., 1968).

In the present studies, DAC-Gly was utilized as a source of supplementary nitrogen. The additional dietary glycine may have limited the utilization of the sulfur amino acids and resulted in the lower growth rates achieved when DAC-Gly was added to the 10.5% milk protein diet. Growth rate was reduced to the same extent following the 20% or 30% substitutions of milk protein nitrogen by both DAC-Gly and NEAA mixture.

Also, in rats given the methionine-supplemented diets containing 10.5% milk protein plus nitrogen, growth rates remained lower than the rate of rats on the 15% milk protein diets with supplementary methionine and cystine. Therefore, in addition to possible alterations in sulfur amino acid metabolism, decreased utilization of all essential amino acids may have been responsible for the re-

Table 7—Effect of 40-day replacement of milk protein by DAC-Glycine on urinary nitrogen excretion in young adult rats (Experiment 7).

Days of expt.[2]	% of Milk protein N replaced[1] 0 (1)	10 (2)	15 (3)	20 (4)
	(Mg urine N per day)[3]			
16–20	40.3 ± 1.2	42.8 ± 2.0	42.4 ± 1.6	40.4 ± 1.5
21–25	40.0 ± 1.0	43.7 ± 2.0	46.7 ± 1.6	45.9 ± 1.3
26–30	38.0 ± 1.4	39.7 ± 1.9	41.1 ± 1.2	44.5 ± 1.1
31–35	37.0 ± 0.9	35.6 ± 1.0	36.0 ± 1.1	36.0 ± 1.3
36–40	38.7 ± 0.8	35.6 ± 1.8	36.6 ± 1.3	37.3 ± 1.0

[1] Group number is in parentheses.

[2] During days 16–20 and 31–40 all rats were given the basal 4% milk protein diet. During days 21–30 groups of rats were fed the basal diet or a diet containing 10, 15 or 20% of milk protein N replaced with DAC-Glycine.

[3] Mean ± standard error for 10 rats per group.

Table 8—Urinary nitrogen excretion in young adult rats fed milk protein diets, supplemented with sulfur amino acids and tryptophan, following partial replacement with diammonium citrate and glycine for 40 days (Experiment 8).

Days of expt.[1]	% of Milk protein N replaced 0	20	30
	(Mg urine N per day)[2]		
16–20	44.0 ± 1.1	42.3 ± 1.6	42.5 ± 1.7
25–30	40.9 ± 2.1	45.5 ± 2.3	47.8 ± 1.2
31–35	39.5 ± 1.6	38.9 ± 2.7	35.5 ± 1.8

[1] During days 16–20 and 31–35 all rats were given the basal diet and during days 25–30, rats were fed the basal diet or diets containing 20 and 30% of total dietary N from DAC-Glycine. All diets contained 0.13% methionine and cystine and 0.06% tryptophan.

[2] Mean ± standard error for 6 rats per group.

duced growth rates following replacement of the milk protein by the nitrogen.

The essential amino acid pattern of the test protein probably is an important factor modifying response to substitution of that protein by supplementary nitrogen. Braham et al. (1968) observed that addition of nonessential amino acids or DAC to egg protein did not influence weight gain of rats. Addition of either or both of these nitrogen sources to casein, soybean, or cottonseed protein decreased weight gain. These authors suggest that the response to nonessential amino acids or DAC depends largely on the essential amino acid pattern of the protein tested.

Moran et al. (1967) observed a depression in egg production and weight gain of hens when DAC was added to a 10% protein diet of which half the protein was supplied by corn meal and half by soybean meal. Recently Daniel et al. (1968) have reported that addition of L-glutamic acid to diets containing 5.0 to 8.5% milk protein adversely affected growth rates of rats. On the other hand, Venkat Rao et al. (1964) showed that, although weight gain was proportional to the quantity of egg protein in the diet, addition of L-glutamic acid to the egg protein diets did not adversely affect growth rates in this case.

The present findings showed that tryptophan supplementation of the 10.5% milk protein diet containing added DAC-Gly and sulfur amino acids resulted in a small improvement of growth rate. Addition of several other essential amino acids suspected as being limiting in the methionine-supplemented 10.5% milk protein diets, with or without added nitrogen, failed to improve growth rates. However, Swendseid et al. (1962), using an 8% casein diet supplemented with methionine, found that the growth depression resulting from addition of several sources of nonspecific nitrogen disappeared after addition of tryptophan and threonine, except when glycine was the source of supplementary nitrogen.

In the present study the higher growth rates of control rats fed the 15% milk protein diet, compared with the controls used by Swendseid et al. (1962), may explain the failure of tryptophan and threonine supplementation of diets containing the nitrogen to restore growth to the maximum rate achieved with the 15% milk protein diet.

A 10% substitution of milk protein N, with or without supplementary sulfur amino acids, only slightly and nonsignificantly reduced the growth rate in the present study. Proportionately greater, significant reductions were observed at higher levels of substitution. These results differ from those in children (Snyderman et al., 1962) and adult men (Kofranyi et al., 1964; Scrimshaw et al., 1969), who apparently are able to tolerate at least a 10–20% replacement of milk protein with supplementary nitrogen.

When urinary nitrogen excretion was the major parameter used for determining the effects of milk protein substitution by nitrogen in young adult rats, the results also indicated lessened N utilization (Tables 7 and 8). The increase in urinary nitrogen excretion was greater with higher levels of substitution. This adverse effect on nitrogen balance was not prevented by maintenance of the S-amino acid and tryptophan content of the diets at the level present in the control 4% milk protein diet.

Substitution of the milk protein nitrogen by supplementary nitrogen sources at the 10 or 15% levels, without additional sulfur amino acids, resulted in increased urinary nitrogen excretion. However, a partial adaptation to protein replacement with DAC-Gly appeared to occur, as indicated by the decreased rate of urinary nitrogen excretion during the latter part of the replacement period. These results with young adult rats are, therefore, generally similar to results obtained with weanling rats.

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