Antibiotic Growth Stimulation of Rats Fed Raw Soybean Oil Meal

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The rate of gain of rats fed raw soybean oil meal was 80%, or less, of gains made by rats fed autoclaved meal. The addition of 0.1% procaine pencillin plus 0.1% streptomycin sulfate has resulted in approximately equal rates of gains by rats fed raw and autoclaved soybean oil meal. These results open a new point of attack on understanding the growth inhibitory effects of raw soybean oil meal and, possibly, on the problem of the growth stimulatory effects of antibiotics.

RATS FED RAW SOYBEAN OIL MEAL, as the protein source in an otherwise complete ration, grow at a slower rate than animals fed a similar ration containing autoclaved soybean oil meal. This effect was first noted by Osborne and Mendel (*24*) in 1917. The same effect on the growth rate of swine was reported by Hayward, Bohstedt, and Fargo in 1934 (*13*) and on chicks by Wilgus, Norris, and Heuser in 1936 (*32*).

Numerous explanations for this inhibitory effect of raw soybean oil meal on the growth rate have been proposed. Differences in digestibility between raw and autoclaved soybean oil meal cannot account for differences in the growth rate (*15, 20*). Almquist, Mecchi, Kratzer, and Grau (*1*) suggested that methionine was not readily available in raw soybean but became more available after autoclaving. Supplements of methionine did not correct the difference in growth rate (*5, 12, 14*). As shown particularly by Hayward and Hafner (*14*), the difference in growth rate was narrowed by the addition of methionine. The lack of effect of the addition of all the essential amino acids on the growth inhibition by raw soybean oil meal has been noted for the chick by Hill, Borchers, Ackerson, and Mussehl (*17*) and the failure of vitamin supplementation for the chick has been reported by Hill and Branion (*18*). A similar ineffectiveness of amino acid or vitamin supplementation has been observed with rats by Borchers (*3*). Interference with digestion by the trypsin inhibitor found in raw soybean by Read and Haas (*26*) was proposed as an explanation by Ham, Sandstedt, and Mussehl (*12*). However, growth was inhibited even though the animal was fed predigested protein (*21, 31*). Borchers, Ackerson, and Mussehl (*5*) fed a purified soybean trypsin inhibitor without affecting the growth rate of rats or chicks. Discussions of these and other explanations have been reviewed, especially by Liener (*22*), Griswold (*11*), and Borchers *et al.* (*6*).

Table I. Composition of Rations

Soybean oil meal (autoclaved or raw)[a], g.	25.0
DL-Methionine, g.	0.6
Salt mixture (*19*), g.	2.0
Starch, g.	51.8
Crisco, g.	20.0
Choline chloride, g.	0.5
Thiamine hydrochloride, mg.	0.5
Calcium pantothenate, mg.	1.25
Nicotinic acid, mg.	2.5
Pyridoxine hydrochloride, mg.	0.125
Riboflavin, mg.	0.5
Folic acid, mg.	0.1
Vitamin B_{12}, γ	2.0
Vitamin A, units	1000
Vitamin D, units	200

[a] The soybean oil meal was prepared by the hexane process with a minimum of heat treatment according to the manufacturer's statement. Autoclaved meal was prepared from this by autoclaving thin layers of the meal at 15-lb. steam pressure for 30 minutes. Protein content was 45.5% (N × 6.25).

A search is being made in this laboratory for a dietary supplement which might favorably affect the growth rate of rats fed raw soybean oil meal. Borchers and Ackerson (*4*) reported only crude trypsin powder was found to be effective, showing that the effect was not due to the trypsin content of the crude trypsin powder. Purification of the factor from crude trypsin powder will be described in a later publication. The lack of effect of yeast, liver, casein, and a crude preparation of streptomycin was also reported (*4*).

During feeding experiments, conducted by Borchers and Mohammad-Abadi (*7, 8*), in a study of the effects of antibiotics on the excretion of indican, it was observed in some experiments that antibiotics stimulated the growth of rats fed raw soybean oil meal. Since the observation that antibiotics increase the growth rates of farm animals (*9*), there have been several publications concerning the effect of antibiotics on the growth rate of rats. That antibiotics do not stimulate the growth of rats fed a complete ration (*23, 27*) seems to be generally accepted, although there are reports to the contrary (*2, 10, 16, 25, 29, 30*).

Hensley, Carroll, Wilcox, and Graham (*16*) reported that 0.02% of aureomycin stimulated the growth of rats fed raw soybean oil meal. However, their data reveal that the growth rate of rats fed the raw soybean, plus methionine and aureomycin, was 78.5% of the growth made on the similarly supplemented autoclaved soybean ration. This value is similar to the difference in growth rates observed in comparing raw and autoclaved soybean rations (supplemented with methionine).

Experimental procedures

Weanling rats (Sprague Dawley strain) of the same litter, sex, and initial weight (35 to 40 grams) were paired and housed in screen-bottomed cages. Experimental rations (Table I) and water were available *ad libitum*. Four females and four males were fed the autoclaved soybean oil meal ration, and a similar group was fed the raw soybean oil meal ration during 20-day feeding periods in each experiment.

The feeding studies compared the growth rate of the rats fed the raw soybean oil meal ration with those fed the autoclaved soybean ration (Table II). The autoclaved soybean oil meal ration was used as a control, rather than the unsupplemented raw soybean ration, for two reasons. The primary interest was to find a factor which would equalize the growth rate of rats fed autoclaved and raw soybean oil meal. The growth rate varied more, from experiment to experiment, on the raw soybean oil meal ration than on the autoclaved soybean ration.

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Table II. Growth of Rats Fed Autoclaved or Raw Soybean Oil Meal Plus Antibiotics

Antibiotic	%	Expt. No.	Gain on Soybean Rations[a] Autoclaved: Grams/day ± SE	Autoclaved: Gram/gram food	Raw: Grams/day ± SE	Raw: Gram/gram food	t Value[b]	%[c]
None		1	3.44 ± 0.14	0.43	2.45 ± 0.21	0.36	3.951[d]	71
Penicillin[e]	0.01	2	3.27 ± 0.21	0.41	2.41 ± 0.28	0.35	2.458[f]	74
		3	3.44 ± 0.09	0.43	3.13 ± 0.06	0.41	2.930[f]	91
	0.1	4	2.94 ± 0.15	0.37	2.21 ± 0.10	0.32	3.987[d]	75
		5	3.34 ± 0.09	0.43	2.88 ± 0.06	0.39	4.249[d]	86
Streptomycin[e]	0.01	6	3.46 ± 0.17	0.45	2.73 ± 0.22	0.35	2.628[f]	79
		7	3.28 ± 0.14	0.42	2.83 ± 0.14	0.38	2.314[f]	86
	0.03	8	3.16 ± 0.11	0.36	2.26 ± 0.13	0.30	5.293[d]	72
	0.1	9	3.05 ± 0.11	0.36	2.15 ± 0.12	0.31	5.595[d]	70
		10	2.96 ± 0.13	0.40	2.85 ± 0.26	0.43	0.380	96
		11	3.19 ± 0.10	0.42	2.96 ± 0.16	0.40	1.218	93
	0.3	12	3.22 ± 0.12	0.36	2.10 ± 0.10	0.28	7.156[d]	65
	0.4	13	3.35 ± 0.13	0.44	3.07 ± 0.21	0.42	1.010	92
Penicillin + streptomycin	0.01	14	3.35 ± 0.22	0.43	2.98 ± 0.26	0.40	1.093	89
		15	3.22 ± 0.19	0.44	2.93 ± 0.25	0.42	0.943	91
	0.1	16	3.49 ± 0.23	0.47	3.23 ± 0.25	0.43	0.756	93
		17	3.01 ± 0.13	0.40	3.20 ± 0.21	0.45	0.769	106
		18	3.55 ± 0.20	0.39	3.32 ± 0.33	0.41	0.603	94
		19	3.13 ± 0.19	0.44	3.11 ± 0.30	0.44	0.056	99

[a] Average daily gain of 4 female and 4 male weanling rats over a 20-day feeding period.
[b] *t* value according to Snedecor (*28*).
[c] % growth on raw ration over growth on autoclaved ration × 100.
[d] Significantly different at 1% level.
[e] Indicates amount of procaine penicillin or streptomycin sulfate, respectively.
[f] Significantly different at 5% level.

Results

The usual difference in growth rate between rats fed the autoclaved and raw soybean oil meal ration, 71%, was noted in experiment 1, Table II. This comparative value was consistently less than 80% when the rats were fed the rations listed in Table I.

Little or no growth stimulation was noted when antibiotics were fed at the 0.01% level; occasional effects were noted at the 0.1% level (*7, 8*). In a preliminary survey made of 16 antibiotics, each fed at the 0.1% level, penicillin and particularly streptomycin showed the most promise, especially when fed in combination. The present report covers only these two antibiotics, but it is not to be concluded that other antibiotics or other combinations are not equal to or more effective than penicillin and streptomycin.

In experiments 3 and 5, when penicillin (procaine) was fed, either at the 0.01 or 0.1% level, an effect on the growth rate of rats fed raw soybean meal was noted, but in experiments 2 and 4, no effect was noted (Table II). The addition of streptomycin (sulfate) at levels of 0.01 to 0.4% gave increased growth rates in four experiments and appeared to be ineffective in four others, as shown in experiments 6 to 13 of Table II. There was no correlation between effectiveness and the level at which streptomycin was fed. The addition of both penicillin and streptomycin, each at the 0.01 and 0.1% level, to the soybean rations gave an increased growth rate in each of six experiments (14 to 19, Table II). The 0.1% level of penicillin plus streptomycin was more effective than the 0.01% level of the combination.

Discussion

This study suggests that the action of intestinal bacteria may be involved in the problem of growth inhibition due to feeding raw soybean, as compared to the autoclaved soybean. However, as no satisfactory mechanism has been proposed for the growth stimulatory action of antibiotics, there may be other factors involved. Also a previous report from this laboratory (*7, 8*) indicated that intestinal bacterial putrefaction, as measured by indican excretion, was not involved in the growth difference between raw and autoclaved soybean.

When an antibiotic increases the growth rate, the ration must be nutritionally inadequate [Lih and Baumann (*23*)], suggesting that the raw soybean ration is lacking in some nutrient. Such a lack might be envisioned either as an unavailable nutrient in the raw soybean meal, such as has been suggested for methionine, or a lack which arises because of increased requirements on a raw soybean ration. If there is an increased requirement for some nutrient, the lack of effect of yeast, liver, casein, crystalline B-vitamins, or essential amino acids (*3, 4, 17, 18*), indicates either that this is a marked increase or that an unidentified factor is required. It is not clear if the factor postulated to be present in crude trypsin powder (*4*), which equalizes the growth rate on raw and autoclaved soybean rations, is such an unidentified factor.

The effect of antibiotics presents a new point of view in attacking the problem of the growth depressing effect of raw soybeans and involves the search for a satisfactory explanation of the effect of antibiotics on the growth rate. A more convenient experimental approach to this latter problem may be available in the raw soybean oil meal ration.

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PROTEIN SUPPLEMENTATION

Influence of Addition of Certain Amino Acids and Vitamin B_{12} to Proteins in Enriched Milled Wheat Flour on Growth, Protein Efficiency, and Liver Fat Deposition

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The influence of addition of certain amino acids and vitamin B_{12} to proteins in enriched milled wheat flour on growth, protein efficiency, and liver fat deposition was investigated. The proteins in milled wheat flour were fed to albino rats at an 8% level for 10 weeks. The optimum gains in body weight and protein efficiency were secured by supplementation with 0.4% L-lysine, 0.2% DL-threonine, 0.4% DL-methionine, and vitamin B_{12}.

THE BENEFICIAL EFFECTS of additions of several essential amino acids and vitamin B_{12} to the proteins in cereal grains have been demonstrated (*7–11, 13, 15*). This study shows the influence of additions of methionine, and increasing amounts of lysine, threonine, and valine—in the presence and absence of vitamin B_{12}—to the proteins in enriched milled wheat flour on growth, protein efficiency, and liver fat deposition. This investigation was stimulated by the recent reports of Harper and associates that certain combinations of amino acids may produce imbalance, toxicities, and antagonisms (*4*). Elvehjem has recently reviewed the findings of his Wisconsin group on the effects of amino acid imbalance on maintenance and growth (*2*).

Recently Deshpande and associates (*1*) found in 2-week experiments that the retardation in growth caused by including 0.4% of L-lysine hydrochloride in a rice diet was prevented by increasing the levels of leucine, isoleucine, valine, and histidine. In longer experiments the growth-retarding effect of additional lysine disappeared without the addition of other amino acids in the diet. Supplementation of the rice diet with various combinations of amino acids, from which one or more of leucine, isoleucine, valine, and histidine had been omitted, resulted in a retardation of growth. In all instances in which the ration contained at least 0.4% of additional L-lysine hydrochloride, the fat content of the liver approached a normal value. Even after 7 weeks an accumulation of liver fat occurred, unless the rice diet was supplemented with this level of lysine.

As the Food and Nutrition Board of the National Research Council is now investigating the matter of protein nutrition in the United States and the advisability of amino acid enrichment of cereal foods, it was thought particularly essential to study the possible injurious effects of increasing concentrations of lysine, threonine, and valine to the proteins in enriched milled wheat flour fed at a higher level of protein intake, 8%, than Deshpande and associates used in their rice diets fed at a lower plane of protein intake, 5.9% (*1*).

Experimental Procedure and Materials

This study was carried out, for 10 weeks, on the Wistar strain of albino rats, which were 30 days old when started on experiments and weighed 50 to 54 grams each. There were 12 animals in each group, equally divided between male and female. The rations contained 8% of proteins (supplied entirely by enriched wheat flour); 2% of Cellu flour for roughage; 4% of Sure's salts No. 1 (*14*); 7% of hydrogenated vegetable shortening; 2% of cod liver oil; 1% of wheat germ oil; and the rest, percentagewise, glucose (Cerelose). The fat-soluble vitamins A, D, and E were supplied by cod liver oil and wheat germ oil in the rations. All rations were supplemented with a liberal supply of the B vitamins, separately, six times weekly with double doses on Saturdays (*12*). The vitamin B_{12} was prepared daily in aqueous solutions and administered to each animal in Petri dishes in a daily dose of 0.1 γ per animal per day. The animals were weighed once weekly and accurate records were kept of food consumption. From these data the protein efficiency ratios were calculated, and expressed as gains in body weight per gram of protein intake.

The amino acids added some nitrogen to the rations and the food consumption was multiplied by 8, which gives the total protein intake, but does not express the total nitrogen intake. However, it has been demonstrated (*9, 10*) that such amino acid additions contribute so little nitrogen that when calculations are made on gains in body weight per gram of nitrogen intake the relative results obtained approximate those of gains per gram of protein intake; hence figures for total nitrogen intake have not been included.

The liver fats were covered with 95% ethyl alcohol, dried overnight at 53° C., and extracted with petroleum ether for