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Comparative Nutritive Value and Amino Acid Content of Different Varieties of Sorghum and Effect of Lysine Fortification

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A study was conducted to evaluate the nutritive value, i.e., PER (protein efficiency ratio), NPR (net protein retention), and amino acid content of different varieties of sorghum (hybrid and old selected varieties). The protein quality index based on PER and NPR at a 8.5% protein level was found to be highest in the variety CSH-1, followed by CSH-2 and Swarna. A chemical score based on the essential amino acid content of egg protein and FAO/WHO provisional pattern of protein (1973) indicated the levels of amino acids which are limiting in three different varieties of sorghum. The EAAI (essential amino acid index) and BV (biological value) were also calculated. Fortification of sorghum grain with lysine at 9% level has increased the PER and NPR values significantly.

Sorghum grain is one of the important crops in the world. This cereal plant is cultivated on all six continents and widely used as food in Africa and Asia. In Western countries the grain is used as feed and the whole plant as forage (Wall and Ross, 1970).

Although sorghum is a staple food in India, the nutritional value of sorghum grain is inferior to that of maize and barley (Xavier Filho, 1974; Howe et al., 1965). The amino acid composition of its proteins shows deficiency of lysine and threonine, and their digestibility is less than that of other cereal grain proteins. The reason for this low digestiblity is not known (Wall and Ross, 1970).

Preliminary data of Miller et al. (1964) on sorghum indicated that wide variation in protein content was associated with location, hybrids, and fertilization. Little work has been reported on the amino acid composition of sorghum (Baumgarton et al., 1945, 1946; Lyman et al., 1956; Deyoe and Shellenberger, 1965; Singh and Axtell, 1973) and on the nutritive value of hybrid sorghum (Breuer and Dohm, 1972; Jambunathan and Mertz, 1973).

It was considered important to study systematically the nutritive value based on PER, NPR, and amino acid content in different varieties of hybrid sorghum and compare it with that of older selected variety, in order to explore the possibility of improving the nutritional quality by breeding and to learn what other amino acids are limiting besides lysine. The effect of lysine fortification at different levels on the nutritional quality was also studied in this investigation. The present paper describes results of such investigation on different varieties of sorghum developed at the Indian Agricultural Research Institute, New Delhi.

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MATERIALS AND METHODS

Sorghum grain samples were collected from a field experiment conducted at Division of Agronomy, Indian Agricultural Research Institute, New Delhi.

Methods. Samples were dried in a hot air oven at 105 °C to determine moisture content. The protein content of the samples was calculated by multiplying the Kjeldahl N by 6.25. The amino acid composition was studied using a Technicon sequential multisample amino acid analyzer (TSM). Defatted samples containing 5 mg of protein were hydrolyzed by refluxing with 5 mL of 6 N HCl acid for 22 h. After removal of acid by evaporation under reduced pressure, the residue was dissolved in 2 mL of citrate buffer (pH 2.875). An aliquot (0.4 mL) was used for determination of amino acids according to the method of (Spackman et al., 1958). Tryptophan was determined by the method of (Spies and Chambers, 1949).

Diets. The diets for all the biological experiments were prepared at a 8.5% protein level. The composition of 100 g of diet was as follows; test sample flour weight calculated to give 8.5% protein; ground nut oil 10 g (containing 1 mg or 100 IU vitamin E; 4% mineral mixture U.S.P. XVII 4) (Sikka et al., 1975); 1 g of a complete vitamin mixture

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Table I. Mean Values of Food Intake, Protein Intake, Gain in Weight by Albino Rats, and PER of Different Varieties of Sorghum and Casein at 8.5% Protein Level

protein source	food intake, g/28 days per animal	protein intake, g/28 days per animal at 8.5%	mean wt gain, g/28 days per animal	PER	F value obsd
Swarna	90	7.65 ± 0.4[a]	2.2 ± 0.03[a]	0.30 ± 0.057[a]	
CSH-1	97	8.25 ± 0.46	5.8 ± 0.23	0.70 ± 0.033	
CSH-2	101	8.59 ± 0.51	4.6 ± 0.15	0.54 ± 0.020	
Casein	351	29.80 ± 6.10	76.6 ± 40.2	2.57 ± 0.450	
Swarna + 6% lysine	104	8.84 ± 0.53	17.1 ± 2.0	1.93 ± 0.260	
Swarna + 9% lysine	88	7.48 ± 0.38	15.6 ± 1.67	2.08 ± 0.30	6.07[b]

[a] Standard error. [b] Significant at $p = 0.05$.

Table II. Net Protein Retention (NPR) of Different Varieties of Sorghum and Casein at 8.5% Protein Level

protein source	protein intake, g/10 days per animal at 8.5%	mean wt gain, g/10 days per animal	meat wt loss in nonprotein, g	NPR
Swarna	3.3 ± 0.34[a]	0.5	4.8	1.60 ± 0.09[a]
CSH-1	3.2 ± 0.34	0.7	4.8	1.71 ± 0.09
CSH-2	3.4 ± 0.42	0.7	4.8	1.61 ± 0.09
Casein	7.5 ± 1.86	22.0	4.8	3.57 ± 0.42
Swarna + 6% lysine	3.3 ± 0.36	6.8	4.8	3.51 ± 0.41
Swarna + 9% lysine	3.0 ± 0.30	7.0	4.8	3.93 ± 0.51

[a] Standard error.

(Manna and Hauge, 1953) and 2 drops of Adoxline containing vitamin A (1200 IU/g) and vitamin D_2 (I.P. 2000 IU/g) was fed orally twice a week. The N content was adjusted by adding the required quantity of N-free potato starch.

Protein Efficiency Ratio (PER). PER was determined by the method of (AOAC, 1975) (except that in place of ten male animals, six animals were used, and fiber content was not equalized, since sorghum grains contain only 3% fiber, which may not influence the relative PER of casein. Weanling albino rats about 22 days old and weighing 30–40 g were divided into six groups. All groups within each experiment had the same average initial weight. Each group consisted of three males and three females.

The rats were placed in individual all-wire cages with a raised platform. Water was available to them at all times. Food intake was measured every day, and spilled food was collected daily and used to correct the amount of food intake. The animals were weighed twice a week for 28 days.

Net Protein Retention (NPR). NPR was determined by the method of (Bender and Doell, 1957). One-month-old albino rats, three females and three males in each group (seven groups in all), having the same initial weight were used. All grain flours in the diet were at 8.5% protein level. A nonprotein diet was prepared by replacing grain flour with protein-free potato starch. Three groups were fed with three different grain flours: one group was given a nonproteinous diet, one group was given a standard casein diet, and two groups were given one grain diet + 6% or + 9% L-lysine (based on total protein), respectively. The weight of each rat was recorded every third day. NPR was calculated as follows:

$$\text{NPR} = \frac{\text{wt gain of TPR + wt loss of NPG}}{\text{wt of protein consumed}}$$

where TPG = test protein group and NPG = nonprotein group.

Biological Value (BV). BV was determined by calculation according to regression equation given by (Duggal and Eggum, 1977) for proteins with lysine as limiting amino acid.

RESULTS AND DISCUSSION

Table I shows food intake, protein intake, weight gain, and PER. The PER values reveal that hybrid variety CSH-1 followed by hybrid variety CSH-2 had better protein quality than that of Swarna, an older selected variety. Similar PER values were obtained by earlier workers (Swaminathan, 1937; Bornstein and Bartov, 1967; Lamb et al., 1966; Daniel et al., 1965) in older or hybrid sorghum varieties. The PER values are lower than those of maize and barley (Howe et al., 1965; Trible, 1971).

Further the PER values of sorghum grain at 8.5% protein level (0.3) increased significantly to 1.93 and 2.08 on addition of L-lysine to diet at the 6 and 9% levels, respectively. The growth of rats with 9% L-lysine supplementation of diet was significant at 5% level when analyzed statistically over all other diets. This further confirms the findings of Daniel et al. (1965) who had also observed a similar increase in PER values from 0.85 to 2.44 on supplementation with L-lysine. Further the values obtained with L-lysine supplementation also approach closely the PER value obtained in case of standard casein diet.

Net Protein Retention. The NPR values of three varieties of sorghum are presented in Table II. The NPR values listed in Table II are higher for the hybrid variety CSH-1. The relative order remains the same as in PER determination. The NPR values appear to magnify the differences in protein quality to greater extent than other biological determination (PER) because the PER measures protein efficiency based on growth only, whereas the NPR measures protein efficiency based on both growth and maintenance. Since determination of NPR is less time consuming (9–10 days) than PER (28 days) and involves less laboratory work than BV, it is a useful test for evaluation of protein quality. According to (Bender and Doell, 1957), NPR is more accurate as a measure of protein value than PER and gives a highly significant correlation with NPU (net protein utilization). But in the case of these sorghum varieties, the variation in PER between the three grains was more marked. The supplementation of diet with L-lysine at the 6 and 9% levels had increased the NPR values of Swarna significantly, as in the case of PER, and the figures are very close to those obtained for standard

Table III. Amino Acid Composition of Different Varieties of Sorghum, FAO/WHO Provisional Pattern (1973), and Egg (g/16 of N)

amino acid	FAO/WHO provisional pattern, 1973	egg	Swarna	CSH-1	CSH-2
essential aa					
isoleucine	4.0	5.8	1.9	4.9	3.9
leucine	7.0	8.9	12.7	12.6	12.5
lysine	5.4	6.7	2.1	2.6	2.1
total aromatic aa	6.1	10.3	7.8	7.4	8.9
phenylalanine	3.0	6.7	4.5	4.2	4.9
tyrosine	3.0	3.6	3.3	3.2	4.0
total sulfur aa	3.5	5.3	4.1	3.9	3.0
cystine		3.0	2.8	2.5	1.5
methionine		2.3	1.3	1.4	1.5
threonine	4.0	5.1	2.2	2.4	2.9
tryptophan	1.0	1.5	1.1	1.2	1.2
valine	5.0	7.5	2.1	5.0	4.9
nonessential aa[a]					
arginine	5.2	6.7	3.5	3.6	3.3
glycine	2.2	3.6	3.8	3.2	2.8
aspartic acid	7.7	10.4	3.9	5.7	6.2
serine	7.7	6.0	5.8	5.2	3.8
histidine	2.5	3.5	2.8	2.3	2.0
alanine	6.1	3.5	9.9	8.6	8.9
glutamic acid	14.7	25.2	24.3	19.5	19.4
proline	10.7		9.0	7.7	8.7
ammonia			0.5	1.9	2.4
nitrogen %			1.5	1.4	1.5

[a] A mixture of nonessential amino acids in FAO/WHO provisional pattern based on the proportion of these amino acids as found in skim milk protein (Eggum, 1968).

Table IV. E:N, E:P, E:T Ratios, Chemical Score, EAAI, and BV of Different Varieties of Sorghum[a]

protein source	E:N, mg	E:P, mg	E:T, mg	EAAI	chemical score, %, egg	BV, egg	chemical score, %, FAO/WHO
Swarna	0.556	0.347	0.357	54.6	30.9	52.2	37.8
CSH-1	0.783	0.420	0.439	69.0	38.9	58.6	47.6
CSH-2	0.780	0.412	0.438	65.1	30.9	51.2	37.8
FAO/WHO pattern 1973							100.0
egg				100.0	100.0	100.0	

[a] EAAI (essential amino acid index) is based on the ratios of the amounts of essential amino acids in a protein relative to their amount in whole egg protein (Oser, 1951). Chemical score is the percentage of the most deficient essential amino acid in the protein as compared to the requirement pattern (Mitchell and Block, 1946). E:N, ratio of essential amino acids to nonessential amino acids. E:P, ratio of essential amino acids to protein, 100 g. E:T, ratio of essential amino acids to total amino acids.

casein and better than those for 9% L-lysine supplementation. It further reveals that L-lysine supplementation of diet improves the protein quality of grain (deficient in lysine) from the very beginning of its feeding and further that 9% increase of lysine in diet is superior to 6% increase of lysine in diet. It has significantly increased the growth of rats over all other diets.

Amino Acid Chemical Score and EAAI. The amino acid composition of three varieties of sorghum FAO/WHO Provisional pattern (1973) and egg is presented in Table III. It is quite evident from Table III that the older selected variety Swarna is deficient in many essential amino acids compared to the hybrid varieties CSH-1 and CSH-2 as well as FAO/WHO pattern (1973) and egg. The amino acid composition obtained in the case of CSH-1 and CSH-2 is well supported by the results of earlier workers (Deyoe and Shellenberger, 1965; Shoup et al., 1970) in the case of hybrid sorghum. The most limiting amino acids were found to be lysine and threonine for the hybrid varieties CSH-1 and CHS-2, whereas in the older selected variety Swarna, lysine, valine, threonine, and isoleucine were found to be limiting. A close correlation was observed between PER, NPR, and amino acid composition. Low PER and NPR values obtained in the case of all the three varieties are due to deficiencies of L-lysine, which is quite evident from the results obtained by supplementation of diet with L-lysine (Table I and Table II), which has improved the PER and NPR values significantly. Lower PER values of the older selected variety Swarna when compared with the hybrid varieties CSH-1 and CSH-2 might be due to excess of leucine in diet and as well as lower isoleucine content. The ratio of leucine to isoleucine is much higher for Swarna than CSH-1 and CSH-2. This is supported by the observation of Rogers et al. (1967) who observed that if given a choice between diets containing 5% leucine and a protein-free diet, rats will select the protein-free diet. It appears that rats will avoid all diets containing 5% or more leucine even if the alternative diet is much inferior.

As observed from Table IV, the ratio of essential amino acids to nonessential amino acids (E:N), as well as essential amino acids to protein (E:P) and total amino acids (E:T), is also higher for hybrid variety CSH-1 over Swarna and CSH-2. This also supports the higher PER and NPR values obtained for hybrid variety CSH-1. The "chemical score percent egg", "BV egg", "chemical score percent FAO/WHO", and EAAI also support the higher PER and NPR values obtained in the case of hybrid variety CSH-1

as compared to the older selected variety Swarna and hybrid variety CSH-2. This may be due to differences between lysine content of those grain proteins and protein of CSH-1.

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Nutritive Value of Two Different Beans (*Phaseolus vulgaris*) Supplemented with Methionine

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Two varieties of *Phaseolus vulgaris*, black and white beans, were supplemented with 0.6% methionine under different conditions: (a) the amino acid added at the beginning of the cooking process, (b) the amino acid added 30 min before the end of the cooking process, (c) the amino acid added at the end of the cooking, and (d) the samples dried with or without broth. Proximate analysis, amino acid determination, available lysine, in vitro digestibility, PER, and apparent digestibility were performed with the samples. Methionine was the limiting amino acid in the supplemented and nonsupplemented samples. The black beans had the lowest in vitro digestibility when they were dried with broth. This result correlated with the in vivo digestibility. PER and apparent digestibility were higher in both beans when they were dried without broth. The utilization of methionine was not affected by thermal treatment, which means that there is no difference if the supplementation is carried out at home or in the industrial processed beans.

Beans are the most consumed legumes in Mexico; however, their nutritive value is poor (Kakade and Evans, 1965; Liener, 1962) mainly due to the low content in sulfur amino acids (Bressani et al., 1961). The addition of methionine increases their nutritive value (Purdon and Brown, 1967), and since this amino acid is chemically synthesized at low prices, it would be convenient to focus its utilization in human nutrition as at the present it is mainly used in animal nutrition. In the present study a preestablished amount of methionine was added to two varieties of beans (Jaffé and Vega-Lette, 1968). The addition was done at different times of the home cooking process in order to determine whether heat affected the utilization of methionine.

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MATERIALS AND METHODS

Two edible beans (*Phaseolus vulgaris*) of different acceptance were studied: white bean, which has very low

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