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-

claved navy bean flour (protein content 24%) was used as the source of protein in diets to supply a level of 10% protein. The autoclaved beans were prepared by heating raw bean flour in the autoclave at 121° for 5 minutes. The isolated fractions were included in the autoclaved bean diet to determine their growth-inhibitory effect. The same fractions were added to the raw bean flour separately, and the mixture was then autoclaved to serve as an appropriate control.

Results and Discussion

Table I shows the hemagglutinating and trypsin inhibitor activities of various

Table I. Hemagglutinating and Trypsin Inhibitor Activities of Various Navy Bean Fractions

Fraction	Hemagglutinating Activity, HU[a]/Mg. Protein[b]	Trypsin Inhibitor Activity, TIU[c] 10^3/Mg. Protein[b]
1	5.3	195
2	3.6	113
3	0	858
4	24.1	143
5	35.5	122

[a] Hemagglutinin unit defined as least amount of hemagglutinin which will produce positive evidence of agglutination (1+) of 0.2 ml. of 4% suspension of washed chicken red blood cells after 1-hour incubation at 37° C.

[b] Determined according to method of Lowry and coworkers (*11*).

[c] Trypsin inhibitor unit expressed in terms of tryptic units inhibited, tryptic unit defined as increase of one unit of absorbance at 280 mμ per minute of digestion under experimental conditions.

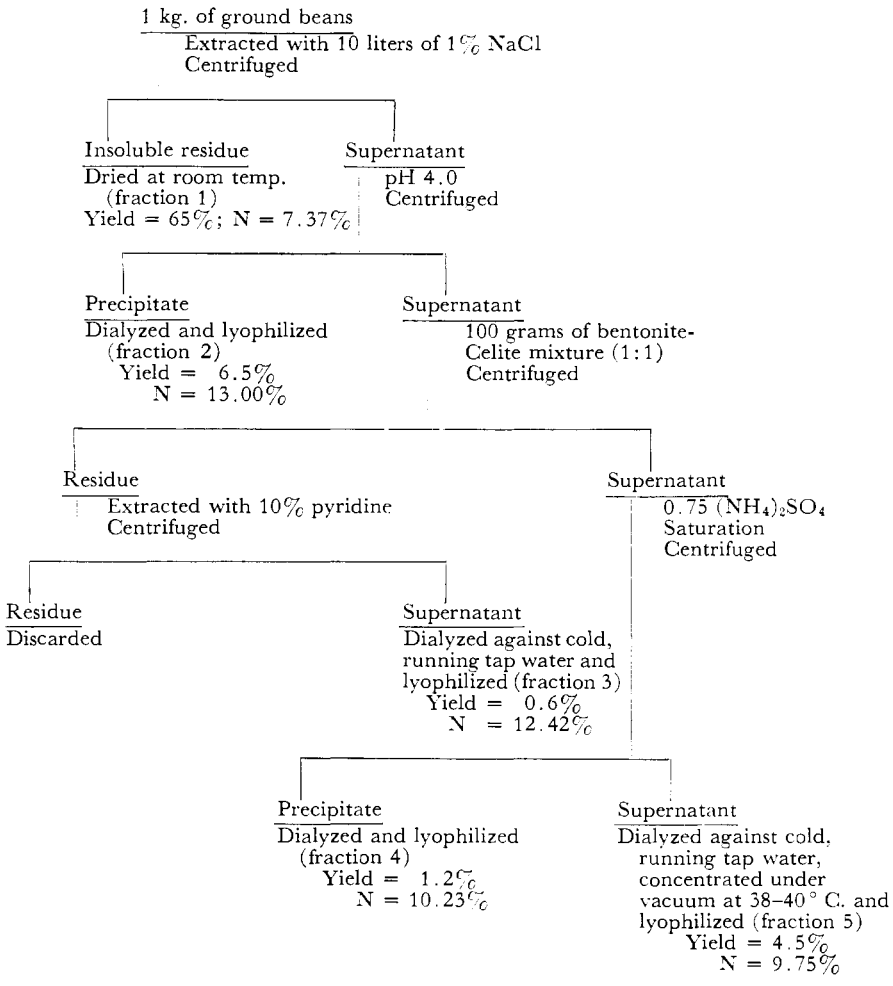

Figure 1. Preparation of navy bean fractions

Table II. Effect of Feeding Navy Bean Fractions[a] on Growth of Rats

Expt. No.	Protein Source (Navy Beans)	Change in Wt.,[b] Grams	Food Intake,[b] Grams	Protein Efficiency Ratio[b] (PER)	Trypsin Inhibitor in Terms of TIU Consumed	Hemagglutinin in Terms of HU Consumed × 10^3
1A	Raw beans	−12.8	88	100% mortality	...	...
B	Autoclaved beans	33.0	220	1.50 ± 0.10	...	...
2A	Autoclaved beans + 30% fraction 1	15.7	125	0.53[c] ± 0.10	3364	92
B	(Raw beans + 30% fraction 1) autoclaved	61.0	258	0.99 ± 0.01	...	...
3A	Autoclaved beans + 5% fraction 2	35.0	240	1.02[c] ± 0.05	1094	35
B	(Raw beans + 5% fraction 2) autoclaved	56.3	266	1.49 ± 0.04	...	...
4A	Autoclaved beans + 1% fraction 3	13.0	184	0.66[c] ± 0.03	1198	0
B	(Raw beans + 1% fraction 3) autoclaved	43.0	247	1.62 ± 0.04	...	...
5A	Autoclaved beans + 1% fraction 4	−4.0	94	−0.40[c] ± 0.05	92	15
B	(Raw beans + 1% fraction 4) autoclaved	41.3	271	1.43 ± 0.01	...	...
6A	Autoclaved beans + 2.5% fraction 5	17.6	179	0.85[d] ± 0.14	264	95
B	(Raw beans + 2.5% fraction 5) autoclaved	30.6	196	1.35 ± 0.16	...	...

[a] Fractions added in excess of actual yield obtained (see Figure 1) to overcome losses during isolation and/or due to incomplete separation.
[b] Expressed as average of six rats over 4-week period; expt. 1A, 3-week period.
[c] Highly significant $P < 0.01$, compared to appropriate treatment.
[d] Significant $P < 0.05$, compared to appropriate treatment.
± Standard error of mean.
... Values not determined.

fractions isolated from raw navy beans. The highest hemagglutinating activity lies in fractions 4 and 5, while fraction 3 is devoid of hemagglutinating activity but contains the highest trypsin inhibitor activity. The low trypsin inhibitor activity in all other fractions and the low hemagglutinating activity in fractions 1 and 2 may be due to incomplete separation during the isolation procedure.

Effects on growth and protein efficiency ratios obtained by feeding the various navy bean fractions to rats are presented in Table II. Rats fed the raw navy bean diet lost weight, consumed less food, and ultimately died within the experimental period of 28 days (experiment 1A). On the other hand, rats fed the autoclaved bean diet gained weight and consumed more food (experiment 1B).

All the fractions when included in the autoclaved bean diet significantly inhibited the growth of rats, as judged by protein efficiency ratio values when compared with rats fed the same fraction autoclaved (experiments 2, 3, 4, 5, and 6, Table II), the major growth-inhibiting fraction being fraction 4. Although fraction 5 contained the highest hemagglutinating activity and was included in the diet in 2.5 times greater quantity than fraction 4, it did not inhibit the growth of rats as significantly (experiment 6; $P < 0.05$) as fraction 4 (experiment 5; $P < 0.01$). It seems probable that hemagglutinating activity is not an essential factor for growth depression in rats fed raw navy beans. It remains to be seen whether the hemagglutinating activity and the growth-inhibiting activity of fraction 4 are due to the same or different factors. The work of Jaffe (*6*) and that of Honavar and coworkers (*5*) indicate that growth inhibition of rats fed black beans or kidney beans is due to their hemagglutinin content. However, it appears that the nutritional significance of hemagglutinins should be established carefully, especially in the light of very recent work of Funatsu (*4*), who separated the toxic activity from the hemagglutinating activity of ricin.

It may be that there are two types of hemagglutinins—one toxic and the other nontoxic. Fraction 5 may be identical to the nontoxic hemagglutinin isolated by Rigas and Osgood (*13*).

Experiment 4 (Table II) indicates that the navy bean trypsin inhibitor has a deleterious effect on the growth of rats. Growth depression observed from the inclusion of fraction 1 (experiment 2) and fraction 2 (experiment 3) in the diet is very difficult to rationalize. It is possible that the residual trypsin inhibitor activity present in them may account for the observed growth inhibition of rats. Recent studies of Saxena and coworkers (*14*) on raw soybean meal indicated that the major growth-inhibiting factor for chicks resides in the water-insoluble residue devoid of trypsin inhibitor activity. It may be that the growth inhibiting factor present in fraction 1 (insoluble fraction) is similar to that of soybean meal. The results of Rackis and coworkers (*12*) also indicated that there is no direct correlation between trypsin inhibitor activity of different soybean fractions and their influence on growth inhibition or pancreatic hypertrophy of rats.

In the light of the above discussion, the results presented in Table II could be explained in a different way. For example, a simple calculation of the trypsin inhibitor and hemagglutinin intake by rats fed various fractions (Table II, experiments 2A, 3A, 4A, 5A, and 6A) indicates that rats fed fraction 1 consumed the greatest while those fed fraction 4 consumed the least amount of trypsin inhibitor and hemagglutinins, and yet fraction 4 was the most growth-inhibitory. Therefore, it can be suggested that neither the hemagglutinin nor the trypsin inhibitor is toxic, but a toxic material was scattered throughout all the navy bean fractions in differing amounts. Fraction 4 appears to contain the highest and fraction 5 the least amount of that toxic material. Work is in progress to isolate and characterize a toxic factor contained in fraction 4.

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FATTY ACID RECOVERY

Recovery of Alpha-Ketoglutaric Acid from Crude Fermentation Liquors

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KOEPSELL, Stodola, and Sharpe (*5*) have found that the fermentation of glucose by certain strains of *Pseudomonas fluorescens* yields significant amounts of α-ketoglutaric acid. The object of this investigation was the development of a method for recovering the acid from such fermentation processes. The fermentation liquors contain pyruvic and α-ketoglutaric acids together with calcium carbonate and small amounts of other inorganic salts, glucose, cells, and other by-products of the fermentation. For this study fermentation liquors were provided by the Northern Utilization Research and Development Division of the Agricultural Research Service, Peoria, Ill. The particulars relative to the fermentation employed in the preparation of the crude fermentation liquors are given in Table I. The