Utilization of Methionine in Fonio by Growing Rats

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ABSTRACT

Fonio (*Digitaria exilis* (Kippist) Stapf.) has been reported to be rich in methionine in its protein fraction. The metabolic availability of methionine from this cereal in methionine-limited diets has been determined by protein efficiency ratio (PER), protein retention (PR), net protein utilization (NPU), and net protein ratio (NPR). Fonio methionine was highly available under the conditions in question. Comparison of NPU and NPR values has led to the conclusion that on methionine-limited diets, nitrogen concentration in newly formed tissues is significantly lower than on normal diets.

Fonio (*Digitaria exilis* (Kippist) Stapf.) is a common West African cereal covering large areas of cultivated land and used as a staple food in many parts of that region (1). This grain, being a cereal, is low in protein, but its proteinaceous fraction is very rich in sulfur-containing amino acids. A crude protein content of 7.2 to 8.8% (N X 6.25) has been reported in whole grain, and 3.2 to 5.6% methionine and 2.8% cystine per 100 g. protein with 16% nitrogen (1,2,3). According to Carbiener et al. (2), the grain is palatable and highly digestible. It should therefore be suitable for supplementing methionine-limited diets (4).

For prediction of the protein nutritive value, specific foods or diets may be scored (5) according to different systems on the basis of the limiting amino acid (6) or all the essential amino acids (7). Such a system makes no allowance for the availability of the amino acids to the body, and this drawback has been stressed by different authors (8,9,10,11,12). This study was undertaken to determine the availability of the methionine present in high concentration in fonio protein.

MATERIALS AND METHODS

Animals

Albino rats (Charles River C. D. strain) from our stock colony were used. The average weanling weight after 21 days was 37 g. Weanling rats were separated according to sex without pre-test feeding, classified on weight blocks differing by not more than 0.5 g., and distributed at random into six groups, five males and five females in each. The animals were housed in individual metal-screen cages in an air-conditioned room (24°C. and 50 to 60% r.h.).

Feed in powdered form, containing colloid-milled fonio, and water were provided *ad lib*. After 10 days animals were killed with chloroform, weighed, and their nitrogen content was determined.

Methods for Evaluation of the Protein Nutritive Value

The protein efficiency ratio (PER) was determined by the method of Osborne et al. (1919), cited by Chick (13), and the protein retention (PR) by the method of

[1]Deceased.

Butterworth (14). For calculation of PR, the nitrogen content of control group 6, killed at the beginning of the experiment, served as reference. The net protein utilization (NPU) was determined by the method of Bender and Miller (15), and calculated according to the modification of Bender and Doell (16); the net protein ratio (NPR) according to the method of Bender and Doell (17). Weight changes, feed consumption, and nitrogen content were recorded separately for each animal. For accurate measurement of consumption, the following procedure was applied: Each cage was placed on a cardboard tray to collect the spilled feed and the feces. At the end of the experiment, the trays were dried overnight at 110°C. and weighed. The scattered feed was freed of feces by means of a vacuum cleaner whose suction end was covered with a piece of window screen. Each tray was then weighed again and the difference in weight was subtracted from the "gross feed intake." The result was the "net feed consumption."

Analytical Methods

Nitrogen was determined by the Kjeldahl procedure on whole carcasses, wet-ashed with 3 g. selenium, 3 g. cupric sulfate, and 36 g. potassium sulfate in approximately 350 ml. sulfuric acid. To prevent frothing, about 3 g. of nitrogen-free solid paraffin was added at the beginning of digestion, and the Kjeldahl flasks preheated at approximately 200°C. for 2 days. Digestion was carried out according to the appropriate method of the Association of Official Agricultural Chemists (18). The digests were diluted with distilled water to 2 liters and two portions of 100 ml. each were taken for distillation.

Proximate analyses of fonio and soya-bean meal were carried out by the methods of the Association of Official Agricultural Chemists (18) and methionine contents determined microbiologically (19). The results are shown in Table I.

TABLE I. PERCENTAGE PROXIMATE COMPOSITION AND METHIONINE CONTENT OF FONIO AND SOYA-BEAN MEAL

	Moisture	Protein[a]	Crude Fiber	Crude Lipids	Methionine
Fonio	11.4	7.6	7.7	4.8	0.291
Soya-bean meal	10.6	45.0	5.9	1.5	0.617

[a]Factors used: fonio 6.25 (according to Carbiener et al. (2)); soya-bean meal 5.71 (according to Orr and Watt (21)).

Diets

The diets were so designed that the limiting amino acid was methionine for all groups. In groups 1 to 3 the methionine content was 147 mg. per 100 g. diet, i.e., approximately two-thirds of the recommended level[2]. All the other essential amino acids, including cystine, were supplemented by synthetic amino acids, in the

[2]The recommended level for each essential amino acid was calculated by averaging the data of various authors: Bender, A. E., Proc. Nutr. Soc. 17: 39 (1958); Heller, B. S., Chutkow, M. R., Lushbough, C. H., Siedler, A. J., and Schweigert, B. S., J. Nutr. 73: 113 (1961); Rama Rao, P. B., Norton, H. W., and Connor-Johnson, B., J. Nutr. 73:38 (1961); Allison, J. B. In: Mammalian protein metabolism, ed. by H. N. Munro and J. B. Allison, vol. II, p. 41. Academic Press: New York (1964).

available L-form, up to the recommended level. All protein diets (given to groups 1 to 4) contained the same amount of commercial toasted soya-bean meal, which supplied half the methionine content of the diets for groups 1 to 3. For group 1 the other half was made up with fonio ("Early Variety," Porto Loko, Sierra Leone) and for group 2 fonio and DL-methionine (product of Fluka A.G., Bucks S. G., Switzerland) in equal proportions. For group 3 the soya-bean meal was supplemented with DL-methionine only and group 4 was given the soya-bean diet without added methionine. Group 5 was given a protein-free diet.

Soya-bean oil was included in all diets in the amounts required to standardize the crude fat content at 8%, as recommended by the Association of Official Agricultural Chemists (18). The crude fiber content of all diets was made equal to that for group 1 (2.4%) by appropriate addition of powdered cellulose. The energy content of all the diets, calculated according to Atwater (FAO, 1947) (20), varied between 370 and 380 kcal. per 100 g. feed. In addition, all diets contained identical amounts of added mineral and vitamin mixtures designed according to the Association of Official Agricultural Chemists (18), and the rest of each diet consisted of maize starch. Table II gives the detailed composition of all diets used.

The essential amino acids contents and their appropriate supplements and the detailed composition of the protein fraction of the protein-containing diets are given in Table III. The supplementary amounts were determined on the basis of the amino acid analyses for fonio reported by Carbiener et al. (2) and for soya-bean meal reported by Orr and Watt (21). The crude protein level (in all protein-containing diets) was made up to 10% with a mixture of five nonessential amino acids consisting of 54.3% L-glutamic acid, 10.5% L-proline, 11.0% DL-serine, 12.1% glycine, and 12.1% DL-alanine.

RESULTS AND DISCUSSION

Table IV summarizes the results of our comparative experiments. Reproducibility of results for the different methods chosen was good. The average coefficient of variance was highest for PR (8.3%), decreased to 6.3% for PER and to 5.7% for NPU, and was lowest for NPR (3.7%).

It is obvious that group 4, whose dietary methionine (74 mg. per 100 g. diet) derived exclusively from the soya-bean meal, showed a significantly inferior performance. The animals in this group almost failed to grow. The net dietary-protein calories percent (NDpCal%) (22) of the diet was about the maintenance level of 4 given by Miller and Payne (5). Since the daily metabolizable energy intake of this group averaged 188 kcal. per kg.$^{3/4}$, substantially more than

TABLE II. PERCENTAGE COMPOSITION OF DIETS

	Group 1	Group 2	Group 3	Group 4	Group 5
Soya-bean meal	12.00	12.00	12.00	12.00	...
Fonio	25.00	12.50	...	...	...
Mineral mixture	4.00	4.00	4.00	4.00	4.00
Vitamin mixture	1.00	1.00	1.00	1.00	1.00
Cellulose powder	...	0.96	1.91	1.91	2.38
Soya-bean oil	6.65	7.25	7.85	7.85	8.00
Essential amino acids	1.85	2.25	2.66	2.59	...
Nonessential amino acid mixture	1.25	2.10	2.88	2.95	...
Maize starch	48.25	57.94	67.70	67.70	84.62

TABLE III. COMPOSITION OF THE PROTEIN FRACTION OF PROTEIN-CONTAINING DIETS

Diet for		His	Arg	Val	Tyr	Phe	Cys	Meth	Lys	Leu	Ileu	Thr	Tryp	Crude Protein %
		mg./100 g. diet												
Group 1	12 g. Soya-bean meal	140	425	308	187	290	104	74	440	450	315	230	83	5.40
	25 g. Fonio	35	65	100	62	87	47	73	67	170	70	70	25	1.90
	Total	175	490	408	249	377	151	147	507	620	385	300	108	7.30
	Supplement	75	...	237	116	163	134	...	493	170	210	200	47	1.57
	Nonessential amino acid mixture[a]													1.13
	Total													10.00
Group 2	12 g. Soya-bean Meal	140	425	308	187	290	104	74	440	450	315	230	83	5.40
	12.5 g. Fonio	17.5	32.5	50	31	43.5	23.5	36.5	33.5	85	35	35	12.5	0.95
	Total	157.5	457.5	358	218	333.5	127.5	110.5	473.5	535	350	265	95.5	6.35
	Supplement	92.5	...	287	147	206.5	157.5	36.5	526.5	255	245	235	59.5	1.87
	Nonessential amino acid mixture[a]													1.78
	Total													10.00
Group 3	12 g. Soya-bean meal	140	425	308	187	290	104	74	440	450	315	230	83	5.40
	Supplement	110	10	337	178	250	181	73	560	340	280	270	72	2.26
	Nonessential amino acid mixture[a]													2.34
	Total													10.00
Group 4	12 g. Soya-bean meal	140	425	308	187	290	104	74	440	450	315	230	83	5.40
	Supplement	110	10	337	178	250	181	...	560	340	280	270	72	2.21
	Nonessential amino acid mixture[a]													2.39
	Total													10.00

[a]The mean nitrogen content of the nonessential amino acid mixture was 12.0%; therefore its protein equivalent was calculated as N-content X 8.33.

TABLE IV. VALUE OF FONIO AS SOURCE OF METHIONINE IN TERMS OF PER, PR, NPU, AND NPR

	Group 1	Group 2	Group 3	Group 4
Average initial weight (g./animal)[a]	35.7	37.3	38.1	36.4
Average weight gain in 10 days (g./animal)[b]	22.2	34.1	39.8	5.4
Average protein intake in 10 days	6.1	8.2	9.2	4.45
PER (mean ± SD)	3.6±0.26	4.2±0.14	4.3±0.20	1.2±0.12
PR (mean ± SD)	43.2±4.1	49.3±3.4	52.5±2.4	18.8±2.3
NPU (mean ± SD)	55.3±3.7	58.1±3.3	60.5±2.9	36.4±1.9
NPR (mean ± SD)	4.7±0.28	5.0±0.12	5.0±0.22	2.7±0.12
NDpCal% (mean)	5.8	6.2	6.4	3.8

[a]Average initial weight per animal for group 5 (nonprotein) was 37.7 g. and for control group 6, 37.0 g.

[b]Average weight loss per animal for 10 days in group 5 was 6.5 g.

suggested by Brody (23) for maintenance, it must be inferred that the amount of methionine given was limiting, and reduced protein and energy utilization.

One may think that the results for groups 1 to 3 suggest that increasing amounts of fonio methionine, as against synthetic methionine, slightly reduced the nutritive values in terms of the parameters used. F-test results showed, however, that the differences between groups 1 to 3 were always statistically insignificant for all the parameters, including PER. The availability of methionine in fonio, therefore, compared well with that of the synthetic supplement.

The average values for NDpCal% of the three experimental diets were very close to each other. This fact is also borne out by the insignificant differences in NPU between the diets containing fonio methionine and those containing an equivalent amount of DL-methionine.

The differences in PER and PR between groups 1 to 3 given equi-methioninic diets from different sources were higher than those for NPU and NPR. The differences between the average for group 3 and group 1 in percent of the former were 16.2, 17.7, 8.6, and 6.0%, respectively. This can be explained by the fact that the former two criteria are influenced to a greater degree by feed consumption than are NPU and NPR (24). The feed consumption of the rats of group 1 was less than that of groups 2 and 3, the diets of which contained little or no fonio. Contrary to the report by Carbiener et al. (2), it seems that fonio was not very palatable to the rats. This could perhaps be attributed to the fact that the fonio used in our study was a raw product, containing 7.7% crude fiber, whereas Carbiener et al. referred to a gruel made with decorticated fonio and fed to infants.

Determination of PR yielded no new information. It seems that the composition of the body gain in groups 1 to 3 was rather constant, at least as far as body nitrogen is concerned. This is borne out by our figures for nitrogen concentration: 2.58% ± 0.03 for group 1, 2.51% ± 0.04 for group 2, and 2.47% ± 0.03 for group 3. It is worth noting that the nitrogen content of group 4 was 2.92% ± 0.03, that of the group given the protein-free diet was 3.17% ± 0.12, and that of control group 6, killed at the beginning, was 2.99% ± 0.07. The differences in nitrogen content between the first three groups on the one hand and the last three on the other are statistically significant. Similar results, as far as the two last-mentioned groups are concerned, have been reported by Bender and Doell (17).

The protein retention efficiency (PRE) correlates NPU (measure of gain in nitrogen) with NPR (measure of gain in weight) (17). Both parameters relate nitrogen and weight gains with nitrogen and protein intakes, respectively, and provide appropriate corrections of the maintenance requirements of the animals. The factor NPU/NPR for calculation of PRE was established by the last-mentioned authors as 16, a figure very close to the protein content of 16.75% found by them for their animals given a normal diet. The existing small difference has been inferred to be owing to the fact that the protein content of the new tissue is not quite the same as that of the whole carcass. Comparable figures given by others are 15.5 (25) and 16.9 (26).

The average protein content of the animals in group 6 (killed at the beginning) was 18.7%; the average of all our experimental animals fed on different methionine-limited diets for 10 days was 15.8%. The factors for transforming NPR to the numerical value of NPU, i.e., the percentage of protein in the new tissues grown during the 10 days' experimental period, were 11.8, 11.7, and 12.0 for groups 1, 2, and 3, respectively. It seems, therefore, that with methionine-limited diets, the nitrogen concentration in newly formed tissues is lower than before the nutritional limitation in our experiment, or under normal diets, as reported in literature. In experiments with dogs, a similar influence of protein quality on the nitrogen concentration in the tissues has been reported (27).

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