Effect of Raw Soybean Meal on Growth of the Chick. (19350)

H. J. ALMQUIST AND J. B. MERRITT.
From the Grange Company, Modesto, Calif.

We have shown(1,2) that in diets containing exclusively raw or cooked soybean meals as sources of protein, approximately 5% of raw soybean meal protein is associated with all or nearly all of the growth inhibition for the chick that may be observed with all-raw soybean protein in the diet. The manner in which the growth response changes with changing proportions of raw to cooked soybean meal in the diet may reveal much concerning the mechanism of the inhibiting effect.

Methods. The soybean meal diets were the same as used previously(1,2) except for the omission of the methionine. The remainder of the diets was composed of vitamins, minerals, and glucose. The diets were fed to New Hampshire chicks which were distributed into groups of 18 containing 9 of each sex. We shall for convenience refer to levels of soybean protein in discussing methods and results, although it is understood that the growth inhibitor is not necessarily an integral part of this protein.

Growth results. Results with the 20% protein diets are given in Table I, and plotted as the lower curve in Fig. 1. Practically all the growth inhibition was attained at the 5% raw protein level. There was only a slight further effect on growth at higher levels of raw protein. These data confirm our previous report(2).

Results with the 30% protein diet are given in Table II. In this case, also, approximately all of the growth inhibition was attained at the lower levels of raw protein. Since the protein increments were 5%, the point of inflection of the response curve is not as accurately established. All the chicks grew faster than the chicks at the 20% protein level.

TABLE I. Effect of Various Levels of Raw Soybean Meal on Growth of Chicks Fed a 20% Protein Diet.

Raw soybean meal protein in diet, %	Cooked soybean meal protein in diet, %	Avg wt of chicks at 14 days on diet, g
20	0	84
17.5	2.5	86
15	5	86
12.5	7.5	86
10	10	90
7.5	12.5	89
5	15	90
2.5	17.5	102
0	20	128

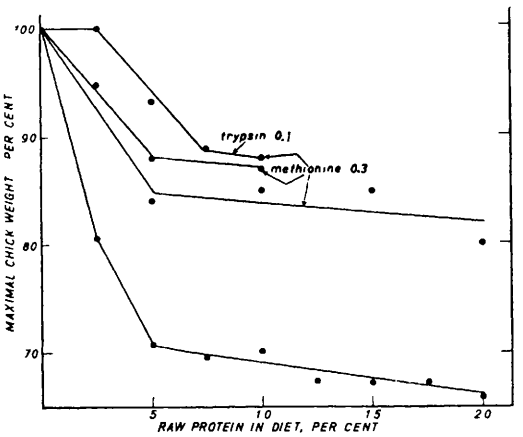

FIG. 1. Growth of chicks on mixtures of raw and cooked soybean meals which supply all the protein in a 20% protein diet. Supplements added as indicated.

TABLE II. Effect of Various Levels of Raw Soybean Meal on Growth of Chicks Fed a 30% Protein Diet.

Raw soybean meal protein in diet, %	Cooked soybean meal protein in diet, %	Avg wt of chicks at 16 days on diet, g
30	0	142
25	5	133
20	10	130
15	15	152
10	20	154
5	25	176
0	30	186

Discussion. If the principal growth inhibition associated with raw soybeans were caused by the reduced availability of some essential nutrient, such as methionine, the growth of chicks should drop progressively over the range from none to all-raw protein. Actually, the inhibition develops rather abruptly at first

and thereafter the growth decreases only slightly as the proportion of raw protein increases. A "toxicity" should become more acute as the threshold of tolerance for the toxic substance is approached, that is, the toxicity should be manifested more distinctly at the higher levels of raw meal. In such case the 30% protein diets would be even more toxic than the 20% protein diets. It is plain that these expectations are not fulfilled.

A mechanism in which a component of the proteolytic system in the chick is inactivated, while the remainder of the system is allowed to function at a reduced but fairly constant efficiency, is concordant with the fact that the growth inhibition is brought to approximately full effect when raw soybean meal supplies only one-fourth of the total protein. More specifically, such an effect would be expected from the antitrypsin of soybeans if this anti-enzyme had little or no influence on the proteolytic enzymes other than the trypsin secreted by the chick.

In Fig. 1, we have plotted the growth results from this study and our earlier studies(1,2) with 20% protein diets. In order to compare data obtained at various times and for slightly different experimental periods, the growth was expressed as a percentage of the maximal obtained in each experiment. Special supplements have been indicated on Fig. 1. The curves show that, as would be expected(4,5), there was a greater range of the inhibitor effect when the moderate methionine deficiency was not corrected (lower curve). The addition of trypsin (1-110) caused a displacement of the growth response curve to the right, after which the curve resumed the same pattern as its control curve. This indicates that the added trypsin preparation was able to neutralize a definite amount of the growth inhibitor.

The effect of trypsin added to raw soybean meal diets may be entirely masked if there is enough inhibitor in the diet to overcome the trypsin added plus that secreted by the animal. This fact is demonstrated further by our results with varying levels of trypsin (1-300) added to the 20% protein diet containing 5% raw protein. The data given in Table III show that 0.15% of the trypsin counteracted all of the growth inhibition while .05% trypsin had no effect. Recently it has been reported that a relatively enormous level of crude trypsin added to a 25% raw soybean meal diet for rats will counteract the growth inhibition(3).

TABLE III. Effect of Trypsin in Counteracting Growth Inhibition Caused by 5% Raw Soybean Protein in Chick Diets.*

Trypsin added to 5% raw protein diet,† %	Avg wt of duplicate chick groups at 14 days, g
.00	115
.05	111
.10	123
.15	161
.00‡	162

* Diets contained a total 20% soybean protein plus .3% methionine.
† Trypsin (1-300).
‡ All-cooked protein diet.

The nature of the flatter portions of the curves in Fig. 1 suggests a proportional growth inhibition of a type different from that involving trypsin. By projecting the flat portions to the left, an estimate may be obtained of the growth that would result if the trypsin effect only were in operation. Subtracting this from the total growth inhibition leaves an estimate of the second effect. This is found to be, without methionine added—6% and with methionine added—4 to 6%. Evidently, this second effect upon growth is relatively small and independent of added methionine. It may be most simply explained perhaps, as a result of a general decrease in availability of nutrients in raw as compared to properly cooked soybean meal.

Summary. Exclusively soybean protein diets, containing varying proportions of raw to cooked soybean meals were fed to young chicks. The growth inhibition associated with raw soybean meal was almost fully developed when one-fourth of the protein of either 20% or 30% protein diets was supplied in the raw form. The results do not fit the concepts of an unavailable essential nutrient or of a toxic component in raw soybean meal. The results are explainable on the basis of an antitrypsin which readily inactivates trypsin but permits the remaining proteolytic system in the digestive tract to function at a lowered degree of

efficiency. The further slight inhibition of growth with increased proportions of raw soybean meal is probably an effect of an entirely different nature and may be a manifestation of generally decreased availability of the nutrients in the raw meal.

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Distribution of Vitamin B_{12} in the Organs and Tissues of the Chick.* (19351)

H. YACOWITZ,† C. H. HILL,‡ L. C. NORRIS, AND G. F. HEUSER.

From the Agricultural Experiment Station and the School of Nutrition, Cornell University, Ithaca, N. Y.

The vit. B_{12} content of various organs and tissues of the rat was first reported by Lewis, Register, and Elvehjem(1). These investigators used a rat growth assay to determine vit. B_{12} content. Several laboratories have published microbiological data on the vit. B_{12} content of some of the organs of the chick (2-4). Only a few organs were tested and no attempt was made to correct for desoxyribosides which interfere in the microbiological assay.

The purpose of this report is to present the results of studies of the vit. B_{12} content of the tissues and organs of the chick using a microbiological assay procedure. Corrections were made for desoxyribosides present in the samples. The rat method was not used because its low sensitivity makes it unsuitable for assaying those tissues which contain small quantities of vit. B_{12}.

Experimental. Thirty day-old Single Comb White Leghorn cockerels, obtained from a commercial hatchery, were used in the experiment. The chicks were housed in electrically heated battery brooders with wire-mesh floors. Food and water were supplied *ad libitum.* An alpha-protein-cerelose diet adequate in minerals and all known vitamins was used(5). The diet was supplemented with 50 μg of vit. B_{12} (Cobione) per 100 g. After 30 days on this regime the chicks were sacrificed and the carcasses frozen. The carcasses were removed from the freezer in groups of 5 and the various organs and tissues removed and weighed. The samples were pooled, homogenized in a Waring Blendor and then autoclaved for 30 minutes in acetate buffer at pH 4.5. After autoclaving, the samples were cooled, adjusted to a convenient volume, filtered and the filtrates assayed. This method of treatment was found to yield consistent results and no further increase in vit. B_{12} values was obtained by enzymatic digestion with pepsin, trypsin, or pancreatin. The assay medium of Peeler *et al.*(6) was employed using *Lactobacillus leichmannii* ATCC 4797. Duplicate assays were conducted on each sample. The alkali treatment used to correct for desoxyribosides was that of Hoffmann *et al.*(7).

Results and discussion. The vit. B_{12} content of the tissues and organs is presented in Table I. The desoxyriboside correction was negligible in most samples. The high level of dietary vit. B_{12} resulted in a very wide ratio between the vit. B_{12} and desoxyriboside content of the organs, thus the interference of

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† Present address: Department of Poultry Husbandry, Ohio State Univ., Columbus, Ohio.

‡ Present address: Department of Agricultural Chemistry, Univ. of Nebraska, Lincoln, Nebr.