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PROTEIN QUALITY OF SELECTED LINES OF SORGHUM VULGARE FOR THE GROWING RAT[1]

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ABSTRACT Evaluation in weanling rats of 10 lines of grain sorghum from the World Sorghum Collection at the 10% protein level showed that two superior lines were approximately half as effective as 10% casein, six were intermediate in quality, and two permitted little growth or nitrogen deposition. Amino acid composition and digestibility differed markedly. Lysine was most deficient but sulfur-containing amino acids and threonine also were limiting. Addition of these three amino acids together or of milk solids to certain sorghums stimulated growth. A slope ratio assay indicated that the relative nutritive value of most sorghums in another group was approximately 30% of that of lactalbumin, and that the combined effects of amount and quality of protein produced at least a two-fold difference in the usefulness of these sorghum lines as sources of protein.

INTRODUCTION

Grain sorghum is an important source of protein for certain population groups in Egypt, India and other developing countries. It is known also as jowar, kaffir corn or milo. Although lysine was the first limiting amino acid in sorghum (1,2), threonine also was low in milo (3) and jowar (4), and the sulfur-containing amino acids were inadequate for young rats (5). Supplementation of sorghum with both lysine and threonine improved nitrogen retention of growing girls (6).

The discovery by Mertz et al. (7) that the recessive mutant gene, opaque-2, increased the concentrations of lysine and tryptophan in maize has stimulated interest in the amino acid composition of other cereals and in improvement of their nutritional value by genetic modification. Selected lines of Sorghum Vulgare Pers. which were obtained from the World Sorghum Collection in Hyderabad, India were grown on the Agronomy Farm of Purdue University for a comprehensive study of yield, stand, disease resistance, and other characteristics. Data pertaining to the protein quality of certain lines are reported here.

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PROCEDURE

The India Series number of each sorghum line is cited. The entire sorghum grain was ground to a fine powder. Two groups of sorghum lines were evaluated with weanling male rats in separate laboratories by different procedures.

Experiment 1

In the first phase of this experiment 10 sorghum lines were tested as the principal component of the diet. The amino acid content of each was determined by ion exchange chromatography on an automatic amino acid analyzer after samples were hydrolyzed in 6N HCl for 24 hours (except that hydrolysis was reduced to 4 hours for methionine and cystine). These data are shown in Table I.

Diets. Sorghum supplied 10% protein (N x 6.25) except that lines 1220 and 0948, which were low in nitrogen, provided 9.4 and 9.3%. Sorghum ranged from 93 to 77% of the diet and dextrin was adjusted so that sorghum plus dextrin equalled 93%. A representative diet contained 91% sorghum, 2% dextrin, 2% cottonseed oil, 1% vitamin-dextrin mixture and 4% mineral mixture[1]. Fat, which is usually low in human diets containing sorghum, was restricted to permit inclusion of the maximum amount of sorghum; also in a preliminary test food intake and weight gain of rats were lower when 7% fat was fed instead of 2%. Diets providing 10 and 20% protein from casein were fed. All diets were fed ad libitum.

Each gram of the vitamin-dextrin mixture contained: vitamin B_{12} 5 mcg, biotin 15 mcg, menadione 15 mcg, folic acid 300 mcg, pyridoxine•HCl 750 mcg, ascorbic acid 1.5 mg, riboflavin 1.5 mg, thiamine•HCl 1.5 mg, niacin 3.0 mg, p-aminobenzoic acid 7.5 mg, calcium pantothenate 7.5 mg, α-tocopheryl acetate 11.0 mg, inositol 49.5 mg, choline chloride 99.0 mg, dextrin 816 mg, vitamin A 4.1 I.U. and vitamin D 0.4 I.U.

In a second phase of the experiment, three of these sorghum lines were supplemented with limiting essential amino acids or with nonfat dry milk[2] after intakes of essential amino acids in the first phase were compared with minimum requirements of the growing rat (8).

Animals. Male rats of the Sprague-Dawley strain weighing between 50 and 55 g were housed under constant environmental conditions. Six were assigned to each sorghum. At the end of a 21-day feeding period, rats were fasted for 3 hours and anesthetized with sodium pentobarbital.

Analyses. Carcass nitrogen was estimated from body water (9). Lipid was extracted from liver samples with anhydrous ethyl ether for 4 hours on a Goldfisch apparatus. The microbiological method of Ghosh et al. (10) was used to determine whether niacin was present in free or bound form.

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[1] Spector, H. J. Biol. Chem. 173, 659 (1948).
[2] From Carnation Co., Los Angeles, California.

Experiment 2

The procedure used in the slope-ratio assay has been described previously (11,12). In the assay, the slopes of the regression curves relating body water to protein intake are compared to that of a standard curve obtained with lactalbumin which is assumed to have maximum nutritive value. The slopes of the curves obtained with the test proteins are expressed as percentages of the slope of the standard curve. The standard curve was obtained by feeding diets containing 0.00, 3.08, 5.39 and 7.70% protein supplied as lactalbumin to 4 groups of 6 rats each. To compare sorghum protein to lactalbumin, sorghum comprised 70, 45 or 20% of the diets.

The diets contained 9.5% hydrogenated vegetable oil, 0.5% cod liver oil, 5.0% salt mixture, 0.5% vitamin mixture, 0.2% choline chloride, and the remainder as test protein or corn starch. After three weeks, during which food consumption was measured, the animals were killed and total body water was determined.

The analysis of variance described by Hegsted and Worcester (11) provides an estimate of the Relative Nutritive Value and the confidence limits of the value obtained. The Net Available Protein in the sorghum is derived by multiplying the Relative Nutritive Value by the protein content of the sorghum.

RESULTS AND DISCUSSION

Experiment 1

Protein and amino acid content. The 10 sorghum lines shown in Table I contained from 9.9 to 12.9% protein (N x 6.25) compared with a range of 9.8 to 13.2% for sorghums in West Africa (1), 7.6 to 12.5% in Guatemala (2), 9.7 to 10.3% in India (4), and 8.7 to 12.5% in the United States (13).

The concentrations of lysine and sulfur-containing amino acids varied widely, whereas isoleucine, threonine and valine fell within a narrow range (Table I). The ratio of leucine to isoleucine was approximately 3:1, as in maize. Arginine ranged from 312 to 149 mg per gram of nitrogen compared with 251 and 242 mg, respectively, in casein and milk.

Digestibility. The wide range of coefficients of apparent digestibility, from 77.3 to 48.9% (Table II), emphasizes the necessity of biological testing of unknown lines of cereals after they are screened for amino acid content.

Intakes of food and essential amino acids. Analysis of variance revealed highly significant differences ($P < 0.01$) in daily food consumption of rats fed different sorghums. The Duncan New Multiple Range Test indicated that rats ate more of diets containing sorghums 2031 and 3552 than of others except 1220, and less of the poorly absorbed 3472. The highest daily nitrogen intake was 16% less than from 10% casein (Table II).

Comparison of mean daily intakes of essential amino acids from sorghums 2031 and 3552 with the minimum requirements reported by Rama Rao et al. (8) showed that lysine intake was only 30% of the requirement, total sulfur-containing amino acids 60% and threonine 75%. The

TABLE I. NITROGEN (mg/g sample) AND AMINO ACIDS (mg/g N) IN SORGHUM, CASEIN AND MILK IN EXPERIMENT I

Sorghum	N	Lysine	Methionine	Cystine	Threonine	Isoleu	Leucine	Valine	Phenalan	Tyrosine	Histidine
2031	19	150	95	80	235	280	900	345	370	270	171
3552	21	170	100	80	225	270	850	350	330	260	137
1220	16	170	110	80	230	265	840	340	320	250	147
2232	20	160	100	75	210	245	780	325	320	250	134
0957	21	135	80	40	210	270	900	320	335	265	135
0083	19	125	85	70	210	285	920	345	350	255	135
0182	18	135	110	85	220	270	890	340	360	260	148
8231	18	125	75	30	210	280	940	340	350	270	134
0948	16	145	75	40	200	230	680	280	290	210	126
3472	17	145	75	30	210	280	910	335	360	265	133
Casein	141	540	195	25	270	365	650	450	340	350	190
Milk	52	470	125	85	310	335	650	520	320	340	183

TABLE II. FOOD INTAKE, WEIGHT GAIN AND BODY COMPOSITION OF RATS IN EXPERIMENT I

Sorghum	Digestibility	Food intake[1]	N intake	Daily gain[1]	Body N stored	Dry liver		
						Fat-free	Total N[1]	Lipid
	%	g/day	mg/day	g	g	g	mg	%
2031	74.3	10.1 ± 0.6	0.16	1.9 ± 0.2	0.95	0.8	102 ± 7	19.7
3552	76.8	10.0 ± 0.7	0.16	1.9 ± 0.1	0.95	0.8	104 ± 6	22.1
1220	67.2	9.6 ± 0.4	0.14	1.1 ± 0.1	0.53	0.8	81 ± 1	25.0
2232	77.3	8.6 ± 1.1	0.14	0.9 ± 0.3	0.43	0.6	80 ± 2	22.4
0957	74.6	8.5 ± 1.0	0.14	0.8 ± 0.1	0.45	0.7	95 ± 3	22.4
0083	60.2	8.5 ± 0.3	0.13	0.8 ± 0.2	0.42	0.5	72 ± 3	26.7
0182	60.3	8.1 ± 0.8	0.13	0.8 ± 0.2	0.32	0.5	68 ± 14	25.0
8231	52.9	8.3 ± 0.7	0.13	0.7 ± 0.1	0.32	0.5	68 ± 3	33.9
0948	50.1	8.4 ± 0.9	0.13	0.5 ± 0.2	0.22	0.6	66 ± 2	30.5
3472	48.9	7.2 ± 0.3	0.11	0.3 ± 0.0	0.11	0.5	59 ± 3	40.5
Casein 10%	87.1	11.8 ± 1.1	0.19	3.5 ± 0.3	2.01	1.2	161 ± 4	14.0
Casein 20%	88.1	12.8 ± 0.5	0.41	5.7 ± 0.5	3.50	1.9	247 ± 4	11.3

[1]Mean ± S.E.

report of Waggle, Parrish and Deyoe (5) relati. to limiting amino acids for the growing rat was thus confirmed.

Weight gain and body composition. Differences in gain (Table II) were highly significant ($P < 0.01$). The sorghums could be arranged in three groups. Lines 2031 and 3552 caused a gain of 1.9 g per day, which was 53% of that resulting from the same amount of protein from casein; 6 lines resulted in gains of 1.1 to 0.7 g per day; but lines 0948 and 3472 produced gains of only 0.5 and 0.3 g, respectively. Mean weights of carcasses of rats fed sorghum ranged from 90 to 57 g compared with 124 and 166 g, respectively, when 10 and 20% casein were fed.

The sorghums ranked in almost the same order on the basis of body nitrogen stored in 21 days (total carcass nitrogen minus nitrogen present at the beginning of the experiment) as by gain in weight (Table II). The two lines that promoted the best growth resulted in storage of 0.95 g of nitrogen, but two allowed storage of only 0.22 and 0.11 g while rats fed a protein-free diet lost 0.13 g of nitrogen in the same interval. Deposition of nitrogen and lipid in the liver varied inversely in most cases (Table II).

Niacin content. This was of interest because pellagra still occurs in certain areas where sorghum is a staple. Free niacin ranged from 6.4 to 5.0 mcg/g and bound niacin from 24.1 to 21.6 mcg/g of sorghum. These values did not differ significantly from each other, but were distinctly higher than those reported by Ghosh et al. (10).

Supplementary effect of certain amino acids and of milk. In the second phase of experiment 1, only the three most promising sorghums were tested. Addition to 9.2% protein from sorghum 1220 of 0.65% lysine alone or with methionine increased daily gain only from 0.7 to 1.1 g, whereas addition of 0.65% lysine plus 0.21% cystine plus 0.16% threonine quadrupled weight gain, tripled body nitrogen stored, doubled hepatic nitrogen and reduced liver lipid by half. Supplementation of sorghum with lysine and threonine only, as reported by Daniel et al. (6) in girls, was not tested because of the limited amount of sorghum available.

When sorghum 2031 was combined with nonfat dry milk to provide the following percentages of the diet, 77:12, 71:18 and 65:24, thereby supplying 13.0, 14.2 and 15.5% protein, the respective daily weight gains were 4.5, 5.0 and 5.8 g and the amounts of body nitrogen stored were 2.5, 2.5 and 3.4 g in 21 days. The combination of 12% milk and 77% sorghum induced as satisfactory weight gain and nitrogen deposition as did 13% protein provided by milk alone as 37% of the diet.

Supplementation of sorghum 3552 with milk also was beneficial. When sorghum alone supplied 12% protein, the daily gain was 2.3 g, whereas the same amount of protein supplied by 63% sorghum plus 12% milk induced a gain of 5.3 g and increased nitrogen storage by almost half. The combination of this limited amount of milk and sorghum stimulated growth to almost the same extent as did 20% casein.

Experiment 2

The estimated nutritive value of most of the sorghums tested by the slope ratio assay was approximately 30% that of lactalbumin, as shown in Table III. When Relative Nutritive Value of the sorghums was multiplied by protein content, the Net Available Protein in most

TABLE III. RELATIVE NUTRITIVE VALUE OF SORGHUM PROTEINS AND NET AVAILABLE PROTEIN IN VARIOUS LINES IN EXPERIMENT II

Sorghum	Protein content	Relative Nutritive Value	95% Confidence limits	Net Available Protein
	%	%	%	%
1220	9.8	31	26-36	3.0
0948	9.3	32	26-37	3.0
3796	10.4	39	30-47	4.1
8120	12.8	26	22-30	3.3
0957	12.4	34	30-37	4.2
0271	11.7	30	25-35	3.5
4328	12.3	32	27-37	3.9
8382	11.1	31	25-37	3.4
7088	11.8	30	26-35	3.5
0718	10.4	21	15-27	2.2

samples was of the order of 3 to 4%. The confidence limits of the assays emphasize the fact that, even when 18 animals are involved in the assays, the estimated value is subject to considerable error.

Sorghum 3796 appeared to be significantly better than lines 8120 and 0718 which were of inferior quality, and somewhat above the other lines tested in this group. Although sample 8120 had a Relative Nutritive Value of only 26%, this was compensated to a considerable extent by the higher protein content so that its Net Available Protein was similar to that of most other samples. The combined effects of amount and quality of protein in these sorghums produced at least a two-fold difference in the usefulness of different samples as protein sources.

When data from both experiments are considered, it is evident that the sorghum lines in each group differed greatly in respect to protein content, amino acid composition, digestibility and ability to promote growth and nitrogen deposition in the young rat. These findings emphasize the importance of considering protein quality as well as other characteristics in the selection and breeding of grain sorghums.

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