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NUTRITIVE VALUE OF GRAINS

Amino Acids and Proteins in Sorghum Grain

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Determination of the protein content and amino acid composition of sorghum grain indicates variations due to hybrids and location. Protein values, N × 6.25, ranged from 8.65 to 12.50%. Analyses by ion exchange procedures of 30 samples of grain sorghums representing 15 different hybrids grown at two locations show variations in amino acid content. Statistical analysis of the amino acid data, per cent of sample and per cent of amino acid in the protein, indicated significant differences ($P < 0.05$) in amino acid level due to hybrids. A significant ($P < 0.05$) location effect was found for the amino acids as per cent of the sample. Of amino acids important from a nutritive standpoint, methionine ranged from 1.22 to 1.97% and lysine from 1.81 to 2.49% of the protein.

PROTEIN is one of the most important portions of animal diets. Since feed grains make up approximately 50% or more of the diet, protein composition and content of the grains are highly important. The importance of grain sorghums has increased materially in the last ten years, during which standard varieties were largely replaced by hybrids. In 1952 United States production was 90,741,000 bushels; 1962 it was 509,137,000 bushels, or 5.6 times more. Studies have indicated that the feed values of grain sorghums and corn are equal. Oklahoma workers (*20*) show that grain sorghums (sorghum) could effectively replace corn in chick diets. Other work has indicated similar replacement values with swine and sheep (*2, 9, 12*). More recent work with laying hens has indicated that sorghum may not be as well utilized as corn. Malik and Quisenberry (*11*) found combinations of corn and sorghum more effectively utilized than sorghum alone.

Research has indicated that hybridization lowered the protein content of corn (*6*) and that quality of protein, as measured by amino acid analysis, varies. Differences in amino acid composition have been ascribed largely to the increasing percentage of zeins in the protein as total protein in the grain increases (*6–8, 16, 17, 22*). Other workers (*14*) have not detected significant differences in methionine, tryptophan, and lysine levels in corn when protein content ranged from 8.9 to 13%. Doty (*5*) reported that amino acid composition of corn might be genetically controlled. Wolfe and Fowden (*21*) found considerable differences in amino acids present in various corn varieties. Largest differences were for arginine, histidine, lysine, leucine, threonine, and valine, and between varieties least related genetically.

Table I. Effects of Hybridization and Location on Average Protein Content of Hybrid Sorghum Grain

	Location			
Hybrid	Hiawatha	Manhattan	Newton	Mean
	% Protein[a]			
59CH5	12.4	11.8	12.2	12.1
61MH233	12.0	10.3	11.3	11.2
60MH173	11.1	10.0	12.3	11.1
60MH172	11.1	9.9	12.1	11.0
59CH71	11.4	10.3	11.2	11.0
61MH235	11.3	10.4	11.3	11.0
KS651	11.3	10.3	11.1	10.9
59MH153	10.8	10.1	11.9	10.9
58MH105	11.5	9.9	11.2	10.9
60MH177	10.8	9.6	11.9	10.8
59MH152	10.6	9.3	11.5	10.5
RS610	10.7	10.0	10.6	10.4
KS652	10.6	9.8	10.6	10.3
60MH212	10.5	9.2	10.6	10.1
KS701	10.2	9.0	10.4	9.9
Mean	11.1	10.0	11.3	

[a] N × 6.25.

Table II. Analysis of Variance of Sorghum Grain Protein Levels

Source of Variance	Degrees of Freedom	Mean Square
Total	134	
Hybrids	14	2.5193[a]
Location	2	23.6627[a]
Replication	2	0.2765
Hybrid × location	28	0.4534[a]
Error	88	0.1410

[a] Significantly different ($P < 0.01$).

Table III. Amino Acid Content of Sorghum Grain

(Amino acid as % of sample)

Amino Acid	Mean (30 Samples), %	Standard Deviation	Extremes, %
Lysine	0.203	0.024	0.152–0.252
Histidine	0.209	0.024	0.164–0.261
Arginine	0.278	0.033	0.218–0.338
Aspartic acid	0.645	0.094	0.461–0.852
Threonine	0.306	0.038	0.235–0.378
Serine	0.420	0.059	0.310–0.531
Glutamic acid	2.170	0.322	1.584–2.933
Proline	0.792	0.112	0.575–1.025
Glycine	0.308	0.036	0.241–0.372
Alanine	0.945	0.131	0.700–1.228
Half cystine	0.105	0.022	0.058–0.153
Valine	0.506	0.065	0.382–0.646
Methionine	0.137	0.024	0.091–0.226
Isoleucine	0.392	0.054	0.275–0.537
Leucine	1.360	0.235	0.978–2.161
Tyrosine	0.172	0.037	0.114–0.283
Phenylalanine	0.490	0.063	0.366–0.635
Protein[a]	10.43	0.939	8.65 –12.50

[a] Protein = N × 6.25.

Table IV. Analysis of Variance of Amino Acids Data

Source of Variance	Degrees of Freedom	Mean Square: For % of sample	Mean Square: For % of protein
Total	509		
Location	1	0.2440[a]	3.0982
Hybrids	14	0.1431[b]	7.2070[b]
Location × hybrid	14	0.0365	1.5185
Amino acids	16	8.4199[b]	795.4630[b]
Amino acids × hybrids	224	0.0103[b]	0.5078[b]
Error	240	0.0054	0.1665

[a] Significantly different ($P < 0.01$).
[b] Significantly different ($P < 0.05$).

Table V. Amino Acid Content of Sorghum Grain

(Amino acids as % of protein)

Amino Acid	Mean (30 Samples), %	Standard Deviation	Extremes, %
Lysine	1.99	0.236	1.57–2.61
Histidine	2.05	0.187	1.65–2.34
Arginine	2.71	0.117	2.07–3.39
Aspartic acid	6.30	0.689	4.80–7.67
Threonine	2.99	0.302	2.38–3.72
Serine	4.10	0.494	3.23–5.54
Glutamic acid	21.16	2.158	17.00–24.85
Proline	7.72	0.632	5.99–8.92
Glycine	3.00	0.301	2.44–3.53
Alanine	9.21	0.932	7.29–10.68
Half cystine	1.00	0.223	0.49–1.38
Valine	4.93	0.453	3.97–5.84
Methionine	1.34	0.223	0.81–1.97
Isoleucine	3.80	0.403	2.86–4.78
Leucine	13.05	1.272	10.19–15.38
Tyrosine	1.64	0.330	1.15–2.46
Phenylalanine	4.77	0.427	3.75–5.51

Preliminary data of Miller *et al.* (*13*) on sorghums indicated that wide variations in protein content were associated with location, hybrids, and fertilization. Little research has been reported on amino acid composition of sorghum protein (*3, 4, 10*). That reported was largely from nonhybrids, therefore more characteristic of older varieties than present hybrids.

Rapid quantitative ion exchange chromatographic methods to determine amino acids of acid hydrolyzates provide techniques for studying effects of various factors on protein quality of sorghum grain.

The increased use of sorghum grain for feeding purposes has indicated that the protein level may vary. To make the best use of this feed grain, more precise information is needed on the factors that affect protein quality. Information on the amino acid composition makes it possible to estimate the nutritive quality of grain sorghum protein.

Methods

Grain sorghum samples were collected from breeding experiments at three locations: Hiawatha, Manhattan, and Newton, Kan. Samples from each of three replications were collected from each of 15 different hybrids grown at each test area. All were assayed for crude protein content by standard methods (*1*) and nitrogen determined by the Kjeldahl method was converted to crude protein by multiplying by a 6.25 factor.

Samples for amino acid determinations were selected from hybrids grown at Hiawatha and Manhattan and prepared by acid hydrolysis. Each sample was weighed and approximately 100 mg. were placed in a 15- × 150-mm. test tube. The top of the tube was then narrowed, the tube was placed in a dry ice bath, and 1.0 ml. of 6*N* HCl were added. The tube was then sealed under vacuum and the sample was hydrolyzed 22 hours at 110° C. All amino acid analyses were conducted by ion exchange chromatography on a Beckman Model 120 amino acid analyzer, using methods of Spackman, Stein, and Moore (*19*). Hydrolyzed samples prepared for analyses were stored in a deep freeze at −20° C. and analyzed within 10 days. Since tryptophan is unstable under acid hydrolysis, no attempt was made to determine it. All data collected on protein and amino acids were analyzed statistically where possible by methods of Snedecor (*18*).

Results and Discussion

Results of protein determinations (Table I) give the average protein content of three replications for each hybrid at each location. Average protein content of hybrids at Hiawatha (11.1%) and Newton (11.4%) exceeded those at Manhattan (10.0%). The protein content within hybrids varied from a high average of 12.1% for 59CH5 to a low of 9.9% for KS701. Analysis of variance (Table II) indicates significantly different ($P < 0.01$) protein levels among hybrids. The hybrid effect noted may indicate genetic effects or may reflect

Table VI. Hybrid Effect on Amino Acid Composition

(% of sample)

	Hybrids														
Amino Acids	59CH5	RS610	60MH 172	61MH 233	61MH 235	59MH 153	59CH71	60MH 177	60MH 173	KS652	KS651	60MH 212	59MH 152	58MH 105	KS701
Manhattan															
Lysine	0.227[a]	0.213	0.209	0.220	0.223	0.177	0.152	0.180	0.183	0.216	0.172	0.206	0.191	0.224	0.183
Histidine	0.261	0.222	0.195	0.224	0.209	0.197	0.166	0.189	0.190	0.204	0.197	0.202	0.207	0.207	0.193
Arginine	0.338	0.281	0.246	0.296	0.281	0.246	0.227	0.261	0.285	0.283	0.250	0.278	0.280	0.305	0.224
Aspartic acid	0.794	0.669	0.629	0.683	0.652	0.592	0.461	0.576	0.602	0.641	0.588	0.618	0.607	0.634	0.529
Threonine	0.378	0.318	0.298	0.372	0.300	0.289	0.235	0.274	0.283	0.291	0.267	0.306	0.297	0.308	0.265
Serine	0.518	0.446	0.407	0.439	0.434	0.407	0.310	0.350	0.380	0.526	0.379	0.412	0.399	0.412	0.352
Glutamic acid	2.839	2.174	2.031	2.272	2.206	2.142	1.632	1.906	1.833	1.990	2.015	1.992	2.081	2.105	1.801
Proline	0.967	0.822	0.742	0.828	0.801	0.757	0.575	0.598	0.724	0.664	0.746	0.810	0.766	0.785	0.666
Glycine	0.363	0.333	0.311	0.337	0.308	0.285	0.241	0.278	0.286	0.335	0.268	0.312	0.292	0.309	0.279
Alanine	1.205	0.958	0.890	0.980	0.959	0.931	0.700	0.837	0.838	0.882	0.868	0.850	0.904	0.919	0.777
Half cystine	0.146	0.110	0.108	0.134	0.099	0.096	0.100	0.124	0.084	0.081	0.107	0.108	0.121	0.106	0.119
Valine	0.644	0.529	0.501	0.584	0.496	0.462	0.382	0.457	0.478	0.497	0.448	0.470	0.487	0.492	0.429
Methionine	0.226	0.152	0.125	0.129	0.141	0.143	0.117	0.132	0.140	0.140	0.126	0.152	0.151	0.150	0.126
Isoleucine	0.537	0.395	0.369	0.392	0.352	0.373	0.275	0.356	0.352	0.371	0.342	0.405	0.377	0.430	0.318
Leucine	1.760	1.346	1.252	1.323	1.337	1.364	0.978	1.188	1.189	1.262	1.208	1.240	1.265	1.304	1.056
Tyrosine	0.283	0.164	0.149	0.211	0.155	0.140	0.164	0.209	0.167	0.166	0.142	0.196	0.201	0.211	0.138
Phenylalanine	0.627	0.504	0.475	0.480	0.501	0.469	0.366	0.445	0.442	0.470	0.459	0.466	0.474	0.496	0.419
Hiawatha															
Lysine	0.233	0.207	0.195	0.249	0.230	0.203	0.176	0.183	0.252	0.200	0.185	0.188	0.212	0.223	0.182
Histidine	0.248	0.216	0.196	0.251	0.255	0.212	0.199	0.186	0.233	0.205	0.205	0.163	0.196	0.229	0.212
Arginine	0.316	0.289	0.276	0.321	0.335	0.304	0.232	0.261	0.322	0.271	0.259	0.218	0.312	0.302	0.240
Aspartic acid	0.852	0.659	0.626	0.794	0.780	0.670	0.574	0.599	0.795	0.657	0.588	0.506	0.721	0.753	0.512
Threonine	0.372	0.312	0.288	0.348	0.362	0.318	0.266	0.278	0.354	0.302	0.280	0.242	0.326	0.354	0.297
Serine	0.531	0.421	0.404	0.482	0.504	0.429	0.368	0.375	0.502	0.406	0.358	0.324	0.446	0.485	0.398
Glutamic acid	2.933	2.108	2.128	2.576	2.678	2.226	2.060	1.985	2.633	2.115	2.029	1.584	2.321	2.569	2.149
Proline	1.025	0.836	0.790	0.951	0.934	0.802	0.726	0.702	0.953	0.866	0.754	0.608	0.869	0.906	0.777
Glycine	0.360	0.312	0.290	0.354	0.356	0.299	0.274	0.270	0.342	0.315	0.291	0.242	0.372	0.351	0.277
Alanine	1.228	0.934	0.895	1.096	1.118	1.004	0.835	0.884	1.126	0.945	0.879	0.742	1.130	1.097	0.927
Half cystine	0.134	0.110	0.121	0.085	0.098	0.074	0.081	0.058	0.153	0.095	0.097	0.076	0.127	0.122	0.090
Valine	0.646	0.505	0.498	0.607	0.600	0.517	0.478	0.464	0.574	0.506	0.473	0.393	0.525	0.570	0.476
Methionine	0.163	0.120	0.136	0.146	0.143	0.138	0.091	0.119	0.162	0.139	0.130	0.104	0.100	0.160	0.122
Isoleucine	0.507	0.391	0.365	0.458	0.453	0.403	0.364	0.364	0.457	0.418	0.355	0.348	0.415	0.434	0.377
Leucine	1.815	1.360	1.301	1.582	1.596	1.394	1.230	1.227	2.161	1.336	1.220	1.150	1.410	1.512	1.477
Tyrosine	0.196	0.184	0.198	0.172	0.164	0.162	0.129	0.147	0.248	0.155	0.131	0.114	0.166	0.176	0.134
Phenylalanine	0.635	0.515	0.480	0.586	0.568	0.510	0.478	0.453	0.579	0.494	0.452	0.372	0.520	0.520	0.432

[a] Values underlined differ from mean by one or more standard deviations.

differences. Location effects also differed ($P < 0.01$) level. These data are similar to those indicating significant hybrid and location effects on the level of protein of sorghum grain (*13*).

Data (per cent of sample) summarized in Table III show that amino acid content of grain sorghums varied considerably. Statistical treatment of the data (Table IV) indicates a highly significant effect ($P < 0.01$) of hybrids and a significant ($P < 0.05$) location effect on amino acid levels. Significant effects were also found for amino acids and amino acid–hybrid interactions.

In Table V, average amino acid composition as percentage of the protein is given. As in Table III, the amino acid levels varied considerably within various

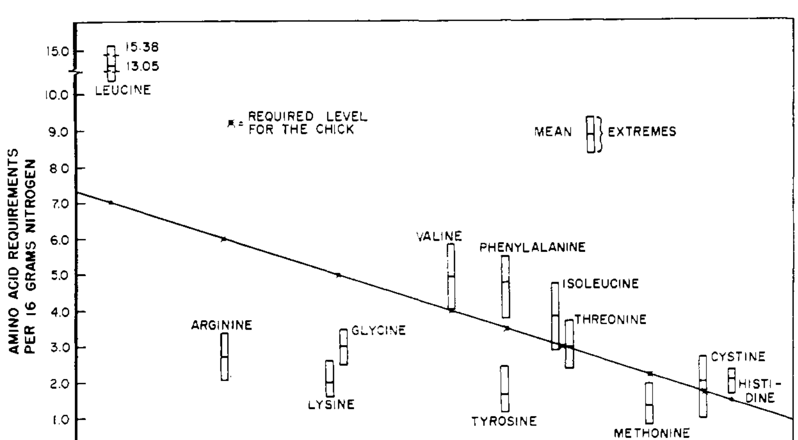

Figure 1. Relation between essential amino acid requirements of chick and amount found in sorghum grain protein

Table VII. Hybrid Effect on Amino Acid Composition

(% of protein)

Amino Acids	59CH5	RS610	60MH 172	61MH 233	61MH 235	59MH 153	59CH71	60MH 177	60MH 173	KS652	KS651	60MH 212	59MH 152	58MH 105	KS701
	Hybrids														
Manhattan															
Lysine	1.97	2.11	2.09	2.20	2.23[a]	1.81	1.58	1.88	1.91	2.27	1.85	2.17	2.12	2.49	2.12
Histidine	2.27	2.20	1.95	2.24	2.09	2.01	1.73	1.97	1.98	2.15	2.12	2.10	2.30	2.30	2.34
Arginine	2.94	2.78	2.46	2.96	2.81	2.51	2.37	2.72	2.97	2.98	2.69	2.76	3.11	3.39	2.71
Aspartic acid	6.90	6.62	6.29	6.83	6.52	6.04	4.80	6.00	6.28	6.75	6.32	6.06	6.74	7.04	6.11
Threonine	3.28	3.15	2.98	3.72	3.00	2.95	2.44	2.86	2.95	3.06	2.87	2.96	3.30	3.42	3.06
Serine	4.51	4.42	4.07	4.39	4.34	4.16	3.23	3.65	3.96	5.54	4.08	3.99	4.43	4.56	4.07
Glutamic acid	24.68	21.52	20.31	22.72	22.06	21.85	17.00	19.86	19.10	20.94	21.67	18.90	23.12	23.38	20.81
Proline	8.41	8.14	7.42	8.28	8.01	7.73	5.99	6.23	7.52	6.99	8.02	8.14	8.51	8.72	7.70
Glycine	3.15	3.30	3.11	3.34	3.08	2.90	2.51	2.89	2.98	3.53	2.88	2.86	3.25	3.43	3.22
Alanine	10.49	9.49	8.90	9.80	9.59	9.50	7.29	8.72	8.72	9.28	9.33	8.13	10.04	10.22	8.98
Half cystine	1.27	1.09	1.08	1.34	0.99	0.98	1.04	1.30	0.88	0.85	1.15	0.98	1.34	1.17	1.38
Valine	5.60	5.23	5.01	5.84	4.96	4.71	3.98	4.76	4.98	5.23	4.82	4.57	5.41	5.52	4.95
Methionine	1.97	1.50	1.25	1.29	1.41	1.46	1.22	1.38	1.46	1.47	1.35	1.46	1.67	1.66	1.45
Isoleucine	4.67	3.91	3.69	3.92	3.52	3.80	2.86	3.71	3.66	3.90	3.68	3.88	4.18	4.78	3.49
Leucine	15.31	13.32	12.52	13.23	13.37	13.92	10.19	12.38	12.38	13.28	12.99	11.97	14.06	14.49	12.21
Tyrosine	2.46	1.62	1.49	2.08	1.55	1.43	1.71	2.17	1.74	1.75	1.53	1.74	2.24	2.36	1.60
Phenylalanine	5.45	4.99	4.75	4.80	5.01	4.79	3.81	4.64	4.60	4.95	4.92	4.79	5.26	5.51	4.84
Hiawatha															
Lysine	1.97	1.97	1.83	2.06	2.07	1.92	1.57	1.74	2.61	1.94	1.71	1.70	1.98	2.03	1.86
Histidine	2.10	2.06	1.79	2.07	2.30	2.00	1.78	1.77	2.21	1.99	1.90	1.65	1.83	2.08	2.16
Arginine	2.68	2.75	2.38	2.60	3.02	2.87	2.07	2.49	2.87	2.63	2.40	2.20	2.92	2.75	2.45
Aspartic acid	7.22	6.28	5.72	6.93	7.03	6.32	5.13	5.70	7.67	6.38	5.44	5.10	6.74	6.85	5.23
Threonine	3.15	2.97	2.57	2.93	3.26	2.99	2.38	2.64	3.29	2.93	2.59	2.44	3.05	3.22	3.04
Serine	4.50	4.01	3.69	4.05	4.54	4.05	3.29	3.57	4.70	3.94	3.31	3.27	4.17	4.41	4.06
Glutamic acid	24.85	20.08	19.79	21.74	24.13	21.36	18.39	18.72	24.64	20.53	18.80	17.00	21.69	23.35	21.93
Proline	8.69	7.96	7.33	8.06	8.41	7.56	6.48	6.68	8.92	8.41	6.98	6.14	8.12	8.23	7.93
Glycine	3.05	2.97	2.63	2.99	3.21	2.82	2.44	2.57	3.28	3.05	2.69	2.44	3.48	3.19	2.82
Alanine	10.41	8.90	8.30	9.34	10.07	9.48	7.45	8.42	10.68	9.18	8.14	7.48	10.56	9.97	9.46
Half cystine	1.13	1.05	1.01	0.49	0.88	0.70	0.72	0.55	0.92	0.93	0.90	0.77	1.19	1.11	0.92
Valine	5.47	4.81	4.53	4.95	5.41	4.88	4.27	4.42	5.26	4.92	4.38	3.97	4.90	5.19	4.86
Methionine	1.38	1.14	1.19	1.19	1.29	1.32	0.81	1.14	1.43	1.35	1.20	1.05	0.94	1.46	1.24
Isoleucine	4.29	3.72	3.16	3.88	4.08	3.78	3.25	3.47	4.13	4.05	3.29	3.52	3.88	3.95	3.85
Leucine	15.38	12.95	12.07	12.77	14.38	13.14	10.98	11.69	14.60	12.97	11.30	11.61	13.18	13.74	15.07
Tyrosine	1.66	1.76	1.33	1.45	1.48	1.53	1.15	1.40	1.65	1.50	1.21	1.16	1.56	1.60	1.36
Phenylalanine	5.38	4.90	4.39	4.89	5.12	4.82	4.27	4.32	5.30	4.80	4.19	3.75	4.85	4.73	4.41

[a] Values underlined differ from mean by one or more standard deviations.

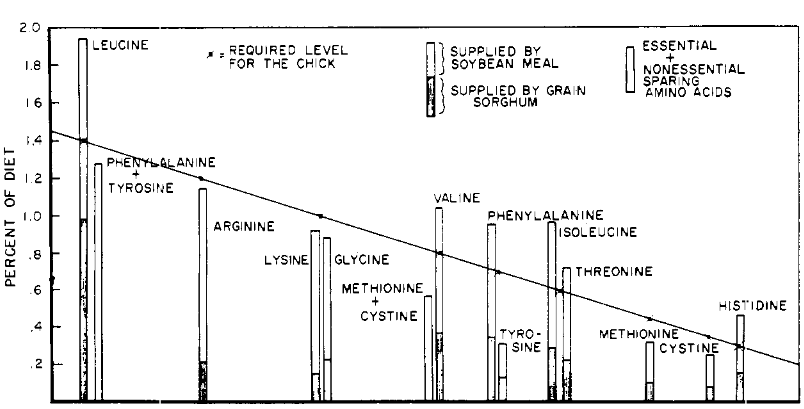

Figure 2. Ability of soybean meal plus grain sorghum to supply required levels of amino acids in 20% protein diet

samples assayed. Analysis of data (Table IV) for amino acids as percentage of the protein shows highly significant ($P < 0.01$) hybrid effects, while the location effect is nonsignificant. The significant hybrid effect indicates that protein composition in grain sorghum varies between hybrids, which agrees with Doty's hypothesis (*5*) based on work with corn. The differences also could be due to changes in the amount of various protein fractions in grain sorghum.

These data indicate lower levels of lysine, histidine, arginine, threonine, valine, methionine, isoleucine, and phenylalanine than are now applied to sorghum grain (*15*).

Data showing differences between hybrids indicate that hybridization may alter amino acid composition of the protein.

Table VI gives amino acid content as per cent of sample for each different hybrid. The data show differences among hybrids and effect of location on amino acids. Similar data (Table VII) for amino acids expressed as percentage of crude protein indicate the effect on protein quality.

Effect of amino acid variation on the ability of sorghum grain to supply nutritional requirements is illustrated in Figures 1 and 2. Sorghum protein used alone is deficient in arginine, lysine, glycine, tyrosine, and methionine. When sorghum grain and soybean oil meal are combined to supply 20% crude protein, methionine is the first limiting amino acid.

Because of the preliminary nature of these results, additional studies to study the effect of various factors on protein content and quality are needed.

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TOXIC FACTORS IN BEANS

Growth Inhibition of Rats Fed Navy Bean Fractions

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Five fractions were isolated from raw navy beans and found to inhibit the growth of rats. Fraction 4 was the major growth-inhibiting fraction. Growth-inhibitory effect of fraction 3 and possibly of fractions 1 and 2 on rats could be attributed to trypsin inhibitor activity. The possibility of the presence of a toxic factor other than hemagglutinin and/or trypsin inhibitor in navy beans is discussed.

Everson and Heckert (*3*) reported that raw navy beans were deleterious to rats when fed at a 10% protein level and that autoclaving the beans destroyed the toxic effect. This would imply the presence of heat-labile toxic factor(s) in raw navy beans. Recently Liener (*9*) reviewed the literature concerning the toxic factors present in edible legumes and indicated the importance of trypsin inhibitors and hemagglutinins as causes of the low nutritive value of legume seeds. Bowman (*2*) has shown the presence of a partially heat-labile trypsin inhibitor in navy beans and suggested that its presence may account for the poor nutritive value of raw navy beans. However, no attempt has so far been made to isolate the navy bean trypsin inhibitor and study its effect on the growth of animals. Rigas and Osgood (*13*) purified the hemagglutinin from navy beans and reported that it is nontoxic to animals. On the other hand, Honavar and coworkers (*5*) observed a definite growth inhibition of rats fed purified hemagglutinins from kidney beans and black beans. In the present investigation, different fractions were obtained from navy beans and feeding experiments were conducted to determine whether a particular fraction having either trypsin inhibitor activity or hemagglutinating activity has any effect on the growth of rats.

Experimental

Fractions were isolated from raw beans by a technique outlined by Honavar and coworkers (*5*) as shown in Figure 1. The isolation procedure was carried out in the cold at 4° C. unless otherwise mentioned. Nitrogen content of each fraction was determined by the micro-Kjeldahl method (*7*).

Trypsin inhibitor activity was determined by the casein digestion method of Kunitz (*8*) and hemagglutinating activity by the method of Liener (*10*).

Preparation of diet and details of rat-feeding experiments were described in a previous publication (*7*). Raw or auto-