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The Effect of Soybean Growth Inhibitors on the Availability of Methionine for Growth and Lipotropism [1]

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INTRODUCTION

The presence of trypsin inhibitors in raw soybeans (4,5,8,14) and the deleterious effect of concentrates of the antitryptic factors on the growth of experimental animals (3,9,13) have been adequately demonstrated, and inactivation of these inhibitors by proper heat treatment has been associated with improvement in the nutritive value of soybean protein (23). It has been suggested (18) that the inhibitor exerts its growth-depressing effect by retarding the enzymic release of methionine in the gastrointestinal tract, thus making this amino acid unavailable for simultaneous utilization with the other essential amino acids. Evidence has been recently presented (6,12,22), however, which indicates that an inhibition of proteolytic hydrolysis cannot be the major causative mechanism. The present report gives the results of an investigation designed to determine more about the nature of this effect, especially in relation to the well-known effectiveness of methionine as a supplement to unheated soybean meal (1,10). The extent to which the trypsin inhibitor interferes with the availability or utilization of methionine as measured by growth (weanling rats) and by the lipotropic action of methionine in low-choline diets has been determined.

EXPERIMENTAL

The approach to the present investigation was based on a recent study by Treadwell (20) wherein a differentiation between the growth and lipotropic requirements

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for methionine in the absence of choline is reported. For optimum growth Treadwell found 1000 mg. of methionine/100 g. of diet to be necessary when the protein level was 18.6%, whereas the total methionine requirement for growth and lipotropism at that protein level was found to be between 1300 and 1500 mg./100 g. diet. For the present study, modification of Treadwell's diet was necessitated in order to incorporate sufficient raw soybean meal to demonstrate growth inhibition, and, at the same time, to minimize the soybean meal's contribution of choline to the basal diet. As a compromise between those two considerations, raw[2] or autoclaved[3] soybean meal (approximately 50% protein) at a level of 10%, and vitamin-free casein at a level of 7.5% were provided as the sources of dietary protein. For the present experiment, a protein level of 12.5% was used because it was believed that this would give the most sensitive measure of protein efficiency (2). In addition to protein, the basal diet contained, in per cent: lard, 40; salt, U.S.P. 2, 5.0; cystine, 0.1; and, as mg.-%: thiamine, 0.25; riboflavin, 0.50; niacin, 2.0; pyridoxine, 0.25; calcium pantothenate, 2.0; inositol, 10.0; *p*-aminobenzoic acid, 5.0; biotin, 0.01; folic acid, 0.10; 2-methyl-1,4-naphthoquinone, 0.10; and sufficient sucrose to make up to 97.5%. Adequate amounts of vitamins A, D, and E were administered twice weekly as a corn oil mixture of α-tocopherol and Haliver oil.

TABLE I

Growth and Lipotropism as Affected by Methionine in the Presence of Soybean Growth Inhibitors

Total methionine in diet[a] (mg./100 g.)	Gain in weight[b] (g./g. protein consumed)		Liver lipides[b] (g./100 g. dry wt.)	
	Raw soybean	Autoclaved soybean	Raw soybean	Autoclaved soybean
600	2.79±.04	3.76±.12	39.2±3.6	41.2±4.8
1000	2.64±.21	3.47±.17	19.9±1.3	20.4±1.3
1500	2.84±.14	3.43±.14	16.2±1.1	19.6±1.4
1500[c]		2.68±.16		14.3±1.0
2500	1.74±.18	2.54±.13	12.9±0.7	11.8±0.6

[a] Includes "effective" methionine content of basal, unsupplemented diet (see text).
[b] Average of each group (7 animals/group) ± the standard error of the mean.
[c] Plus 1.8% trypsin inhibitor preparation.

Calculation of the amount of supplemental methionine to be added was based on the methionine and choline values of the basal diet, which were found to be 257 and 31 mg./100 g., respectively, by the methods of Lyman, *et al.* (17), and of Engel (7). Using Treadwell's data (20), it was estimated that the choline requirement of 200 mg. could be met by about 600 mg. of methionine; hence, the total "effective" methionine content of the basal diet was considered to approximate 350 mg./100 g. The calculated quantities of DL-methionine necessary to provide final levels of 600, 1000, 1500, and 2500 mg./100 g. of the raw or autoclaved soybean diets were adjusted to 2.5 g. with

[2] Nutrisoy XXX, Archer-Daniels-Midland Co., Minneapolis, Minnesota.
[3] Autoclaved for 20 min. at 15 lb. pressure.

sucrose and added to 97.5 g. of the basal diet. An additional diet composed of autoclaved soybean meal plus 1500 mg. methionine/100 g. diet was further supplemented with 1.8% trypsin inhibitor concentrate prepared according to the procedure of Klose *et al.* (13) without an equivalent reduction in protein or sucrose. There were, therefore, nine diets in all (see Table I).

Nine groups of weanling male rats (Sprague-Dawley), consisting of 7 animals per group and having initial weights of 42–48 g., were fed the diets described above *ad lib.* for a period of 18 days. All animals were individually caged so that individual records of food consumption could be obtained. At the end of the experimental period, the animals were anesthetized, and the livers excised and weighed on previously tared aluminum foil dishes. After thorough maceration, the moist tissues were dried to constant weight under vacuum at 70°C. The dishes containing the dried tissues were inserted directly into fat extraction thimbles, and extracted overnight with petroleum ether. After careful evaporation of the solvent on a steam bath, the receiving flasks were dried to constant weight.

Diets containing the autoclaved soybean meal consistently supported better growth[4] than the unheated meal even in the presence of excessive amounts of methionine (2500 mg.-%) to the point where growth was adversely affected. The addition of the trypsin inhibitor concentrate to the diet containing the heated meal also produced the expected inhibition of growth. Methionine in excess of 600 mg.-% produced no further improvement in growth of rats receiving raw or autoclaved soybean meal.

Liver lipides, however, decreased progressively as the level of methionine was increased. The most marked lipotropic effect occurred when the methionine was increased from 600–1000 mg.-%. The lipotropic effect of 1500 mg.-% methionine was not significantly greater than that of 1000, although increasing the methionine to 2500 mg.-% produced a more significant decrease in liver lipides. The consistent failure to obtain significant differences in liver lipides between diets containing raw and autoclaved soybean meal at any level of methionine was of special interest. Adding the trypsin inhibitor to the autoclaved soybean meal diet containing 1500 mg.-% to methionine not only failed to increase the liver lipides above that of a similar diet without added inhibitor, but actually reduced the lipide content to some extent.

Discussion

Comparison of the results obtained using raw and autoclaved soybean meal diets with respect to growth and lipotropism shows that

[4] Apparent differences were analyzed for significance by the t test (18), and only those t values with a P value of less than 0.01 were considered significant.

heating caused a substantial improvement in growth of protein efficiency without a concomitant effect on liver lipides. Similarly, the added trypsin inhibitor depressed the growth of rats receiving autoclaved soybean meal without affecting the availability of methionine for lipotropism. It may be concluded, therefore, that the growth-depressing effect of the trypsin inhibitor is not related to an interference with the amount of methionine absorbed from the gastrointestinal tract, a finding which is in complete agreement with the observation that the actual absorption of sulfur (11) or methionine (18) from the intestinal tract of rats fed raw or autoclaved soybean is the same.

Although groups of animals receiving the basal, unsupplemented diets were not included in this study, sufficient evidence is available from studies reported elsewhere (1,10) that the nutritive value of such diets is improved by supplementation with methionine. Thus, the following typical results (16) were obtained when diets containing 12.5% protein derived from raw or autoclaved soybean meal were supplemented with methionine. Inasmuch as Treadwell (20) has reported that methionine up to 1000 mg.-% produced a progressive increase in growth, the failure of methionine in excess of 600 mg.-% to effect further improvement in growth was somewhat unexpected. This difference in the growth-promoting effect of supplemental methionine is probably related to the higher level of protein in Treadwell's diet (18.6%) compared with that in this study (12.5%). As subsequently pointed out by Treadwell (21), the effectiveness of supplemental methionine may be limited by the availability of other amino acids when the protein intake is at a lower level.

Diet	Protein efficiency
Raw soybean meal	1.33
Raw soybean meal+0.6% methionine	2.42
Autoclaved soybean meal	2.62
Autoclaved soybean meal+0.6% methionine	3.01

The addition of the trypsin inhibitor concentrate to the autoclaved soybean diet caused a marked decrease in growth even when methionine was present in amounts far in excess of the level adequate to satisfy the need for methionine for growth at the level of protein fed. In an analogous manner, excessive amounts of methionine when added to diets containing raw soybean meal did not restore their nutritive value to the corresponding level of methionine-supplemented diets containing autoclaved soybean meal. This finding may be interpreted as verification of the results of *in vitro* digestion studies previously reported showing that a decreased rate of release of methionine cannot be the only factor involved (15) and that the soybean trypsin inhibitor exerts an effect through a mechanism unrelated to the availability or utilization of methionine. The improvement in the nutritive value of raw soybean effected by supplemental methione may be, to a certain extent, a reflection of the fact that even properly heated soy protein is charaterized by a deficiency of methionine (1). The mode of action of the trypsin inhibitor in relation to the effectiveness of methionine supplementation of raw soybean meal will be the subject of a future detailed report.

Summary and Conclusions

1. The presence of methionine at effective levels of 600, 1000, 1500, and 2500 mg.-% in a low-choline, 12.5% protein diet containing raw or autoclaved soybean meal caused a progressive decrease in liver lipides of weanling rats.

2. The levels of liver lipides were equal for the same level of methionine intake regardless of whether the soybean protein was raw or autoclaved.

3. Differences in protein efficiency were not parallel to the changes in liver lipides described above. The growth-promoting value of diets containing raw soybean meal was consistently less than similar diets containing autoclaved soybean meal. This difference persisted in spite of the presence of more than adequate amounts of methionine necessary for growth.

4. Addition of trypsin inhibitor concentrate to the autoclaved soybean diet containing 1500 mg.-% methionine caused a definite decrease in rate of growth which was not accompanied by any significant change in liver lipides.

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