Growth and liver enzyme response in growing rats to graded levels of methionine plus cystine in fortified-barley diets. Response at constant methionine : cystine

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1. Twenty-eight male rats of initial age 27 d were fed on fortified-barley diets for 3 weeks. In all experimental diets, both crude protein (nitrogen × 6·25) and methionine:cystine were constant at 120·0 g/kg dry matter (DM) and 2:1 respectively. The basal diet contained 4·5 g methionine plus cystine/kg DM with L-methionine plus L-cystine (2:1, w/w) added in increments of 0·5 g/kg DM to a final level of 7·0 g methionine plus cystine/kg DM. A 'positive-control' diet of barley plus 193·7 g soya-bean meal/kg DM contained 6·0 g methionine plus cystine/kg DM.

2. Weight gain, food conversion efficiency (FCE), urinary urea-N excretion, carcass composition and activities of liver cystathionine synthase (*EC* 4.2.1.22) and N^5-methyltetrahydrofolate-homocysteine-methyltransferase (*EC* 2.1.1.13) were determined.

3. Weight gain, food consumption, FCE and carcass composition measurements of rats showed either small or no differences between the experimental diets containing 4·5–7·0 g methionine plus cystine/kg DM. For the over-all period, weight gain and FCE of rats receiving the 'positive control' diet were significantly higher than values obtained with rats receiving any of the experimental diets.

4. Cystathionine synthase activity (μmol/mg protein per 60 min; units) increased from 13·38 at 4·5 g dietary methionine plus cystine/kg DM to 18·81 at 5·0 g dietary methionine plus cystine/kg DM. The activity was then inhibited to reach a minimum value of 10·16 units at the 6·0 g/kg DM dietary level. Thereafter the activity increased to a value of 30·00 units at 7·0 g dietary methionine plus cystine/kg DM.

5. The activity of N^5-methyltetrahydrofolate-homocysteine-methyltransferase was constant at 0·70–0·74 nmol/mg protein per 60 min between dietary levels of 4·5 and 5·0 g methionine plus cystine/kg DM. The activity then increased to a maximum value of 2·32 nmol/mg protein per 60 min at the 6·0 g/kg DM level. Thereafter the activity decreased, reaching a minimum value of 0·70 nmol/mg protein per 60 min at the 7·0 g methionine plus cystine/kg level.

6. Urinary urea-N excretion decreased significantly from 1·07 g/kg DM intake at the 4·5 g dietary methionine plus cystine/kg DM level to 1·05 g/kg DM at the 5·0 g/kg dietary level, then dropped significantly to a level of 1·01–1·00 g/kg DM intake for the higher levels of dietary methionine plus cystine.

Results of a previous experiment (Ngwira & Beames, 1978) indicated that the requirement of dietary methionine plus cystine for optimal growth of weanling rats was 4·5–5·0 g/kg (dry matter (DM) basis), when the cystine level was held constant at 2·0 g/kg DM. The experiment also showed that the activities of liver cystathionine synthase (*EC* 4.2.1.22) and N^5-methyltetrahydrofolate-homocysteine-methyltransferase (mTHF Enz; *EC* 2.1.1.13) were constant between the dietary methionine plus cystine levels of 3·5 and 5·0 g/kg DM. The activities of both enzymes indicated metabolic disturbances in methionine metabolism when dietary methionine plus cysteine was increased to levels higher than 5·0 g/kg DM.

Byington *et al.* (1972) showed that the growth of young rats was optimal when methionine:cystine was 70:30 but not when the value was less than 50:50. Finkelstein (1967) and Shannon *et al.* (1972) found the activity of cystathionine synthase to be inhibited by low proportions of methionine relative to cystine. In our earlier experiment (Ngwira & Beames, 1978) where enzyme disturbances were detected beyond dietary methionine plus cystine levels of 5·0 g/kg DM, the ratio, methionine:cystine varied from treatment to treatment.

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With the basal diet containing 1·5 g methionine and 2·0 g cystine/kg DM, methionine was added to provide a maximum level of methionine plus cystine of 7·0 g/kg DM, resulting in methionine:cystine varying from 1·5:2 in the basal diet to 5·0:2 in the diet with the maximum level of sulphur amino acids. Because of a possible effect of this confounding of the methionine:cystine value and the level of methionine plus cystine, it was decided essentially to repeat the experiment of Ngwira & Beames (1978) but with a constant methionine:cystine of 2:1 as shown by Wretlind and Rose (1950) to be satisfactory and later recommended by Byington *et al.* (1972).

MATERIALS AND METHODS

Experimental design

The design incorporated seven dietary treatments, with four individually housed weanling rats per treatment. Treatments and replicates were randomly assigned to individual cages in a room with temperature kept constant at 27°.

Animals and cages

Twenty-eight male rats (Woodlyn–Wistar strain, Woodlyn Laboratories, Guelph, Ontario) 27 d of age at the start of the 21 d feeding period were used. The rats were randomly allocated to the stainless-steel cages, with urine collected as outlined by Ngwira & Beames (1978).

Diets

The diets (Table 1) were based on hammer-milled (0·75 mm screen) barley grain and included a 'positive-control' diet (diet 1) of barley plus soya-bean meal. All experimental diets had a methionine:cystine value of 2:1. A minimum level of dietary methionine plus cystine of 4·5 g/kg DM (diet 2) was chosen for the present trial, as any further reduction would have required this basal diet and all the other experimental diets to be virtually completely synthetic. This would have been in conflict with the basic aim of the experiment which was to test the relationship on barley-based diets.

The basal diet (diet 2) provided (/kg DM) 1·5 g cystine and 3·0 g methionine. Methionine:cystine was held constant at 2:1 in all experimental diets, with 0·5 g/kg DM increments of methionine plus cystine (2:1) added to provide a maximum level of 7·0 g methionine plus cystine/kg DM in diet 7.

The experimental diets were isonitrogenous with 120 g crude protein (nitrogen × 6·25)/kg DM and isoenergetic with 15·80 MJ gross energy/kg DM. Threonine inclusion was based on the net requirements estimated by Aw-Yong & Beames (1975). The requirements for all other nutrients were based on the (US) National Research Council (1972) values for the growing rat. Amino acids were added to the L-isomers (Ajinomoto Co., Japan). Amino acid, protein and gross energy contents of the barley, soya-bean meal, positive-control diet and basal diet were the same as those given by Ngwira & Beames (1978).

Methods used in the feeding of the rats, the determination of daily body-weight gain and food consumption, the extraction of livers for enzyme assays and the preparation of carcasses for chemical analyses were the same as those described by Ngwira & Beames (1978).

Analytical methods

The activity of cystathionine synthase was assayed using the method of Kashiwamata & Greenberg (1970). The mTHF Enz was extracted and assayed using the method of Mudd *et al.* (1970). Both these methods and those used for urea-N, total amino acids, and carcass N, fat and ash were described in an earlier paper (Ngwira & Beames, 1978).

Table 1. *Composition (g/kg dry matter (DM)) of rat diets with a constant methionine:cystine value of 2:1*

	Diet no.						
	1	2	3	4	5	6	7
Methionine + cystine*	6·0	4·5	5·0	5·5	6·0	6·5	7·0
Ingredients							
Barley	774·9	555·6	555·6	555·6	555·6	555·6	555·6
Soya-bean meal	193·7	—	—	—	—	—	—
Maize starch	—	351·4	351·4	351·4	351·4	351·4	351·4
Methionine + cystine†	—	1·9	2·4	2·9	3·4	3·9	4·4
NEAA	—	30·4	29·9	29·4	28·9	28·4	27·9
Threonine‡	—	3·4	3·4	3·4	3·4	3·4	3·4
Valine§	—	3·8	3·8	3·8	3·8	3·8	3·8
Isoleucine§	—	3·2	3·2	3·2	3·2	3·2	3·2
Leucine§	—	3·2	3·2	3·2	3·2	3·2	3·2
Phenylalanine + tyrosine§	—	2·7	2·7	2·7	2·7	2·7	2·7
Histidine§	—	1·7	1·7	1·7	1·7	1·7	1·7
Lysine§	—	6·8	6·8	6·8	6·8	6·8	6·8
Arginine§	—	3·2	3·2	3·2	3·2	3·2	3·2
Tryptophan§	—	0·6	0·6	0·6	0·6	0·6	0·6
Defluorinated rock phosphate ‖	15·2	16·4	16·4	16·4	16·4	16·4	16·4
Calcium carbonate¶	6·2	5·7	5·7	5·7	5·7	5·7	5·7
Iodized salt**	5·0	5·0	5·0	5·0	5·0	5·0	5·0
Trace mineral + vitamin premix††	5·0	5·0	5·0	5·0	5·0	5·0	5·0

NEAA, non-essential amino acids (incorporated as a 1:1:1:1 mixture of alanine–aspartic acid–glycine–glutamic acid (Abernethy & Miller, 1965; Womack, 1969) to bring the crude protein (nitrogen × 6·25) level to 120·0 g/kg dry diet).

* Expressed as total amino acids, not residues.

† Methionine and cystine were supplied as L-isomers (Ajinomoto Co., Tokyo, Japan). The 1·9 g/kg DM added to the basal diet (diet 2) was from L-methionine only, to bring the methionine:cystine value to 2:1. All other experimental diets contained a methionine:cystine value of 2:1.

‡ Incorporated according to Aw-Yong & Beames (1975).

§ All amino acids were incorporated as L-isomers (Adjinomoto Co.) to meet the (US) National Research Council (1972) recommendations.

‖ Estimated to contain 300 g calcium/kg and 140 g phosphorus/kg.

¶ Estimated to contain 400 g Ca/kg and 0 g P/kg.

** Estimated to provide 0·15 mg iodine/kg dry diet.

†† Provided (/kg dry diet) 44 mg manganese as $MnSO_4 . H_2O$, 110 mg zinc as $ZnSO_4 . 7H_2O$, 500 mg butylated hydroxytoluene, 20 μg cyanocobalamin, 2·9 mg riboflavin, 11 mg nicotinic acid, 5 mg calcium pantothenate, 3·6 mg pyridoxine, 0·2 mg D-biotin, 925 μg retinol, 10 μg ergocalciferol, 1 g choline chloride.

Statistical procedures

All values were subjected to analysis of variance with the MFAV programme of Halm & Le (1975). When F was significant ($P < 0.05$), differences between means were tested using the Newman-Keul's multiple range test. Specific single degree of freedom comparisons were also made using the same programme.

RESULTS

Growth, food intake and food conversion efficiency (FCE) results are presented in Table 2 and carcass composition, enzyme activity and urinary urea-N results in Table 3. Table 4 gives the results of single degree of freedom comparisons of measurements for which treatment means showed significant differences.

Average daily body-weight gain

The average body-weight of the rats at the start of the trial was 101 ± 0.9 g. Neither for the individual weeks nor for the over-all 3-week feeding period was there any difference in body-weight gain between experimental diets. However, the barley plus soya-bean meal 'positive-control' diet produced a growth rate for each period which was consistently greater than the mean of all the experimental diets (Table 4). Also, except for week 2, growth on the 'positive-control' diet was consistently greater than that obtained on diet 6, as assessed by the Newman-Keul's test.

Average food consumption

During both the first and second weeks of the trial, there were no significant differences between treatments in food consumption. During the third week, rats fed on the 'positive-control' diet consumed significantly ($P < 0.05$) more food than those receiving the diets containing 5·5–7·0 g methionine plus cystine/kg DM. The rats receiving 4·5 and 5·0 g dietary methionine plus cystine/kg DM consumed significantly ($P < 0.05$) more food than those receiving the 5·5–7·0 g/kg DM dietary levels.

For the over-all trial period, the rats fed on the 'positive-control' diet consumed significantly ($P < 0.05$) more than those given 5·5–7·0 g dietary methionine plus cystine/kg DM. The food consumption of rats receiving 4·5 and 5·0 g dietary methionine plus cystine/kg DM was significantly ($P < 0.05$) higher than that of rats receiving 5·5–7·0 g dietary methionine plus cystine/kg DM (Table 5). Mean consumption of diets containing 6·0 and 6·5 g dietary methionine plus cystine/kg DM was significantly ($P < 0.05$) less than the mean for all other diets.

Average FCE

During the first week of the trial period there were no significant differences in FCE between any of the experimental diets. However, the Newman-Keul's multiple range test indicated the FCE of rats receiving the 'positive-control' diet to be significantly ($P < 0.05$) higher than that of rats receiving 4·5, 5·5 and 6·5 g dietary methionine plus cystine/kg DM. There were no significant differences in FCE between treatments during the second week. During the third week there were no significant differences in FCE values between any of the experimental diets but single degree of freedom comparisons (Table 5) showed the FCE of rats receiving the 'positive-control' diet to be significantly ($P < 0.05$) higher than the mean of rats receiving the experimental diets.

For the over-all trial period, the FCE obtained on the 'positive-control' diet was significantly ($P < 0.05$) greater than that obtained on any of the experimental diets (Tables 3 and 5), between which there were no significant differences.

Table 2. *Average daily body-weight gain, food consumption and food conversion efficiency of rats fed on varying levels of dietary methionine plus cystine*

	Period	Diet no. 1	2	3	4	5	6	7	SE of mean	Test of statistical significance of difference between means ($P < 0.05$) F test	Newman-Keul's test†
Methionine + cystine (g/kg DM diet)		6·0	4·5	5·0	5·5	6·0	6·5	7·0			
Average daily body-wt gain (g)	Week 1	5·54	3·30	4·05	3·79	4·53	3·29	4·32	0·21	*	6 2 4 3 7 5 1
	Week 2	6·54	5·30	6·04	4·54	4·80	5·54	5·30	0·20	NS	
	Week 3	7·31	6·28	5·81	4·31	5·77	4·27	5·80	0·30	*	6 4 5 7 3 2 1
	Over all	6·54	4·81	5·30	4·06	5·27	4·29	5·06	0·19	*	4 6 2 7 5 3 1
Average daily food consumption (g)	Week 1	14·79	14·54	14·28	14·77	15·03	13·28	14·30	0·23	NS	
	Week 2	20·06	18·30	18·82	16·28	17·29	16·05	17·81	0·41	NS	
	Week 3	22·52	21·80	20·54	16·81	17·81	16·55	19·02	0·57	*	6 4 5 7 3 2 1
	Over all	19·05	18·29	18·03	15·79	16·33	15·52	17·04	0·35	*	6 4 5 7 3 2 1
Average food conversion efficiency (g gain/g food consumed)	Week 1	0·39	0·25	0·31	0·28	0·32	0·27	0·34	0·01	*	2 6 4 3 5 7 1
	Week 2	0·34	0·31	0·33	0·30	0·30	0·36	0·31	0·01	NS	
	Week 3	0·35	0·30	0·30	0·28	0·32	0·27	0·32	0·01	*	6 4 3 2 7 5 1
	Over all	0·36	0·29	0·31	0·29	0·31	0·30	0·32	0·01	*	4 2 6 5 3 7 1

DM, dry matter; NS, not significant.
* $P < 0.05$.
† Values for individual diets not having a common underline are significantly different ($P < 0.05$).

Table 3. *The effect of varying levels of dietary methionine plus cystine on carcass composition, live cystathionine synthase* (EC 4.2.1.22), *N^5-methyltetrahydrofolate-homocysteine-methyltransferase (mTHF Enz; EC* 2.1.1.13) *and urinary urea-nitrogen excretion*

	Diet no.							SE of Mean	Test of statistical significance of difference between means ($P < 0.05$)	
	1	2	3	4	5	6	7		F test	Newman-Keul's test†
Methionine + cystine (g/kg DM diet)	6·0	4·5	5·0	5·5	6·0	6·5	7·0			
Dry carcass wt (g)	54·55	57·29	54·27	51·02	53·80	55·03	55·29	0·71	NS	
Fat in whole carcass (g/kg)	147·8	230·3	195·3	185·4	175·7	175·7	195·4	7·0	NS	
Total carcass fat (g)	8·04	13·04	10·30	9·29	9·09	9·56	10·56	0·45	NS	
Crude protein in fat-free carcass (g/kg)	830·5	818·1	810·2	800·3	835·5	825·5	827·9	4·2	NS	
Crude protein in whole carcass (g/kg)	705·4	628·0	648·1	645·4	682·9	673·2	660·7	8·0	NS	
Total carcass crude protein (g)	38·55	35·81	35·06	32·81	36·80	37·05	35·56	0·58	NS	
Ash in fat free carcass (g/kg)	135·5	140·9	145·5	163·0	137·9	125·6	143·0	3·2	NS	
Ash in whole carcass (g/kg)	115·4	110·4	110·9	130·6	112·9	105·2	115·2	2·7	NS	
Total carcass ash (g)	6·05	6·06	6·27	6·55	5·81	5·31	6·28	0·15	NS	
Cystathionine synthase activity (μmol/mg protein per 60 min)	12·79	13·28	18·81	17·88	10·16	19·95	30·00	1·29	*	5 1 2 4 3 6 7
mTHF Enz activity (nmol/mg protein per 60 min)	2·65	0·70	0·74	1·59	2·32	0·92	0·70	0·15	*	7 2 3 6 4 5 1
Urinary urea-N excretion (g N/kg DM food)	nd	1·07	1·05	1·01	1·01	1·00	1·00	0·06	*	7 6 5 4 3 2

DM, dry matter; nd, not determined; NS, not significant.
* $P < 0.05$.
† Values for individual diets not having a common underline are significantly different ($P < 0.05$).

Table 4. *Results of single degree of freedom comparisons of variables showing significant differences between treatments in Tables 2 and 3*

	Period of experiment	Contrasts of diet nos.					
		1 *v.* 2–7	1 *v.* 2 and 3	1 *v.* 4–7	2 *v.* 3	2 and 3 *v.* 4–7	5 and 6 *v.* 2–4 and 7
Average daily body-wt gain	Week 1	*	*	*	NS	NS	NS
	Week 3	*	NS	*	NS	NS	NS
	Over all	*	*	*	NS	NS	NS
Average daily food consumption	Week 3	*	NS	*	NS	*	NS
	Over all	*	NS	*	NS	*	*
Average food conversion efficiency	Week 1	*	*	*	NS	NS	NS
	Week 3	*	*	*	NS	NS	NS
	Over all	*	*	*	NS	NS	NS
Cystathionine synthase (*EC* 4.2.1.22) activity		*	NS	*	*	*	*
mTHF Enz activity		*	*	*	NS	*	*

NS, not significant ($P > 0.05$); mTHF Enz, N^5-methyltetrahydrofolate-homocysteine-methyltransferase (*EC* 2.1.1.13).
* $P < 0.05$.

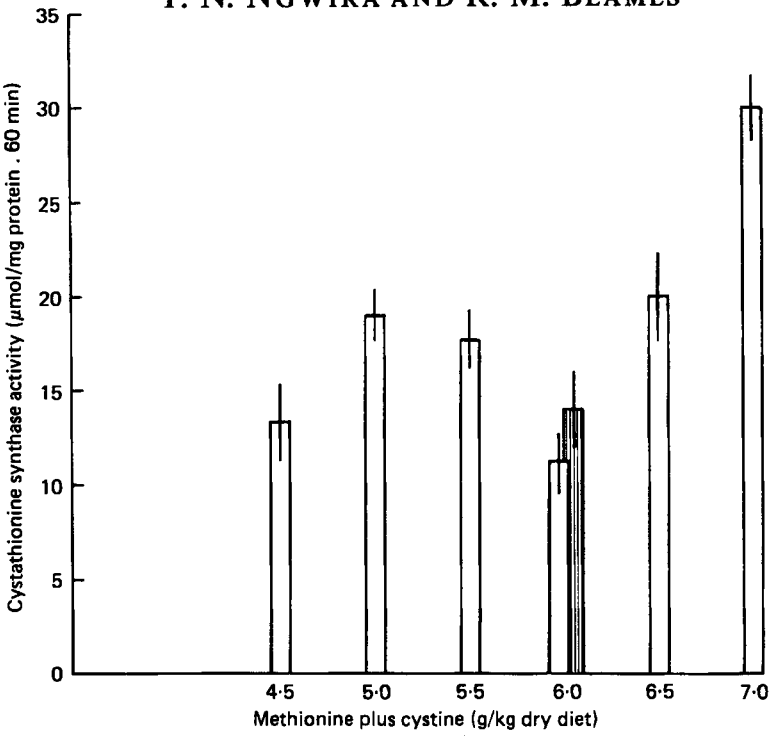

Fig. 1. Response of liver cystathionine synthase (*EC* 4.2.1.22) activity (μmol/mg protein per 60 min) in rats to varying levels (g/kg) of dietary methionine plus cystine. Values are means with their standard errors for four rats per dietary treatment. □, Experimental diets; ■, 'positive control' diet (diet no. 1). For details of diets, see Table 1.

Carcass composition

None of the carcass composition measurements (Table 3) showed any significant differences between treatments.

Liver enzyme activities

The activity of liver cystathionine synthase (Table 3, Fig. 1) shows a significant ($P < 0.05$) rise in activity from 13·38 μmol/mg protein per 60 min at 4·5 g dietary methionine plus cystine/kg DM to 18·81 μmol/mg protein per 60 min at the 5·0 g/kg DM level. The activity then decreased, reaching a minimum value of 10·16 μmol/mg protein per 60 min at 6·0 g dietary methionine plus cystine/kg DM and thereafter increased to a value of 30·00 μmol/mg protein per 60 min at 7·0 g dietary methionine plus cystine/kg DM. This latter value was significantly ($P < 0.05$) higher than that at any other level of dietary methionine plus cystine, including the 'positive-control' diet.

The activity of mTHF Enz (Table 3, Fig. 2) rose only slightly (from 0·70 to 0·74 nmol/mg protein per 60 min ($P < 0.05$)) when the dietary methionine plus cystine level was increased from 4·5 to 5·0 g/kg DM. The activity thereafter significantly ($P < 0.05$) increased, reaching an average maximum value of 2·32 nmol/mg protein per 60 min at 6·0 g dietary methionine plus cystine/kg DM. The activity then decreased at the 6·5 and 7·0 g/kg DM dietary levels to values similar to those obtained in rats receiving 4·5 and 5·0 g dietary methionine plus cystine/kg DM. The activity of mTHF Enz in rats receiving the 'positive-control' diet was significantly ($P < 0.05$) higher than the activity in rats receiving any of the experimental diets.

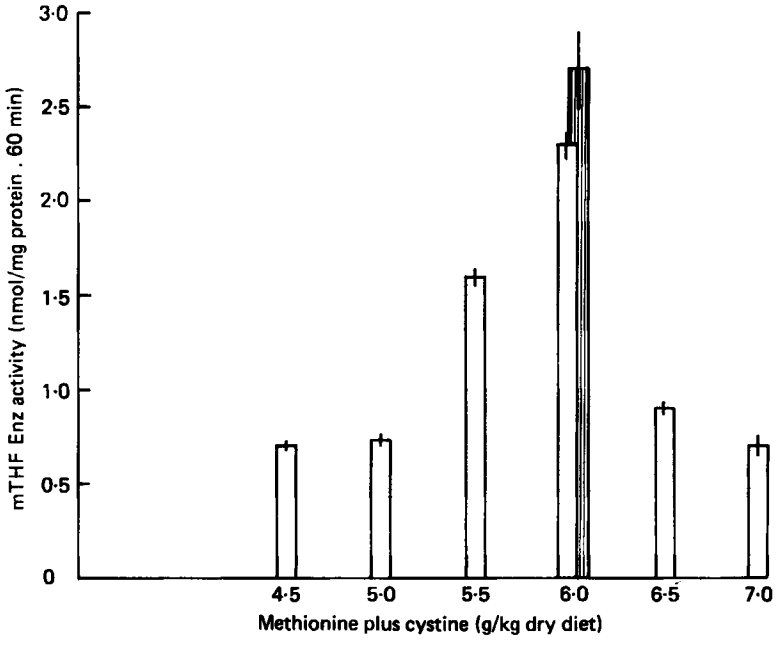

Fig. 2. Response of liver N^5-methyltetrahydrofolate-homocysteine-methyltransferase (mTHF Enz *EC* 2.1.1.13) activity (nmol/mg protein per 60 min) in rats to varying levels (g/kg) dietary methionine plus cystine. Values are means with their standard errors for four rats per dietary treatment. □, Experimental diets; ▥, 'positive control' diet (diet no. 1). For details of diets, see Table 1.

Urinary urea-N excretion

Urinary urea-N excretion (Table 3) showed a significant ($P < 0.05$) reduction from 1·07 g/kg DM food intake to 1·05 g/kg DM food intake as the dietary methionine plus cystine level increased from 4·5 to 5·0 g/kg DM. There was a further significant ($P < 0.05$) reduction to 1·01 g urea-N/kg DM intake when the dietary methionine plus cystine level was raised to 5·5 g/kg DM. The excretion then stabilized with no further significant reduction as the dietary methionine plus cystine level rose to the highest level of 7·0 g/kg DM.

DISCUSSION

In this trial, neither the growth measurements (average daily body-weight gain, food consumption and FCE) nor carcass composition values, were sufficiently uniform within treatments to demonstrate any significant difference in the performance of rats receiving dietary levels of methionine plus cystine ranging from 4·5 to 7·0 g/kg DM. The few significant results associated with these measurements tended to conflict with each other. This observation is in agreement with the results over this range reported by Ngwira & Beames (1978) where the dietary level of cystine was held constant at 2·0 g/kg DM. In that experiment no growth response was detected to the addition of 0·5 g methionine to the 3·5 g methionine plus cystine/kg DM diet although both the fat concentration and total fat in the carcass were significantly ($P < 0.05$) reduced. However, there were no significant growth or carcass

responses to a further increase in the methionine plus cystine level from 4·0 to 4·5 g/kg diet. Thus it is not unexpected that no growth or carcass response was able to be measured in the present trial when the lowest dietary level of methionine plus cystine was 4·5 g/kg DM.

Although the carcass composition results (Table 3) did not show any significant differences between the rats receiving the 'positive-control' diet and those receiving any of the experimental diets, the growth measurements (average daily body-weight gain, and FCE) indicated that rats receiving the 'positive-control' diet performed better than those on the other diets (Tables 3 and 4). Although this could be interpreted as an indication of an amino acid inadequacy in the experimental diets (2–7) such an interpretation is not valid in view of the greater consumption of the 'positive-control' diet. FCE is closely associated with food intake (Vanschoubroek *et al.* 1967) which itself is determined by many factors (Le Magnen, 1976) only one of which is amino acid balance (Harper *et al.* 1970). The fact that the 'positive-control' diet contained a digestible energy content of only 0·92 that of the other diets, as estimated from values tabulated for swine ((US) National Research Council, 1969) and assuming complete digestion of starch (Davidson & Passmore, 1967), when considered in association with the inverse relationship between digestible energy concentration and food intake in the rat (Sibbald *et al.* 1957) further clouds interpretation. In spite of the above, the use of a 'positive-control' diet is considered highly desirable, as it allows a comparison between experimental values and values obtained on a conventional diet.

Although the growth and carcass results in this experiment were analysed on a weekly basis because of the rapidly decreasing methionine requirements of the rat over this timespan (Hartsook & Mitchell, 1956), differences between treatments in growth measurements did not diminish as the trial progressed. No ready explanation can be advanced for this low sensitivity to treatments during the first week. It certainly was not due to a greater within treatment variation during the first week, as might be expected if acclimatization were a problem, as the standard errors of the means for the first week for body-weight gain and FCE were similar to and, for food intake, less than, standard errors for corresponding means for the over-all period.

The activity of liver cystathionine synthase (Table 3) followed the same pattern as that obtained in the rat trial reported by Ngwira & Beames (1978) in which cystine incorporation in the diets was held constant at 2·0 g/kg DM, and total methionine plus cystine varied from 3·5 to 7·0 g/kg DM. However, two differences should be pointed out. First, in the present trial, the activity of cystathionine synthase at 4·5 g dietary methionine plus cystine/kg DM was lower than that at 5·0 g/kg DM, whereas in the trial reported by Ngwira & Beames (1978) the activity at these two dietary methionine plus cystine levels was constant. Since the transsulphuration pathway via cystathionine formation is obligatory (Finkelstein, 1967), it is expected that any increase in dietary methionine level below the level for optimal metabolism should result in an increase in cystathionine synthase activity. However, Ngwira & Beames (1978) showed a constant cystathionine synthase activity when the methionine content was increased from 2·5 to 3·0 g/kg DM in diets containing a fixed cystine level of 2·0 g/kg DM. It is possible, therefore, that the methionine:cystine value plays a major role in the control of cystathionine synthase activity at these dietary levels of methionine plus cystine. The present results, and those reported by Ngwira & Beames (1978), lead to the conclusion that a value of 1·25:1 for methionine:cystine in a 4·5 g methionine plus cystine/kg DM diet activates cystathionine synthase more than a value of 2:1 for methionine:cystine. The second difference between cystathionine synthase activity in the present trial and that in the trial reported by Ngwira & Beames (1978) was the larger increase in the present trial in the enzyme activity at levels above the 6·0 g methionine plus cystine/kg DM. This difference was especially evident at 7·0 g methionine plus cystine/kg DM, where the cystine content was 2·0 g/kg DM in the trial reported by Ngwira & Beames (1978) and

2·3 g/kg DM in the present trial. It thus appears that the higher cystine level resulted in a greater activation of cystathionine synthase.

The activity of cystathionine synthase in the present trial, when compared with the results of Ngwira & Beames (1978), has shown that a value for methionine:cystine of 2:1 leads to a lower relative enzyme activity at 4·5 g methionine plus cystine/kg DM, and a higher relative activity at 6·5–7·0 g methionine plus cystine/kg DM than diets of similar total methionine plus cystine content containing a fixed 2·0 g cystine/kg DM. This confirms the findings of Shannon *et al.* (1972) and Finkelstein (1967) that the value for methionine:cystine affects the activity of this enzyme.

The activity of mTHF Enz obtained in the present trial showed a similar pattern to that reported by Ngwira & Beames (1978) where cystine concentration was held constant at 2·0 g/kg DM diet. This means that the pattern, and presumably the rate, of remethylation of homocysteine is not markedly sensitive to methionine:cystine.

The results of the activities of both cystathionine synthase and mTHF Enz indicate 5·5 g methionine plus cystine/kg DM to be the level beyond which disturbances in enzyme activity occur. It could be concluded, therefore, that 5·0–5·5 g dietary methionine plus cystine/kg DM is the range for optimal methionine plus cystine intake for growing rats.

Urinary urea excretion was greater at 4·5 and 5·0 g dietary methionine plus cystine/kg DM than at the 5·5–7·0 g/kg DM levels. Quantitatively, the difference in urea excretion between 1·07 and 1·00 g N/kg DM food consumed at 4·5 and 7·0 g dietary methionine plus cystine/kg DM respectively, which was found in the present trial, was not as pronounced as that between 1·41 and 0·90 g N/kg DM food consumed at 4·5 and 7·0 g methionine plus cystine/kg DM respectively, obtained by Ngwira & Beames (1978) where cystine concentration in the diets was held constant at 2·0 g/kg DM. The fact that the dietary levels of 3·5 and 4·0 g methionine plus cystine/kg DM could not be included in the present trial (because of the desire to retain barley in the diet at levels above 500 g/kg), meant that no comparisons could be made with the sharp urea excretion decline observed by Ngwira & Beames (1978) between 3·5 and 4·5 g dietary methionine plus cystine/kg DM. As calculated from this measurement, in the present trial, 5·0–5·5 g dietary methionine plus cystine/kg DM would be the requirement for optimal rat growth.

In summary, the growth rate and carcass composition measurements could not be used with any precision to estimate the requirements of methionine plus cystine within the 4·5–7·0 g methionine plus cystine/kg DM range in diets containing methionine:cystine at 2:1. The same diets yielded results that indicated normal activities of liver cystathionine synthase and mTHF Enz when dietary methionine plus cystine levels were at or below the 5·0 g/kg DM level, but a disturbed methionine metabolism when the dietary methionine plus cystine levels were equal to or greater than 5·0 g/kg DM. The patterns of the two liver enzyme activities obtained in the present trial when methionine:cystine was fixed at 2:1 were almost the same as those reported by Ngwira & Beames (1978) where the dietary cystine concentration was held constant at 2·0 g/kg DM in all diets.

The results may indicate 5·0–5·5 g/kg DM to be the optimal range for dietary methionine plus cystine concentration for the weanling rat when the dietary gross energy concentration is 15·80 MJ/kg DM.

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