increase was responsible for the decreased renal function that finally became evident at 0.3% dietary adenine. Dietary adenine in *Azolla* probably did not influence weight gain in this experiment, but the high level of adenine may represent a possible limitation to the use of *Azolla* for some species of animals.

As there were no significant differences with respect to weight gain or food intake of rats given diets 5 and 6, it appears unlikely that *Azolla* contains a toxic substance inhibiting growth in the short term.

The high NDF and possibly adenine of *Azolla* limit its usefulness as a food for simple-stomached animals. However, the in vitro dry matter digestion coefficent for *Azolla* using the two-stage Tilley and Terry method (1963) was 77%, indicating that the cell walls are readily digested by ruminants. This digestion coefficient is considerably greater than that of alfalfa which averaged 55.6%. Therefore, it would appear that ruminants may be the logical species which could effectively utilize both the high protein and energy of *Azolla*.

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Methionine and Cystine Contents of Bean (*Phaseolus*) Seeds

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An improved microbiological procedure for determination of methionine and cystine in bean seeds was developed. Finely ground beans were hydrolyzed with 20% HCl by heating in the autoclave for 30 min at 121 °C. The hydrolysates were neutralized and assayed for methionine and cystine with *Leuconostoc mesenteroides*. Growth was measured by increased turbidity after 20 h incubation. Five hundred and forty-four bean samples were analyzed for crude protein (N × 6.25), methionine, and cystine. Methionine contents of air-dried whole seeds ranged between 0.16 and 0.33%, methionine plus cystine between 0.29 and 0.56%, and crude protein between 16.1 and 33.9%. Calculated on a basis of 16% nitrogen, methionine in bean proteins was between 0.51 and 1.24%, and methionine plus cystine was between 1.17 and 2.49%. Correlation coefficients between protein content of beans and methionine content of bean protein and between protein content of beans and methionine plus cystine content of bean protein were calculated for 198 samples of *Phaseolus vulgaris* from around the world. A correlation coefficient of −0.439 between protein and methionine was obtained, which is significant at the 1% level. A correlation of −0.531 between protein and methionine plus cystine was obtained.

Legume seeds are used extensively in the poorer nations of the world as a source of dietary protein because of a shortage of animal proteins and because legume seeds are a relatively rich source of protein compared to cereal grains. For example, dry edible beans (*Phaseolus vulgaris*) are used extensively in Mexico and in Central and South America. Legume seeds, however, are deficient in the sulfur-containing amino acids, methionine and cystine (Evans and Bandemer, 1967).

The objectives of the present investigation were to develop a rapid method for determination of methionine and cystine in small quantities of bean seeds and to analyze a broad range of bean seeds to identify any lines which

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might contain high levels of methionine and cystine and, thus, be useful in developing bean lines containing high levels of methionine and cystine along with other desirable characteristics.

EXPERIMENTAL SECTION

Beans were finely ground in a micro-mill. The microbiological assay for methionine and cystine was carried out as described by Evans et al. (1974). However, in this earlier publication, no data were given for the development and reliability of the method.

Microbiological assay for methionine with *Streptococcus zymogenes* was carried out as described by Kelly et al. (1971).

Methionine was also determined on the Technicon Amino Acid Analyzer by the single-column procedure of Piez and Morris (1960), and cystine was determined by chromatography after oxidation to cysteic acid and hydrolysis of the seeds (Bandemer and Evans, 1960). The colorimetric procedure of McCarthy and Sullivan (1941) as modified by Lunder (1973) was also tried for determination of methionine.

Nitrogen was determined by the micro-Kjeldahl procedure, digesting with H_2SO_4 containing $CuSO_4$ and K_2SO_4 and with H_2O_2, and crude protein was calculated as N × 6.25.

Bean seeds were obtained from many sources, and 544 samples were analyzed for methionine and cystine by the rapid microbiological procedures and for protein by the micro-Kjeldahl procedure. Seeds were obtained from commercial sources, from the Plant Introduction (PI) Collection of the United States Department of Agriculture, from South America and Mexico, and from plant breeding experiments by the Department of Crop and Soil Sciences at Michigan State University.

Correlation coefficients were calculated as described by Snedecor (1959).

RESULTS AND DISCUSSION

Microbiological Assay Procedure for the Determination of Methionine and Cystine in Bean Seeds. Cystine is partially destroyed by the long hydrolysis procedures required to free all amino acids from proteins and foods when carbohyrates are present (Lugg, 1933). Horn and Blum (1956) observed that refluxing for 2 h with 20% hydrochloric acid liberated cystine in a form available for *Leuconostoc mesenteroides* P-60 with minimum destruction. Heating in the autoclave at 121 °C with 20% hydrochloric acid hydrolyzes proteins more rapidly than reluxing does and gives comparable results (Schweigert et al., 1944; Riesen et al., 1946). One-gram samples of finely ground beans were hydrolyzed by heating in the autoclave with 20-mL portions of 20% hydrochloric acid for 15, 30, or 45 min, or for 1, 2, 4, 6, or 8 h. The hydrolysates were assayed for both cystine and methionine with *L. mesenteroides*. The results of the assays are presented in Table I. Maximum cystine and methionine values were obtained with beans that had been hydrolyzed for 30 min. Cystine values were lower for longer hydrolysis times. The values agreed reasonably well with values obtained by column chromatography for methionine and by the cysteic acid procedure for cystine. Methionine values on other samples were similar to values obtained in the samples using the *S. zymogenes* assay procedure. There was too little methionine in the beans to use the colorimetric procedure, because the bean hydrolysate contained an interfering color.

Methionine and Cystine Content of Bean Seeds. The 544 bean samples ranged in methionine content between 0.16 and 0.33% (average 0.23%), in methionine plus cystine content between 0.29 and 0.56% (average 0.37%), in crude protein content between 16.1 and 33.9%, in methionine in the bean proteins between 0.51 and 1.24% (average 0.94%), and in methionine plus cystine in the bean proteins between 1.17 and 2.49% (average 1.54%). Some selected data are presented, and the tables contain data for crude protein in whole beans, for methionine in whole beans, for methionine plus cystine in whole beans, for methionine in bean protein (N × 6.25), and for methionine plus cystine in bean protein (N × 6.25). Complete data are presented in the Supplementary Material (see Supplementary Material Available paragraph at the end of the article).

The methionine content of the whole bean is important because methionine is the first limiting essential amino acid of bean proteins (Kakade and Evans, 1965). Beans having the highest level of methionine will furnish the most dietary methionine per unit amount of beans consumed. Table II lists five of the samples which contained the highest levels of methionine. Asgrow number 2511, a snap bean seed, contained 0.33% methionine and 0.11% cystine, which was the highest level of methionine in the bean seeds analyzed. It contained 1.16% methionine in the bean protein.

The cystine content of bean seeds is also important, because cystine can spare methionine, in that an animal requires both methionine and cystine, and can convert methionine to cystine, but cannot convert cystine to methionine. Table II lists the two legume seeds which contained the most methionine plus cystine. *Phaseolus atropurpureus* seeds contained the highest level of methionine plus cystine, 0.56% of the seed. Several samples of *Phaseolus vulgaris* seeds contained 0.47% of methionine plus cystine and one contained 0.49%.

The beans contained a wide range of crude protein in the seed. Ten samples contained over 30% crude protein (N × 6.25). These are listed in Table II. Only one of these, M.S.U. Seafarer 302-542-7-1975, contained over 0.30% methionine, and none contained over 0.49% methionine plus cystine. One would have expected, if the amino acid composition of the bean was fairly constant, that the high protein bean seeds would also have been those with high levels of methionine and cystine, but this was not always true. There were large differences in both methionine and methionine plus cystine contents of the bean proteins. Legume seeds which contained protein with levels of methionine of 1.16% or higher, and those whose protein contained 2.10% or more of methionine plus cystine, are presented in Table II. Only Asgrow No. 2511 contained both high methionine in the seed (0.13%) and in the seed protein (1.16%). A Tepary (*Phaseolus acutifolius*) seed

Table I. Influence of Hydrolysis Time on Methionine and Cystine Liberated from Bean Seeds in a Form Available for *Leuconostoc mesenteroides*[a]

Hydrolysis time	Cystine, %	Methionine, %
15 min	0.23	0.19
30 min	0.25	0.21
45 min	0.24	0.20
1 h	0.23	0.20
2 h	0.20	0.20
4 h	0.17	0.20
6 h	0.14	0.20
8 h	0.14	0.18
Chromatography[b]	0.24	0.20

[a] Hydrolysis was with 20% hydrochloric acid at 121 °C. [b] After oxidation of methionine to methionine sulfone and cystine to cysteic acid.

Table II. Methionine, Methionine plus Cystine, and Protein Contents of Bean Seeds

Sample name	In seed: Protein, %	In seed: Methionine, %	In seed: Methionine plus cystine, %	In seed protein: Methionine, %	In seed protein: Methionine plus cystine, %
	High in Methionine Content of Seed				
Asgrow No. 2511	28.7	0.33	0.44	1.16	1.54
Asgrow No. 2514	28.9	0.31	0.42	1.06	1.46
MSU Seafarer-302-542-7-1975	30.8	0.31	0.45	1.00	1.42
P. vulgaris 175-869-Turkey	24.7	0.30	0.43	1.01	1.52
P. vulgaris 180-739-Germany	26.6	0.30	0.45	0.94	1.50
	High in Methionine plus Cystine Content of Seed				
P. atropurpureus	24.7	0.26	0.56	1.03	2.26
P. vulgaris 169-764-Turkey	24.7	0.26	0.49	1.06	1.96
	High in Protein Content				
Rojo Chirripo	33.9	0.27	0.41	0.81	1.21
33-13	33.7	0.27	0.40	0.80	1.20
MSU-169-764-HP-1971	32.4	0.26	0.46	0.79	1.43
Ahumado Chirripo	32.0	0.29	0.44	0.90	1.38
MSU-136-691-HP-1969-1	31.5	0.28	0.42	0.90	1.34
S-64-P	31.4	0.28	0.42	0.88	1.48
Chimbolo Rojo	31.2	0.27	0.41	0.85	1.33
Carnita-1	31.2	0.27	0.40	0.85	1.27
Mexico-80-R	31.1	0.26	0.37	0.82	1.17
MSU Seafarer-302-542-7-1975	30.8	0.31	0.45	1.00	1.42
	High in Methionine Content of Seed Protein				
P. acutifolius Tepary-D	16.1	0.20	0.40	1.24	2.49
P. coccineus Scarlet Runner	18.7	0.23	0.43	1.23	2.29
P. vulgaris Asgrow No. 2511	28.7	0.33	0.44	1.16	1.54
	High in Methionine plus Cystine Content of Seed Protein				
P. acutifolius Tepary D	16.1	0.20	0.40	1.24	2.49
P. coccineus Scarlet Runner	18.7	0.23	0.43	1.24	2.49
P. atropurpureus	24.7	0.26	0.56	1.03	2.26
P. vulgaris Mercado de Puntarenas	19.6	0.20	0.41	1.00	2.10

sample and a Scarlet Runner (*Phaseolus coccineus*) seed sample contained proteins with methionine contents of 1.23–1.24%. Only *Phaseolus atropurpureus* contained over 0.49% methionine plus cystine in the seed and 1.92% or more methionine plus cystine in the seed protein.

Not all of the seed samples analyzed were of the common bean, *Phaseolus vulgaris*. Of the non-*Phaseolus* legume seeds studied, the cowpea, *Vigna unguiculata*, contained the most methionine and cystine, both on a seed (0.43%) and on a seed protein (1.72%) basis, and the dry pea seed, *Pisium sativum*, contained the least (0.30 and 1.09%). Three species of *Paseolus* other than *Phaseolus vulgaris* and 13 different varieties of *Phaseolus vulgaris* were analyzed. The results of this analysis are available in the Supplementary Material. The kidney and Asgrow beans were high in crude protein contant (over 28%), and the Asgrow beans were high in methionine (0.32%) and in methionine plus cystine (0.43%). Samples of navy bean strains were analyzed, but no conclusions can be made because of the small number of samples involved; however, the Monroe beans appeared to be superior to the other strains of navy beans with regard to methionine (0.27 and 1.11%) and methionine plus cystine (0.43 and 1.73%) both in the seed and in the seed protein.

Seeds from 198 *Phaseolus vulgaris* samples obtained from a number of different countries were analyzed (see Supplementary Material). Only one sample of seeds was obtained from most countries, although two or three were obtained from several countries. Four samples were obtained from Germany, seven from Mexico, 24 from India, and 131 from Turkey. There were differences in composition of the beans from the various countries, but no conclusions can be made because of the small number of samples from most countries and the variability in the samples from each country. The single sample from New Hampshire contained the most protein (28.4%) and the one from Colombia the most methionine (0.29%), and these same two samples contained the most methionine plus cystine (0.47%). The single seed sample from Colombia contained the most methionine (1.14%) and methionine plus cystine in the bean proteins (1.83%). Over half of the *Phaseolus vulgaris* seeds came from Turkey, and the average values for these beans were close to those for the entire 198 seed samples. Two bean seed samples from Turkey contained more protein (28.8 and 30.7%) than the one from New Hampshire (28.4%), one contained more methionine in the seed (0.30%) than the sample from Colombia (0.29%), one contained as much and one more methionine plus cystine in the seed (0.47 and 0.49%) as the Colombian sample (0.47%), and one contained the same amount of methionine (1.14%) and more methionine plus cystine (2.01%) in the protein than did the Colombian sample (1.14% methionine and 1.83% methionine plus cystine). Several of the Turkish bean seeds contained more than 1.83% methionine plus cystine in the protein.

Individual bean samples from Turkey also contained less protein (18.9% with several below 20%), less methionine in the seed (0.19%), less methionine plus cystine in the seed (0.30%), less methionine in the protein (0.80%), and less methionine plus cystine in the protein (1.31%) than any of the average values given for other countries. The 24 *Phaseolus vulgaris* seed samples from India also varied in protein content between 19.6 and 28.8%, in methionine content between 0.17 and 0.27%, in methionine plus cystine content between 0.29 and 0.44%, in methionine content of the protein between 0.86 and 1.02%, and in methionine plus cystine content of the protein between 1.39 and 1.92%.

Jaffe and Brucher (1974) obtained a negative correlation between protein content and the content of cystine and methionine in the protein of 70 pure lines of beans which was significant at the 5% level. Adams (1975) reported a correlation of −0.84 between the protein content and the methionine in the protein of eight commercial bean types grown in the United States. The data for protein content and methionine content of the protein for the 131 *Phaseolus vulgaris* samples obtained from Turkey gave a correlation coefficient of −0.447, which is significant at the 1% level. Correlation coefficients of −0.439 between protein and methionine in the protein, and of −0.531 between protein and methionine plus cystine in the protein were obtained for the 198 samples of *Phaseolus vulgaris* from around the world. Thus, although there were some bean samples with high protein and high methionine or high methionine plus cystine in the protein, generally the methionine content of the protein was less for those bean seeds containing high protein content. Waterman et al. (1923) isolated two globulins from bean seed, an α- and a β-globulin. The less abundant α-globulin contained much more of the sulfur amino acids than did the more abundant β-globulin. This has been confirmed by Evans et al. (unpublished data). Increased protein content of beans is probably due to increases in the β-globulin.

Beans have been analyzed previously for methionine and/or cystine contents by colorimetric (Vigayaghaven and Srinivasan, 1953), microbiological assay (Bressani et al., 1961; Chang and McAnelly, 1961; Jaffe and Brucher, 1974), Amino Acid Analyzer (Bandemer and Evans, 1963, methionine; de Moraes and Angelucci, 1971; Hackler and Dickson, 1973), and oxidation of methionine to the sulfone and cystine to cysteic acid and separation by column chromatography (Bandemer and Evans, 1963, cystine; Baldi and Salamini, 1973) procedures. Values for methionine and for methionine plus cystine contents of *Phaseolus vulgaris* or the other beans in the present study agreed well with those obtained by the others mentioned using microbiological assay or colorimetric techniques. Methionine and methionine plus cystine values obtained with the Amino Acid Analyzer or by column chromatography after oxidation to methionine sulfone and cysteic acid also agreed well with the present study except for the data obtained with the Amino Acid Analyzer by de Moraes and Angelucci (1971), who reported methionine contents of Brazilian bean proteins ranging from 0.35 to 1.86% and methionine plus cystine contents ranging from 1.33 to 8.22%. None of the beans which were analyzed in the present study had such high levels of cystine. Evans et al. (1976) analyzed cowpeas for methionine by ion-exchange, colorimetric, and microbiological (*Streptococcus zymogenes*) techniques. Their values of 1.18% by ion-exchange, 1.08% by colorimetric, and 1.22% by microbiological procedures compare with 1.15% methionine in cowpea protein reported in the present work.

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Supplementary Material Available: Data for the methionine, methionine plus cystine, and protein content of bean seeds (32 pages). Ordering information is given on any current masthead page.

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