CEREAL PROTEINS

Amino Acid Composition and Lysine Supplementation of Teff

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Six samples of Teff and a sample of pearl millet were analyzed for their essential amino acid content using ion exchange chromatography. In addition, the Teff and millet were fed with and without supplemental lysine to weanling rats for 4 weeks. Analysis indicated that both cereals have a good balance among essential amino acids, except for being markedly first limiting in lysine. By rat feeding, lysine supplementation of Teff and millet was found to raise the protein efficiency ratio of each approximately to that obtained with Animal Nutrition Research Council (ANRC) casein.

It is generally realized that in much of the world, cereal grains furnish the major portion of dietary protein intake. Although corn, rice, and wheat are the main staples, in some areas of the world millet is eaten to a considerable extent. Senecal has pointed out that in Dakar, French West Africa, millet is an important cereal crop (*9*). It has been reported by Rao *et al.* that in South India, where protein malnutrition of infants is common, the diet consists mainly of rice and millet (*8*). Recently, a nutrition survey of Ethiopia was made (*2*). This survey reported a strong preference for and almost universal use of the milletlike cereal grain Teff (*Eragrostis abyssinica*) by the Ethiopians. The seeds of this plant, which are half the size of a pinhead, are used primarily in making a sour dough, pancake-type baked product called "injera."

A typical, daily Ethiopian diet was calculated to contain 65 grams of protein, of which 41 grams would come from Teff. The total amount of animal protein in such a diet would be only 6 grams daily. Teff is a good source of vitamins and minerals including iron, according to the nutrition team which made this survey. It is evident that the amino acid composition and biological value of Teff are of considerable practical as well as academic interest. This report presents data on the essential amino acid content of six varieties of Teff and on the biological evaluation of a combined sample of Teff by rat growth procedures. Corresponding data are also included for pearl millet (*Pennisetum glaucum*).

Experimental

All of the essential amino acids except tryptophan were determined by ion exchange chromatography. For analysis of the six varieties of Teff, the standard ion exchange procedures of Moore and Stein were followed using a fraction collector (*7*). The combined sample of Teff and pearl millet was analyzed by the continuous amino acid analysis procedures of Spackman, Stein, and Moore (*10*) on a commercial instrument (Spinco Division, Beckman Instrument Co.). Hydrolysis was effected by heating, in an autoclave, samples in 6*N* HCl for 20 hours at 120° C.

Tryptophan and cystine were determined, except for minor modifications, by standard microbiological assay procedures (*5*, *11*). For tryptophan, the assay organism used was *Lactobacillus plantarum*.

The protein quality of the Teff and millet samples was biologically evaluated by rat growth procedures with and without varying amounts of supplemental lysine (added as the monohydrochloride). The lysine monohydrochloride used contained 95% of the L-isomer. In the first experiment, Teff, and in the second, pearl millet, was studied. The cereals were incorporated into test diets at as high a level as was allowed by the other necessary dietary ingredients. Under these conditions, Teff or millet made up about 92% of the diets, salts 3%, vitamins 2%, and corn oil 3%. Salt mixture (USP XIV), Vitamin Diet Fortification Mixture (Nutritional Biochemicals Co.), and Mazola corn oil were used. In the case of millet, because of a slightly higher oil content, corn oil was added only as 1% of the diet.

The diets furnished protein at 10% and fat at 5%, dry weight. In both experiments, control diets were made up in which the protein source was Animal Nutrition Research Council (ANRC) casein. These diets contained 10% of casein, 3% of salts, 2% of vitamins, and 5% of corn oil, and were made up to 100% with corn starch. The diets were fed to groups of 22-day-old male weanling Charles River C-D rats for 4 weeks, and weight gains, food consumptions, and protein efficiency ratios (PER's) were recorded weekly. The rats were 21 days old on arrival and were kept overnight on stock ration and water ad libitum prior to being placed on the experimental diets. For the millet experiment, there were 10 rats in each group, but in the Teff experiment each group consisted of six rats because of a limited supply of Teff. The average initial weights of the rats in experiments 1 and 2 were 48.6 and 40.6 grams, respectively. The rats were housed in single, screen-bottomed cages in an air-conditioned room maintained at 72° to 76° F. Food and water were supplied ad libitum.

At the conclusion of the experiments, the rats were anesthetized, and heparinized samples of blood were obtained directly from the right atrium of the heart. Plasma protein concentrations were then determined by the biuret method as described by Goldstein and Scott (*4*).

Results

The nitrogen contents of several varieties of Teff and the sample of pearl millet are shown in Table I. The amino acid composition of six varieties of Teff is shown in Figure 1. All six varieties are closely similar in their essential amino acid content. All are limiting in lysine, with Kay Teff Wolliso containing the least and Red Teff Jimma the most of this amino acid. Except for lysine, the balance among essential amino acids is excellent and compares favorably with egg. Even the absolute amounts of essential amino acids in the protein, except for lysine, exceed rat growth and the Food and Agricultural Organization, U.N. (FAO) pattern, although histidine appears bor-

Table I. Nitrogen Content of Teff and Millet

Sample	Nitrogen Dry Wt., %	Protein[a] Dry Wt., %
Kay Teff Wolliso	1.93	12.06
Teff Gondar	1.79	11.19
Teff flour Wolliso	2.01	12.56
Teff Kolla Duba	1.91	11.94
White Teff Jimma	1.55	9.69
Red Teff Jimma	1.75	10.94
"Combined" Teff	1.68	10.51
Pearl millet	1.68	10.51

[a] Protein = N × 6.25.

derline for rat growth and isoleucine borderline compared with the FAO pattern. The ratio of essential to nonessential amino acids in Teff is high for a cereal product, although, of course, it is considerably lower than that in whole egg.

The sample of Teff fed was a combined sample of several varieties, and its amino acid composition is shown in Table II. Here, its pattern is compared with the FAO and whole egg patterns and a sample of pearl millet purchased locally. The composition of Teff is similar to that of millet, although it contains generally higher amounts of the essential amino acids including the most limiting amino acid, lysine. The analysis for pearl millet is in good agreement with the data reported by Baptist and Perera for five other varieties of millet (*1*).

The good amino acid balance in Teff is demonstrated by the growth and PER that result when the unsupplemented Teff is fed to weanling rats (Table III). However, supplementation with lysine converts the good quality protein into a high quality protein source. Supplementation of Teff with 0.25 and 0.42% of L-lysine monohydrochloride (LMH) raised the PER from 1.95 to 2.78 and 3.27, respectively. Casein gave a PER of 3.47. Rats on Teff + 0.42% of LMH gained considerably more weight than those on casein, although the PER was slightly lower. The authors have consistently observed that, if the quality of a cereal protein is made equal to that of a high quality animal protein such as ANRC casein by supplementation, weight gains are higher on the cereal diet. It is possible that cereal diets have a higher degree of palatability for the rat, and consequently he may eat more and gain more, although the efficiency of the diet is not greater. Under these conditions, the PER would appear to be a more accurate indicator of protein quality than weight gain.

The results of experiment 2 in which pearl millet was supplemented with lysine are also shown in Table III.

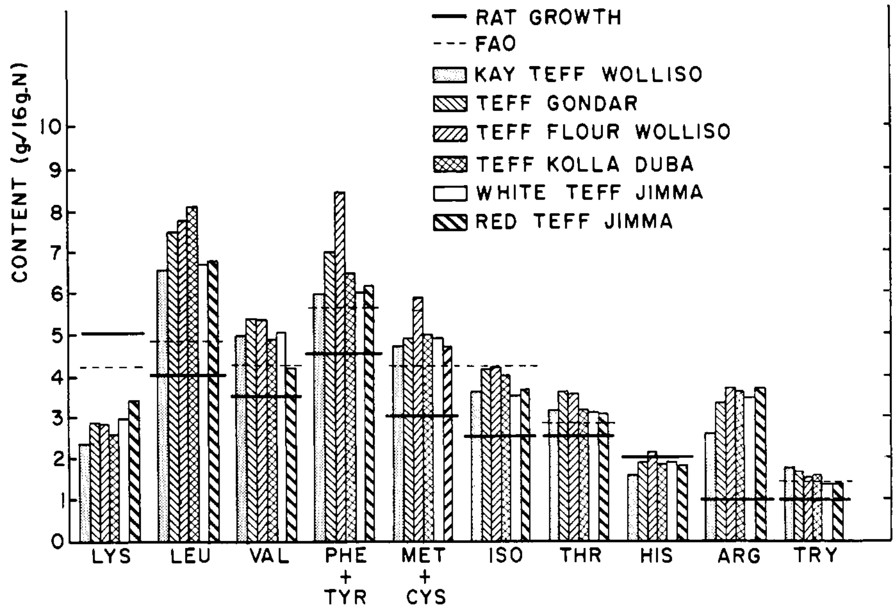

Figure 1. Amino acid composition of Teff compared to rat growth and FAO patterns

Table II. Comparison of the Amino Acids of Pearl Millet and Teff with the FAO and Whole Egg Patterns[a]

Amino Acid[b]	Pearl Millet	Combined Teff	FAO Pattern	Whole Egg
Lysine	2.89	3.11	4.2	6.6
Histidine	2.08	2.14	...	2.1
Arginine	3.48	3.54	...	6.9
Threonine	2.50	3.34	2.8	4.2
Methionine	1.35	2.79	2.2	3.8
Cystine	3.19	2.50	2.0	2.4
Valine	4.49	5.25	4.2	7.2
Leucine	7.29	7.73	4.8	9.4
Isoleucine	3.09	4.07	4.2	7.5
Phenylalanine	3.46	4.87	2.8	5.8
Tyrosine	1.41	2.20	2.8	4.4
Tryptophan	1.62	1.30	1.4	1.4

[a] Grams/16 grams N.

[b] All amino acids determined by column chromatography, except cystine and tryptophan which were determined by microbiological assay.

They are generally similar to those obtained with Teff, although the increase in weight gain on addition of lysine is not as large as that observed with Teff. Starting weights of rats used in the millet experiment were less than those used in the Teff study, and this may have been a factor. However, the PER's obtained at the highest supplementation levels were identical for both cereals. The unsupplemented millet did not give as high a PER as Teff, and this is probably a result of its lower lysine content. The improvement in weight gain and PER reported for pearl millet on supplementation with lysine is in good agreement with recent literature reports by Mangay, Pearson, and Darby (*6*) in which the PER of Italian millet (*Setaria italica*) was raised from 1.06 to 3.07 by lysine supplementation. It has also been observed by studies on nitrogen retention that lysine improves utilization of the protein in Italian millet (*3*).

Changes in plasma protein concentration that took place as a result of lysine supplementation are shown in Table IV. Rats fed unsupplemented Teff had lower plasma protein concentrations than those on millet. On lysine supplementation, an improvement for both Teff and millet was observed with supplemented Teff giving a slightly higher level than supplemented millet. In both cases, plasma protein concentrations were less than that found on casein, thus paralleling the PER data closer than the weight gain data.

Discussion

The dietary survey, referred to earlier, found that protein deficiency exists in Ethiopia among infants and young children, and also among older children and adults, particularly where there also is a caloric shortage. Protein malnutrition was frequently seen in all the pediatric clinics visited. A typical daily

Table III. Rat Growth Evaluation of Teff and Pearl Millet[a]

Diet	Grams Lysine/16 Grams N	Weight Gain, Grams	Protein Efficiency Ratio[b]
	Teff		
Teff	3.1	50.3	1.95
+0.25% LMH[c]	5.0	104.3	2.78
+0.42% LMH	6.3	138.5	3.27
ANRC casein	...	91.2	3.47
	Pearl millet		
Pearl millet	2.9	36.2	1.83
+0.25% LMH[c]	4.8	103.9	2.93
+0.50% LMH	6.7	118.0	3.28
ANRC casein	...	91.7	3.55

[a] Four weeks' growth.

[b] $PER = \frac{\text{grams weight gained}}{\text{grams protein consumed.}}$

[c] Percentage of L-lysine monohydrochloride added, based on weight of Teff or millet.

Table IV. Effect of Lysine Supplementation on Plasma Protein Concentration

Diet	Grams Protein/100 Ml. Plasma
Teff	4.01
+0.25% LMH[a]	4.45
+0.42% LMH	5.14
Pearl millet	4.50
+0.25% LMH	4.99
+0.50% LMH	5.01
ANRC casein	5.39

[a] Percentage of L-lysine monohydrochloride added, based on dry weight of cereal.

Ethiopian diet was calculated to contain 65 grams of protein, of which 41 grams would be supplied by Teff. From analysis of food supplies, it was estimated that 87 grams of protein were available per person per day, although the survey team felt that the calculated "typical diet" agreed better with biochemical and clinical findings. Deficiencies of many essential nutrients were found in the Ethiopian diet. Probably the most immediate and serious is total calories. The following discussion is restricted to protein deficiency, recognizing that it is only one part of the problem.

An improvement in the quality or quantity of the protein supply should help to alleviate any existing protein deficiency condition. Increasing the availability of animal protein sources in Ethiopia, particularly increasing the milk supply for infants and children, would be very desirable. Potential problems in sanitation could be serious, however, if production and distribution of whole milk were contemplated. The universal preference for Teff and its amino acid composition indicate that substantial improvements in the protein quality of the over-all diet of many Ethiopians could be made by balancing the first limiting amino acid, lysine, against the second. This can be accomplished either by supplementation with a lysine-rich protein or with lysine as the monohydrochloride.

The advantages of a protein source are that it raises the quantity of protein in the diet as well as the quality, and it also could be important as a supplemental source of vitamins and minerals. In connection with the use of supplemental protein, it should be remembered that only excess lysine is available for balancing lysine deficiency in Teff, not total lysine in supplemental protein.

In the case of supplementation with synthetic lysine monohydrochloride, data presented in this report show the efficiency of this method in converting Teff protein into a high quality protein. Advantages in the use of lysine monohydrochloride are that it does not change the taste and textural characteristics of the diet or the use of the basic foods that are commonly used by habit and tradition.

Economics at the present time would probably favor the use of relatively lysine-rich protein foodstuffs that could be grown locally such as fenugreek, pulses, and soybean. However, large-scale commercial production of lysine monohydrochloride could possibly lower the price sufficiently that this method could become the method of choice.

Conclusions

All varieties of Teff contained a good content and balance of essential amino acids, except for being markedly first limiting in lysine. Pearl millet had an amino acid pattern similar to Teff, but was generally lower in essential amino acids, including the first limiting amino acid, lysine. Its composition fell within the range reported for six varieties of Teff, being lower only in threonine, isoleucine, and aromatic, and sulfur amino acids.

Supplementation of Teff with 0.4% of LMH raised the 4-week weight gain and PER from 50.3 grams and 1.95 to 138.5 grams and 3.27, respectively. Similarly, supplementation of pearl millet with 0.50% of LMH increased weight gain and PER from 36.2 grams and 1.83 to 118.0 grams and 3.28, respectively.

Plasma protein concentration was 4.01 grams per 100 ml. for the unsupplemented Teff diet. Supplementation of Teff with 0.4% of LMH resulted in a plasma protein concentration of 5.15 grams per 100 ml. Similarly, in the case of pearl millet, lysine supplementation was associated with a change from 4.50 to 5.01 grams per 100 ml.

Acknowledgment

The authors gratefully acknowledge the cooperation of Lester J. Teply and Arnold E. Schaefer, members of the nutrition team which surveyed Ethiopia, who supplied the samples of Teff used in these studies. Amino acid analysis of Teff protein was originally made at the suggestion of A. E. Schaefer.

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Received for review December 27, 1960. Accepted May 22, 1961. Presented at Fifth International Congress on Nutrition, Washington, D. C., September 1960.