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Effect of Methionine, Vitamin B_{12} and Antibiotics Supplementation on Protein Nutritive Value of Navy Beans.* (29067)

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The effectiveness of methionine, Vitamin B_{12} and antibiotics supplementation in improving the nutritive value of soybean protein has been reported by a number of investigators(1,2,3,4,5). Borchers(6) observed an apparent increased requirement for methionine in rats fed raw soybean meal. Barnes *et al*(7) suggested that the growth inhibitor in raw soybean specifically interferes with tissue utilization of methionine. This would explain why supplementary methionine is necessary for proper utilization of raw soybean protein. The role of Vit. B_{12} may be attributed to its methionine sparing action as suggested by Liener and Schultze(8). However, the mode of action of antibiotics in overcoming the growth depression of rats fed raw soybean meal is not known. Since raw navy beans fed to rats have a low protein quality, similar to raw soybean, this study was undertaken to determine the effectiveness of methionine, Vit. B_{12} and antibiotics supplementation in overcoming growth inhibition of rats fed raw navy beans(9).

Experimental. Navy beans of the Sanilac variety† were ground into fine flour. Heated samples were prepared by autoclaving the finely ground beans in shallow pans at a thickness not exceeding 1.0 cm at 121°C for 5 minutes after this temperature was reached. After autoclaving the samples were placed before a fan and allowed to dry at room temperature.

Preparation of diet. The percentage composition of the basal diet was as follows: sucrose, 30; corn oil, 6; Hegsted salt mixture,‡ 4; vitamin diet fortification mixture,§ 2. Raw or autoclaved navy beans or vitamin free casein‡ was incorporated into the basal diet to provide a level of 10% protein (N × 6.25). The chemical composition of navy beans is presented in Table I. The essential amino acid values for navy beans are given elsewhere(10). Corn starch‡ was added to make the total to 100.

TABLE I. Chemical Composition of Navy Beans.*

(expressed in g per 100 g of beans)	
Moisture	8.96
Ash	3.71
Crude fiber	4.18
Carbohydrate	58.07
Protein (N × 6.25)	24.00
Fat (ether extractable)	1.02
Calcium	.15
Phosphorus	.46
Iron	.007

* Determined according to standard procedures recommended by A.O.A.C.(21).

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† Courtesy of Dr. M. Wayne Adams, Farm Crops Dept., Mich. State Univ.

‡ Obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

§ Purchased from Nutritional Biochemicals Corp., Cleveland,Ohio. Supplies the following vitamins in mg/100 g of diet: Vit. A concentrate (200,000 units per g), 9.0; vit. D concentrate (400,000 units per g), 0.5; alpha tocopherol, 10.0; ascorbic acid, 90.0; inositol, 10.0; choline Cl, 150.0; menadione, 4.5; P-aminobenzoic acid, 10.0; niacin, 9.0; riboflavin, 2.0; pyridoxine HCl, 2.0; thiamine HCl, 2.0; Ca pantothenate, 6.0; biotin, 0.04; folic acid 0.18; vit, B_{12}, .0027.

TABLE II. Effect of Supplementing Raw Navy Beans with Methionine, Vitamin B_{12} and Antibiotics on Growth of Rats.

Group No.	Protein source	Total change in wt*	Total food intake*	Protein efficiency ratio* (PER)	±	SE†
		(g)	(g)			
1	Raw beans	−14.4	98	100% mortality		
2	Raw beans plus methionine	−14.8	102	50% "		
3	Raw beans plus vit B_{12}	−13.0	116	33% "		
4	Raw beans plus methionine plus vit B_{12}	− 6.5	135	−.53	±	.17
5	Raw beans plus antibiotics	.3	129	.03	±	.10
6	Raw beans plus vit B_{12} plus antibiotics	1.0	124	.09	±	.10
7	Raw beans plus methionine plus antibiotics	5.2	130	.44	±	.14
8	Raw beans plus methionine plus vit B_{12} plus antibiotics	14.0	131	1.07	±	.13

* Expressed as average of 6 rats. † Standard error of mean.

Protein quality was evaluated using weanling albino rats, 21 to 24 days old. Rats were divided into groups of 6 animals, each housed in an individual wire-bottom cage, and each group was equalized as nearly as possible with respect to sex and weight. Food and water were supplied *ad libitum*. Rats were weighed weekly and accurate records were kept of food consumption over the 4-week experimental period. Protein efficiency ratio (PER) was calculated as change in body weight in grams per gram of protein consumed.

All supplements were added at the expense of starch at the following levels:

Methionine: 0.6% DL-methionine

Vitamin B_{12}: 50 mg of 0.1% triturated Vitamin B_{12} per kilo of diet

Antibiotics: 0.1% procaine penicillin + 0.1% streptomycin sulfate.

Results and discussion. Table II shows the effect of supplementing raw navy beans with methionine, Vit. B_{12}, or antibiotics on the growth of rats. Rats fed raw navy beans lost weight and died within 20-26 days. They were emaciated, debilitated, had rough hair coats and were wet around the genitalia. Post mortem studies of rats used in these experiments will be reported later. Supplementation with methionine and/or Vit. B_{12} failed to correct the growth depression, but did reduce mortality (Table II).

Although the basal diet contained Vit. B_{12}, it appears that this level was not sufficient to prevent the deleterious effects of the raw bean diet. The supplementation of additional Vit. B_{12} to the raw bean diet, however, reduced mortality (Table II). The importance of supplementing Vit. B_{12} at higher levels in improving the nutritive value of plant proteins has been shown by various workers(3,11). Supplementation with antibiotics prevented growth depression and mortality and when fed in combinations with methionine and Vit. B_{12} promoted limited growth. In all these experiments food intake was considerably reduced.

Results of feeding autoclaved beans supplemented with methionine, Vit. B_{12} and antibiotics are presented in Table III. Rats fed autoclaved beans gained weight although not at a rate comparable to those fed casein. Rats fed the autoclaved bean diet supplemented with methionine grew as well as those fed casein. The PER value obtained with autoclaved beans supplemented with Vit. B_{12} was not significantly different from that with autoclaved beans alone; however, the PER values for autoclaved beans supplemented with antibiotics were significantly higher than that of autoclaved beans. There was no significant difference between the PER values for autoclaved beans plus methionine and autoclaved beans plus methionine, Vit. B_{12} and/or antibiotics. It is unlikely therefore that Vit. B_{12} or antibiotics had any sparing effect on methionine in this investigation. The slight beneficial effect of antibiotics sup-

TABLE III. Effect of Supplementing Autoclaved Beans with Methionine, Vitamin B_{12} and Antibiotics on Growth of Rats.

Group No.	Protein source	Total change in wt*	Total food intake*	Protein efficiency ratio* (PER)	±	SE†
		(g)	(g)			
1	Casein	127.0	360	3.52	±	.07
2	Autoclaved beans	60.0	320	1.88	±	.04
3	Autoclaved beans plus methionine	120.0	350	3.43	±	.07
4	Autoclaved beans plus vit B_{12}	68.0	330	2.06	±	.08
5	Autoclaved beans plus antibiotics	71.0	330	2.13	±	.08
6	Autoclaved beans plus vit B_{12} plus antibiotics	74.0	320	2.32	±	.08
7	Autoclaved beans plus methionine plus vit B_{12}	121.0	354	3.39	±	.18
8	Autoclaved beans plus methionine plus antibiotics	136.0	360	3.78	±	.13
9	Autoclaved beans plus methionine plus vit B_{12} plus antibiotics	134.0	360	3.70	±	.13

* Expressed as average of 6 rats.

† Standard error of mean.

plementation on PER over that of unsupplemented autoclaved bean diet might be caused by an effect on digestibility or absorption of the nutrients as a result of its action on intestinal microflora(5) or on intestinal cell walls(12).

In the light of above results an explanation for the beneficial effect of antibiotic supplementation in overcoming the growth depression of rats fed raw navy beans remains to be determined. Several investigators have reported the presence of hemagglutinin in navy beans(13,14). Honavar *et al*(15) observed growth inhibition of rats fed a purified hemagglutinin fraction isolated from *Phaseolus vulgaris*. It has been suggested (16) that the action of hemagglutinin might be to combine with the intestinal cell wall thus interfering with absorption of nutrients. If this is the case the role of antibiotics may be by action on the intestinal cell linings, thereby increasing the absorption of nutrients. However, the above explanation does not hold true for soybean meal as no significant difference in digestibility was observed between raw and heated soybean meal(17, 18). Therefore other possibilities still exist for explaining the mode of action of antibiotics. Borchers(19) reported that the soybean growth inhibitor exists in a "bound" form and suggested the possibility that an enzyme or microorganisms associated with the raw soybean meal liberates the "bound" form of inhibitor. It is possible that the role of antibiotics is to inhibit the enzyme responsible for the liberation of "bound" growth inhibitor. The inhibition of enzyme systems by antibiotics is not uncommon in the literature. Hartsook *et al*(20) reported the inhibition of kidney xanthine dehydrogenase in the chick and suggested that antibiotics cause increased nitrogen retention.

Summary. Supplementation with methionine and/or Vit. B_{12} failed to correct growth depression but reduced mortality in rats fed raw navy beans. Antibiotics supplementation prevented weight losses and in combination with methionine and Vit. B_{12} promoted limited growth. Rats fed autoclaved beans supplemented with methionine grew as well as rats fed casein. Protein efficiency ratio (PER) values of autoclaved beans supplemented with Vit. B_{12} and/or antibiotics were significantly lower than of those beans supplemented with methionine. Antibiotics may act to overcome growth depression by increasing the digestibility or absorption of nutrients and it is suggested that antibiotics may also act by inhibiting an enzyme involved in liberating a "bound" growth inhibitor from raw beans.

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Raw Soybean Feeding Decreases Transamidinase Activity.* (29068)

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A number of assay systems have been investigated in our laboratory in rats fed a raw soybean ration, a dietary regime which reduces the growth rate, with the hope that a more rapid assay for the "growth inhibitor" might be found. These systems have ranged from endogenous oxygen uptake through transaminases and liver protein regeneration. None of these revealed a difference between rats fed raw and heated soybean meal rations.

Walker(1) reported that creatine feeding depressed kidney transamidinase activity in rats. His data indicated that recovery of normal activity required several days after return to the stock ration. This suggested the hypothesis that a slower regeneration rate of transamidinase activity might occur on a raw soybean ration than on heated soybean. Instead of investigating the initial hypothesis, a comparison of kidney transamidinase activity was made on rats fed heated and raw soybean meal rations, without creatine pre-feeding, with the following results.

Methods and results. Weanling rats of the Holtzman strain were fed rations prepared as previously(2). Kidney transamidinase activity was represented by hydroxyguanidine formation from arginine and hydroxylamine according to Walker(1). Feeding of raw soybean meal resulted in a decrease of kidney transamidinase activity to less than 70% of that on the heated soybean meal in 4 days. The results are presented in Table I. Data were obtained on 2 additional soybean meal samples after a 4-day feeding period to 4 animals. The values were as follows from the heated and raw meal rations, respectively, on sample 2: transamidinase, 0.244 ± 0.014 and 0.182 ± 0.013 μmoles; gain, 15 ± 2 and 14 ± 3 g/4 days; and food consumption, 28 ± 0.3 and 30 ± 1.0 g/4 days and on sample 3: transamidinase, 0.224 ± 0.019 and 0.148 ± 0.002 μmole; gain, 15 ±

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