THE FEEDING OF UNHEATED SOYBEANS TO POULTRY

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Jerome Douglas Yates

1963

This is to certify that the

thesis entitled

THE FEEDING OF UNHEATED SOYBEANS

TO POULTRY

presented by

Jerome Douglas Yates

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Poultry Science

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Date December 20, 1963

ABSTRACT

THE FEEDING OF UNHEATED SOYBEANS TO POULTRY

By Jerome Douglas Yates

Including unheated soybeans in the rations of monogastric animals usually causes very poor growth or production. The exact cause(s) of the poor results has not been elucidated. These studies were conducted primarily to find ways of overcoming this problem so that raw soybeans could be used in practical-type poultry rations.

Nine experiments using chicks, poults, growing pullets, or laying hens were conducted. Different varieties of soybeans were evaluated. Also, rations containing raw soybeans were supplemented with amino acids, antibiotics, a thyroxin-like compound (Protamone), an oral hypoglycemic (Orinase), as well as different energy sources. The effects of pelleting and heat treatment on the nutritional value of the soybeans were also evaluated. Rates of growth, egg production, feed consumption, and hatchability were determined. Histological examinations were made of many of the body organs. Blood-sugar levels were determined and fatty acid analysis of livers were also made.

Differences in rates of growth were produced by the different varieties of soybeans.

Supplementing raw soybean chick rations with methionine was beneficial, but vitamin B_{12}, folic acid and choline at relatively high levels did not prove beneficial. Likewise, hormone-like compounds having activity to increase insulin and thyroxin-like effects did not improve the performance of chicks. The recycling of feces from chicks receiving the raw soybeans did not affect the rate of growth.

In experiments with laying hens, pelleting of raw soybean rations was not beneficial. Likewise, whole grain soybeans, either cooked or autoclaved, did not produce satisfactory results when used in combination with a pelleted concentrate. The selection by the hens of the pellets and the upsetting of the nutritive balance was thought to be part of the cause for these poor results.

Overcoming the growth depression of raw soybeans by amino acid and antibiotic supplementation did not prevent pancreatic hypertrophy; this may indicate that at least two factors are affecting body growth and pancreas size.

Sensitivity of poultry to injury by raw soybeans decreased with age up to the onset of egg production. An adjustment period was apparently needed in order to allow pullets to effectively use raw soybeans for egg production. If raw soybeans are to be used, they should be started before egg production so as not to cause excessive stress. A protein deficiency may have occurred when switching to raw soybean rations after egg production commenced.

The major conclusions of these studies are as follows: Raw soybeans were used in poultry feeds with satisfactory results when they were properly supplemented. The best performance in these experiments occurred with supplements of methionine and certain antibiotics.

THE FEEDING OF UNHEATED SOYBEANS TO POULTRY

By

Jerome Douglas Yates

A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Department of Poultry Science

1963

ACKNOWLEDGMENTS

The author is extremely grateful to Dr. Philip J. Schaible, Professor of Poultry Science, for his guidance and special interest in this study and his many helpful suggestions on the preparation of this manuscript.

The author also appreciates the guidance and help provided by Professors H. C. Zindel, J. A. Davidson and T. H. Coleman of the Department of Poultry Science, Dr. Dorothy Arata of the Department of Foods and Nutrition, and Dr. R. J. Evans of the Department of Biochemistry.

Thanks also go to Michigan State University, the Department of Poultry Science and the people of Michigan for providing laboratory and farm facilities used in this study.

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INTRODUCTION

The soybean, Glycine Max, is indigenous to Asia and one of the oldest crops known. Before becoming an important oriental food, soybeans were grown for their purported medicinal values (Shennung, About 1900 B.C.). Soybeans were first brought to America early in the nineteenth century but more than a hundred years passed before they were produced commercially for oil and meal.

Commercially produced soybean oil meal is the most widely used source of protein for animal feeds in the United States. The protein quality or amino acid balance of soybean meal is probably the best of any plant protein available for adding to a feed mixture. In the processing of soybeans, heat, pressure, and solvents are used to extract the oil. The 44- and 50-percent protein, toasted meals remaining after oil removal are the products used in animal feeding.

In recent years, a surplus of soybean oil has been produced while soybean meal consumption has kept up with production. The current trend is of production of more soybeans due to acreage limitation to grains. If this trend continues, the price of the oil may be lowered to the extent that it will not be economical to process the soybeans for fat removal. Since raw soybeans are poorly utilized unless heated, soybeans for monogastric animal feeding could be heat treated, leaving the oil in the feedstuff. As animal fats or vegetable oils are widely used to produce high-energy poultry feeds in this country, the fat-containing, toasted soybeans should be suitable as a feedstuff. Even more desirable would be the feeding of unheated soybeans since this would eliminate the transportation costs from the farm to the processor and back to the farm, as well as the heating costs. However, feeding unheated soybeans to monogastric animals usually produces

very poor results and this has prevented their general use. The exact cause(s) of the poor results obtained from feeding raw soybeans have not been elucidated although much research has been conducted on this problem. Thus, finding ways to efficiently feed raw soybeans is not only a problem of academic interest but of economic importance as well.

Therefore, the objectives of this study were to delve further into the exact causes for the poor results obtained from feeding raw soybeans and to find combinations of feedstuffs, additives, and conditions whereby it would be economically feasible to use raw soybeans with satisfactory results in poultry rations.

REVIEW OF LITERATURE

That raw soybeans are poorly utilized by monogastric animals was reported many years ago and many studies on the problem have been made. Most of the research with uncooked soybeans has been with either rats or chicks as the experimental animal. In studies with rats, Osborne and Mendel (1917) reported slower growth with raw than with cooked soybeans. That autoclaving soybeans greatly increased the utilization of the protein by chicks was reported by Evans, McGinnis and St. John (1947).

Several inhibitory factors have been found in raw soybeans. A proteolytic inhibitor was extracted from raw soybeans which would greatly retard in vitro activity of trypsin (Ham and Sandstedt, 1944). From his work with rats, Liener (1953) estimated that half of the growth inhibition from raw soybeans was due to the soyin (hemagglutinin) content and half to an effect counteracted by crude trypsin. Soyin depression was attributed to decreased feed consumption. Also, Liener and Pallansch (1952) purified from soybeans a toxic substance remaining after the anti-tryptic activity was destroyed in the crude trypsin inhibitor. It was found to depress rat growth when injected intraperitoneally. The substance was heat-labile and, during its purification, a loss of antitryptic activity and an increase in hemagglutinating activity was observed.

A report by Westfall et al. (1948) indicated that soybeans contain fractions which have lipoxidase, hypotensive, red-blood-cell agglutinating and peroxidase activity as well as the ability to destroy adrenaline.

Much of the research in raw-soybean feeding has been directed toward the study of a trypsin inhibitor. Almquist and Merritt (1953) reported that crystalline trypsin (0.05%) improved growth greatly in chicks receiving raw soybeans. They suggested that enough trypsin must be used to neutralize the antitrypsin so that the proteolytic enzymes secreted by the animal might have freedom of action.

On the other hand, Brambila, Nesheim, and Hill (1961) reported that with chicks, trypsin was not effective in overcoming the growth depression due to raw soybeans. However, trypsin improved nitrogen efficiency and lowered pancreas weights slightly. They suggested that the improved nitrogen utilization may have been due to increased digestibility of dietary nitrogen or inhibition of pancreatic secretion. Raw soybean oil meal greatly depressed the absorption of added soybean oil but this was partially overcome by the trypsin supplement. Raw soybeans in the feed caused a marked hypertrophy of the pancreas which was partially overcome by crude but not crystalline trypsin.

Working with rats, Haines and Lyman (1961) studied the soybean trypsin inhibitor (SBTI). The SBTI depressed growth and feed efficiency but not as much as did the feeding of raw soybeans. The depressions were not attributable to lack of intestinal protease activity, anti-proteolytic action or pancreatic insufficiency. Thus, they concluded that reduced protein hydrolysis was not the depressing factor. The addition of crude SBTI to readily available proteins in feeds reduced the nitrogen absorption while excess proteolytic enzymes were present. They concluded that the nitrogen loss was due to secretion and excretion of pancreatic enzymes which cause deficiencies of the most limiting amino acids.

Recent work by Inamdar and Sohonie (1961) indicated that the growth depression in rats which were fed raw soybeans was due to a definite protein or amino acid deficiency. In their studies with rats, a great decrease in the activity of several enzymes occurred. These enzymes were xanthine oxidase, lactic and succinic dehydrogenases and glucose-6-phosphatase in the liver. In addition, they found that the levels of glycogen, riboflavin, flavin adenine dinucleotide and also total SH compounds were greatly decreased. All of these decreases of enzymes and other compounds in the liver are usually indications of a protein deficiency.

Work by Saxena et al. (1962) revealed that certain other physiological changes take place in chicks which receive raw soybeans. They observed a decrease in the glycogen content of the liver and of muscle and also an increased oxygen consumption. They reported that these changes could be prevented by the addition of a mixture of essential amino acids to the ration.

The effects of amino acid supplementation of rations containing raw soybean-protein have been widely studied. Hayward and Hafner (1941) concluded from experiments with chicks and rats receiving raw soybean protein that their rations were low in sulfur-containing amino acids.

Almquist et al. (1942) found that methionine was the principal growth limiting factor in chick rations containing raw soybeans as the sole source of dietary protein. Choline or L-cystine produced little or no benefit.

Hill et al. (1953) reported that the addition of a mixture of all essential amino acids did not overcome the growth depression of chicks receiving raw soybeans. They, therefore, concluded that the growth depression was not due to unavailability of essential amino acids. However, later research revealed that their mixture was deficient in some essential amino acids.

In growth studies with broiler-type chicks up to two weeks of age, Fisher and Johnson (1958) found that both methionine supplementation and increased levels of raw soybeans improved growth. When a mixture of amino acids or egg albumin was added to a ration containing 15-percent of raw-soybean protein, most of the depression was overcome. Neither of these supplements alone produced good growth but each was apparently adequate to complement the raw soy protein. They concluded that amino acid availability was a major factor in the depressing effect of raw soybeans.

From their research with chicks, Lepkovsky, Bingha, and Peucharz (1959) concluded that raw soybeans contain at least two inhibitory factors -- the anti-trypsin to which the pancreas responds with hypertrophy and the methionine inhibitor which methionine counteracts. They found a gradual decline in proteolytic activity (PA) per unit of feces of chicks receiving heated soybeans. With added methionine, the PA did not so decline with age. When the chicks were fed raw soybeans, a sharp drop of PA occurred on the same day. Fecal output was also lower than that of other groups. When both methionine and raw soybeans were fed, sharp first-day declines in PA occurred. Then, PA gradually increased to normal after four weeks. The proteolytic enzymes were present in feces partially as insoluble complexes and were mostly of pancreatic origin. Measurements of PA varied greatly between assays with casein and hemoglobin substrates. When PA from beef pancreas was introduced into chicks, great losses of activity occurred in the digestive tract.

Saxena et al. (1963a) also found that the major growth-inhibiting factor was in a water-insoluble residue and was devoid of anti-trypsin activity. Other fractions having ahigh anti-trypsin activity had an innocuous effect on chick growth. In all cases of growth depression, the pancreas was markedly hypertrophied.

Experiments with laying hens were conducted by Fisher, Johnson and Ferdo (1957). Feeding raw soybeans at a higher level to increase protein level of feeds greatly increased growth and permitted normal egg production in hens when the ration was supplemented with adequate vitamin B_{12} and methionine. However, the vitamin B_{12} supplement was apparently started after the birds had been on the raw soy protein for four weeks and the effect observed may have been of adjustment to the ration. They suggested that the older birds were insensitive to the inhibitors of soybeans that are detrimental to chicks fed raw soybeans.

Borchers (1961) reported that supplementation of rat rations (12% protein from raw soybeans) with methionine, threonine, and valine improved growth to near the rate of that produced by cooked beans. He theorized that the raw soybeans caused an increase in requirements for amino acids and the poor growth was not due to decreased availability of the amino acids in raw soybeans.

In rats fed 14-percent protein from raw soybeans, slow growth, reduced feed efficiency, and pancreatic hypertrophy were found (Booth, et al., 1960). Additions of four amino acids (0.6% L-tyrosine, 0.6% DL-methionine, 0.6% DL-threonine, and 0.2% DL-valine) reversed the growth depression but not the enlarged pancreas. This supported the concept that slow growth on raw soybeans was due to direct pancreatic stimulation causing excessive amino acid loss in the enzymes excreted in the feces. In the abnormal pancreas, acini had lost regular circular outlines and were jumbled without distinct lumens. The basilar portion of the hyper-plastic acinar epithelium was intensely basophilic and increased at the expense of the zymogenic portion. Ribonuclease digestion studies indicated that this basophilic cytoplasm consisted mostly of RNA. Acini which were measurable were smaller ($P < 0.01$) than normal, apparently due to squeezing by adjacent hyperplastic cells. Nucleoli were larger ($P < 0.05$); nuclei appeared larger (not significant). Pancreatic islets appeared unaffected.

Much additional study has been given to pancreatic function and the effects of raw soybeans upon the pancreas. The secretion of digestive enzymes has been reported to be influenced by a variety of dietary conditions in non-soybean containing rations.

Grossman, Greengard, and Ivy (1943) reported that casein feeding to rats for three weeks did not increase pancreatic amylase but starch feeding did.

Studies on the effects of amino acids on pancreatic enzymes were made by Magee and Hong (1956). Rat rations containing seven percent casein as the only protein source were supplemented with one percent of several amino acids for a three-week period. Rats were then killed after a 24-hour fast and the pancreatic enzyme activity determined. When compared to controls, none of the amino acid supplements studied increased amylase, methionine increased lipase and protease while phenylalanine and isoleucine increased protease activity alone. Only methionine increased body weights. Whether these effects were due to the liberation of pancreozymin and thus increased pancreatic stimulation was discussed. Evidence indicated that in the long term, amylase manufacture is independent of other enzymes.

Kaufman, Klavins, and Kinney (1960) reported that with male rats receiving 18 percent protein, supplements of two or four percent methionine induced destruction of ascinar cells of the pancreas. The destruction was similar to that caused by feeding ethionine. The livers of the methionine treated rats were normal as contrasted to the liver damage produced by ethionine.

In studies into the controls of pancreatic functions, Nishioka (1959) used ten percent solutions of amino acids for introduction into the stomach and found that methionine, tyrosine, histidine, and tryptophan were effective in stimulating the gastric peptic cells; other amino acids had negligible effects. The stimulating effect occurred after release of the gastric hormone from the gastric surface cells. Tyrosine and tryptophan had more effect than histidine on the discharge of zymogen granules from the pancreatic cells. Also, some amino acids which had no effect on the gastric peptic cells caused considerable activity in pancreatic discharge. It was suggested that these amino acids may have caused the release of the duodenal hormone, secretin, which caused enzyme release from the pancreas. In further work with feeding amino acids, Nishioka reported that only histidine promoted the production of secretion granules in the peptic and pancreatic cells of animals on a non-protein diet.

Lyman and Wilcox (1960) studied functional pancreatic damage in rats. Soybean trypsin inhibitor (SBTT) increased pancreatic secretion while methionine caused an accumulation of enzymes in the pancreas. An acute pure methionine deficiency caused less intestinal enzyme activity but apparently had no effect on discharge of the enzymes from the pancreas.

The enzymatic activity of the intestines of the chicks receiving raw and cooked soybeans was evaluated by Alumot and Nitsan (1961). Proteolytic enzyme activity of chicks fed raw soybeans was affected. Up to three weeks of age proteolysis was almost completely inhibited. After the fourth week, it increased and at six weeks approached normal. Pancreas weights were about the same but birds receiving raw soy weighed much less, so the ratio of pancreas to body weight was affected. Pancreatic enlargement was thought to be due to increased enzyme production needed to overcome the inhibitor. Growth depression was explained as being due to unavailability of proteins the first few weeks coupled with increased protein requirement by the stimulated pancreas.

Lyman (1957) reported that rats receiving raw soybeans in their diets had a higher concentration of net unabsorbed nitrogen in the intestine than rats fed the same quantity of heated soybeans. There was a corresponding decrease in pancreas nitrogen in the rats fed raw soybeans. The elevated nitrogen in the fecal material was not due to the undigested inhibitor or a failure of intestinal proteolysis. It was suggested that the increased nitrogen was from the stimulated pancreatic secretions.

Lyman and Lepkovsky (1957) further studied the influence of raw soybean and crystalline trypsin inhibitor on pancreatic enzyme secretion in the small intestine of the rat. Immediately after eating a raw soybean meal diet, intestinal amylase and lipase activity increased. In three hours, the concentration was three or four times that produced by heated soybean meal. The pancreas was depleted of these enzymes. Intestinal trypsin activity was low soon after the raw meal was fed but by six hours it was three times the normal concentration. The initial activity was throught to be due to inactivation by the inhibitor while the later rise was due to pancreatic secretory stimulation. A crude soybean antitrypsin and crystalline soybean inhibitor produced similar results. Pepsin secretion was not affected by the raw soybeans, which indicated a specific action on the pancreas. They concluded that the high level of trypsin produced was evidence against impaired intestinal proteolysis in the rat. The rats used in this experiment had, however, received the heated soybean ration up until the day on which the experimental feedings were made.

In work with chicks, Saxena et al. (1963b) observed pancreatic hypertrophy in 72 hours after the birds were put on raw soybeans. The hypertrophy was reversible in three days when cooked soybeans were fed. Pilocarpine injection reduced amylase activity significantly in pancreas of chicks on heated meal but had no appreciable effect on pancreatic amylase activity in raw meal-fed chicks. Histological examinations after ten hours of starvation revealed an accumulation of eosin-stainable secretory material in the apex of the acinar cells. Pancreatic enlargement of chicks fed raw meal appeared to be due to an increased amount of the zymogen accumulation in each cell. The enlargement was apparently hypertrophy and not hyperplasia since mitotic figures were seldom seen. Pilocarpine injection caused depletion of the secretory material in the control pancreas but not the hypertrophied pancreas. So, apparently the hypertrophy was due to an accumulation of zymogen material in the acinar cells. They concluded that raw soybeans may have interfered with the basic mechanism by which zymogen is released. The slowed growth would be due to the failure of the pancreas to supply the necessary digestive enzymes. They discounted the theory of excess pancreatic secretion and thus protein loss in that way.

The hormonal control of the enzymatic secretions of the pancreas in hypophysectomized rats was studied by Baker et al. (1961). They reported that treating hypophysectomized rats with somatotropin, corticosterone, and L-thyroxin increased the body weights to near normal. Hypophysectomy caused the total volume of both protease and amylase to be lowered but the concentration of only amylase was reduced. With this evidence of depressed protein synthesis was the depletion of RNA (as revealed by basic staining and biochemical analysis) and the reduced quantity of zymogenic granules. The proteolytic activity was partially restored by somatotropin, corticosterone and thyroxine and was further facilitated by insulin. The thyroid hormone played a key role in the maintenance of normal structure of the pancreatic acini.

Effects of raw soybean rations on the thyroid gland and its hormone secretion have been studied. Beck (1958) and also Van Middlesworth (1957) reported an increased fecal loss of thyroxine in rats fed soybean protein. It was assumed that this might cause thyroid hypertrophy by depleting body stores of thyroid hormones. The fecal thyroxin was not apparently due to increased biliary secretion or the addition of thyroxin to the gut from any other source.

Block _et al_. (1961) studied goiter produced by raw soybeans in iodine deficient rations and the curative effect of iodine on the condition. With rats, they found that enlarged thyroids were produced from feeding soybean protein in the low-iodine diets. Although there was more iodine in the raw soybeans, they produced larger thyroids than the heated soy protein, indicating that a true goitrogen may be present in the raw beans. They cited the work of McCarrison (1933) who had reported that goiters produced by soybeans were not cured with high levels of iodine. McCarrison had fed about 100 times the amount of iodine found adequate by Block and co-workers.

In regard to the fat utilization from unheated soybean meal, Nesheim _et al_. (1962) observed that the fat was poorly absorbed in young chicks as compared to the fat from heated soybean meal. However, this defect occurred only during the first two weeks of the chicks' life. At later ages, the decreased fat absorption was slight as compared to the first two weeks of life. However, after two weeks of age the growth continued to be depressed even though the fat absorption was not adversely affected. The work by Nesheim was further interpreted to indicate that absorption and not digestion was affected. The Kunitz inhibitor did not affect fat absorption but this inhibitor did cause pancreatic hypertrophy (Nesheim, 1963).

Carew and Nesheim (1962) reported that pelleting of heat treated ground soybeans increased the availability of soybean oil to chicks. The heat of the pelleting process was not adequate to improve the nutritive value of raw soybeans.

Carew _et al_. (1961) reported that flaking of soybeans improved the availability of soybean oil as compared to the availability of the oil from ground soybeans. They thought that the flaking process caused a greater disruption of the cellular structure than was obtained by grinding of the soybeans. Thus, the flaked soybeans provided more metabolizable energy than did the ground soybeans.

More recently, Bornstein and Lipstein (1963) have studied the effect of age of chicks to sensitivity to rations containing improperly heated soybeans. The period of time required for chicks to adapt to improperly heated soybeans decreased with age but an initial depressing effect was always present up to eight weeks of age. Age *per se* did not affect the degree of sensitivity to the raw soybeans but favored a more rapid adaptive process. Growth inhibition at 7 - 8 weeks of age was not due to decreased intake as was observed at very early ages.

GENERAL EXPERIMENTAL PROCEDURES

Preparation of experimental rations

Efforts were made to mix experimental feeds in which each different ration was different in only one aspect, that of the ingredient or supplemental product being evaluated. To accomplish this, a basal ration was prepared, whenever possible, using only one mix. That is, all of the basal products, the vitamins, alfalfa meal and sources of vitamins and other products were mixed into one large batch up to the amount in which all rations were alike. Then, the basal amounts needed were pulled out and the test product was added to make the ration up to 100 percent.

When raw soybean rations were used, the whole soybeans were ground in a hammermill. When the cooked product was needed, the ground raw soybeans were placed in cotton bags which were spread into a thin layer (about one inch) over a wire rack on the shelf of an autoclave. The autoclave was turned on and the time required to reach the 15-pound pressure was usually from 30 to 45 minutes. Once the pressure had reached 15 pounds, which represents a temperature of 248° F., the pressure was maintained for 15 minutes and then the heat was turned off and the pressure allowed to go down slowly, taking 15-30 minutes. Thus, the total heating time was about 1½ hours.

In all experiments, rations which contained either commercially-produced soybean oil meal or autoclaved ground soybeans were formulated and fed as a practical control to determine normal performance. The objective was to improve the nutritional quality of the rations containing the raw soybeans to a level more comparable to the positive control rations.

With positive control rations containing commercial soybean oil meal, soybean oil or animal fat was incorporated so that all rations in each experiment were isocaloric and also isonitrogenous. In this manner, the percentage of

raw soybeans in one ration would be equal to the percentage of the combination of soybean meal (44 percent protein) and the added fat in the positive control ration. When small amounts of test ingredients such as antibiotics, amino acids, hormone-like compounds and drugs were used in rations, they were simply added to the complete basal ration with no adjustments in other dietary components for the very low amounts of materials which were added.

Supplemental methionine was used in many of the experiments. In all cases, it was feed-grade DL-methionine[1]. In experiments II through IX, the Chippewa variety of soybeans was used.

Experimental rations were analysed for protein, fat, fiber, calcium, phosphorus and water. The composition of the vitamin-trace mineral mixes that were used are shown in appendix Table 1.

<u>Statistical procedures</u>

Certain data from the experiments were subjected to statistical analysis. The most common analysis used was the analysis of variance as outlined by Snedecor (1956). Data for individual animals were used in most cases; e.g., the final body weights of all birds were used and evaluated in the statistics. In most experiments, a two-way classification was made with treatments and replications being the two classifications. In these cases, the variance for subclass for each of the pens was determined. Then the variance due to treatment and replication was determined and these two sums of squares were subtracted from the subclass sum of squares in order to get a value for the interaction between treatments and replications. If the F value for the interaction term was significant, this was the mean square value used as an error term instead of the residual error term as usually used. If the interaction value was not significant, the error mean squares was used to divide into the other mean squares in order to obtain the F values.

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[1] DL-methionine (98-99% pure) produced by Dow Chemical Company, Midland, Mich.

In experiments where two sexes or two varieties -- as in the case of turkeys -- were used, the second classification was sex or variety and the interaction between sex and treatment or variety and treatment was determined.

Once the analysis of variance had been completed, the standard error of a treatment mean was obtained and Duncan's (1955) multiple range test was used to determine which means were significantly different at both the .01 and .05 levels of probability. In tables where means are listed in a column, alongside each mean occurs a large letter and also a small letter if the analysis of variance and multiple range tests were applied to the data. Those means having the same capital letter are not significantly different at the .01 level of probability; whereas, those means not having the same capital letter are significantly different at this level. Likewise, the small letters indicate significance at the .05 level of probability.

Egg production data were usually determined by the use of trapnests with the birds being trapped five days per week. However, when birds were in individual cages, production records were available seven days per week for each individual bird. The rate of production for a treatment is usually listed in the tables as "percent production on a hen-day basis" and the letters indicating significance between different means are listed alongside. However, these percentages were not used in the actual statistical analysis. Rather, the number of eggs laid during a given number of days in which production records were available over the experimental period were used. The actual number of eggs each hen laid over this period was subjected to an analysis of variance and then Duncan's multiple range test. Then the letters indicating significance were placed alongside the respective percentage where a percentage was listed. Percent production was listed as this is the usual method for expressing rate of egg production per hen as compared to the number of eggs over a period of time.

PART I - EXPERIMENTS WITH RATIONS FOR YOUNG CHICKS

Experiment I -- The Use of Different Varieties of Autoclaved and Raw Soybeans and Amino Acid Supplementation in Chick Rations

Since reports of different workers do not always agree as to the nutritive value of raw soybeans, a decision was made to compare the value of several different varieties of soybeans in both autoclaved and raw forms as protein sources for chicks. For this purpose, certified seed of four different varieties of soybeans were obtained from a seed producer[1]. In addition, soybeans from a non-seed source were used as a control. In addition, one of the varieties of soybeans was supplemented with methionine, glycine or lysine, as well as combinations of these. One complete ration was autoclaved for one of the different experimental feeds (diet 1-20). In addition, rolled oats was used as a carbohydrate source of energy instead of corn in one of the rations (diet 1-18) to determine if there was a difference in the supplementary value of the protein of corn and oats.

For this experiment, Cobbs' White Rock cockerels which were two weeks of age were allotted into 15-gram weight groups. Birds weighing from 185 to 275 grams were used. From these weight groups, the birds were randomly distributed into each of 60 pens in five chick starting batteries. There were nine of these chicks in each pen and the average weight for each pen was approximately the

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[1] Myron Jacob, Riga, Michigan

same. Each of the 20 experimental rations was fed to three pens of chicks. The composition of the basal rations used in this experiment is shown in Table 1-1, while the experimental design, body weights and feed efficiencies and analysis of variance are shown in Tables 1-2 and 1-3.

The differences between the different varieties of soybeans as a protein source for chicks were significant in some cases. For example, in the raw form, Chippewa beans produced significantly ($P < .01$) larger birds than did Lindarin; at the .05 level of probability, both Harosoy and Chippewa were significantly better than Lindarin which was not significantly different from Shelby. There was some interaction between the different varieties when cooked versus raw soybeans were compared. In the autoclaved form, none of the four varieties produced significantly different bird weights at the .01 level of probability; whereas, at the .05 level, Lindarin and Shelby performed significantly better than Chippewa. Although Chippewa was the best performer in the raw form, it was the poorest growth producer in the autoclaved form of the four varieties. Thus, some beans which did better in one form were poorer in the other form. All varieties were significantly ($P < .01$) improved by the heat treatment. The storage of beans for two years had no apparent effect on the inhibiting factors in the soybeans.

The methionine supplementation significantly improved the rate of growth of birds receiving the raw soybean-containing rations. The addition of glycine in combination with the supplemental methionine significantly ($P < .05$) increased the body weights over those produced by supplementation with methionine only. Lysine or glycine alone was not beneficial. There was little or no effect from the replacing of corn by the rolled oats or from autoclaving the whole ration as compared to only autoclaving the soybean components. None of the rations was as good as the positive control ration (diet 1-19).

Feed efficiency, in general was closely correlated with rate of gain with the faster rate of gain producing the best feed efficiency. The birds receiving the rations containing the raw ground soybeans consumed much less feed than did the birds receiving rations that produced faster growth.

Table 1-1. Composition of Basal Rations Used in Experiment I

Ingredients	Percent of rations	
	Diets 1-1 & 1-2	Diet 1-19
Soybeans, raw or autoclaved, ground	38.0	--
Soybean oil meal, 44% protein	--	31.3
Corn oil, crude	--	6.7
Corn, ground yellow	50.8	50.8
Fish meal & solubles, 57% protein	3.0	3.0
Alfalfa leaf meal, 20% protein	2.0	2.0
Dried distillers solubles, corn	2.0	2.0
Whey, dried	2.0	2.0
Vitamin-trace mineral mix, Nopcosol M-7	.25	.25
Salt, iodized	.50	.50
Limestone, ground	.90	.90
Dicalcium phosphate, ground	.60	.60

Calculated analyses for both rations:

Protein	%	21.92
Fat	%	9.22
Fiber	%	3.64
Calcium	%	.82
Phosphorus	%	.61
Arginine	%	1.26
Glycine	%	1.17
Methionine	%	.43
Cystine	%	.33
Lysine	%	1.18
Tryptophan	%	.25
Productive energy, Cal/lb		1024

Table 1-2. Results of Chick Experiment at 4 and 6 Weeks of Age for Determining the Effects of Autoclaving, Amino Acid Supplementation, Variety and Age of Soybeans on Growth and Feed Efficiency

Diet	Composition	Av. wt. (gms)		Feed/gain		
		4 wks.	6 wks.*	2-4 wks.	4-6 wks.	2-6 wks.
1-1	Raw Harosoy (King)	432	732 DEFgh	3.11	3.03	3.06
1-2	Autoclaved Harosoy (King)	510	861 Cde	2.49	2.43	2.46
1-3	Raw Harosoy (Jacob)	416	714 EFGgh	3.24	3.13	3.16
1-4	Autoclaved Harosoy (Jacob)	515	918 Bbc	2.45	2.51	2.48
1-5	Raw Chippewa (Jacob)	438	739 DEFfgh	3.11	2.97	3.02
1-6	Autoclaved Chippewa (Jacob)	505	889 BCcd	2.77	2.50	2.74
1-7	Raw Chippewa (stored 2 yrs.)	413	684 FGi	3.46	3.13	3.25
1-8	Autoclaved Chippewa (stored 2 yrs.)	510	933 Bb	2.50	2.68	2.60
1-9	Raw Lindarin (Jacob)	408	673 Gi	3.41	3.23	3.31
1-10	Autoclaved Lindarin (Jacob)	537	932 Bb	2.35	2.43	2.40
1-11	Raw Shelby (Jacob)	412	702 EFGhi	3.38	3.03	3.16
1-12	Autoclaved Shelby (Jacob)	536	937 Bb	2.44	2.59	2.52
1-13	Diet 1-1 + .1% Methionine	469	842 Ce	2.68	2.78	2.73
1-14	" " + .1% Glycine	438	757 Dfg	3.07	3.08	3.07
1-15	" " + .1% Glycine + .1% Methionine	492	889 BCcd	2.65	2.63	2.63
1-16	" " + .1% Lysine	431	746 DEfgh	3.36	3.06	3.17
1-17	" " + .1% each of glycine, methionine and lysine	476	852 Cde	2.74	2.70	2.72
1-18	Rolled oats replacing corn-soy	448	777 Df	3.08	3.08	3.06
1-19	Positive control (commercial soy + corn oil)	564	1013 Aa	2.19	2.32	2.26
1-20	Diet 1-2 Autoclaved	473	851 Cde	3.08	2.44	2.67

* Means having common capital or small letters are not significantly different while means not having common letters are significantly different at the .01 and .05 levels of probability, respectively.

Table 1-3. Analysis of Variance of Body Weights of Six-week-old Cobbs' Cockerels in Experiment I

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	530	8,045,370		
Subclass	59	5,509,741	93,385	17.34**
Treatment	19	5,057,804	266,200	49.44**
Replications	2	129,328	64,664	12.01**
T X R (Int.)	38	322,609	8,490	1.58*
Error	471	2,535,629	5,384	

Standard error of mean = 14.3

* Significant at the .05 level of probability

** Significant at the .01 level of probability

Experiment II -- Supplementation of Raw Soybean Rations With Methionine, Choline, Folacin and Vitamin B_{12}

Since it was apparent that methionine was very beneficial when added to the diet of chicks receiving raw soybean meal, further studies into its exact mode of action were needed. Since methionine serves as a source of methyl groups in addition to its use in protein synthesis, this aspect of its role in nutrition was investigated. Choline will also supply methyl groups to the animal; therefore, choline chloride was used at higher than normal levels in certain experimental rations. Also, since vitamin B_{12} and folic acid are involved in methyl synthesis, these vitamins were investigated. Therefore, to a raw soybean control diet, methionine, choline, folic acid and vitamin B_{12} were added singly and in all possible combinations. Rations containing commercially-produced soybean oil meal as well as autoclaved ground soybeans were used as positive controls.

Single Comb White Leghorn cockerels which were five days of age were weighed into 3-gram weight groups and from these groupings were randomly distributed into the various experimental pens in chick starting batteries. This distribution was made in such a way that the average weight of the chicks in each pen was approximately the same. Three pens of chicks with eight chicks per pen received each of the experimental rations.

Formulations of the two basal rations which were used in this experiment are shown in Table 2-1. The experimental outline, body weights and feed efficiencies are shown in Table 2-2 and 2-3. The analysis of variance of body weights is shown in Table 2-4.

The statistical analysis revealed that the growth responses observed were due to the methionine supplementation. This was the only single component which was added that produced a significant response in growth. The supplementation

Table 2-1. Composition of Basal Rations Used With Raw Soybean Experiment Number II

Ingredients	Percent in Rations Diet 2-1	Diet 2-5
Soybeans, raw or autoclaved, ground	--	37.0
Soybean oil meal, 44% protein	30.4	--
Soybean oil, crude	6.6	--
Corn, ground yellow	51.5	51.5
Fish meal & solubles, 57% protein	3.0	3.0
Alfalfa leaf meal, 20% protein	2.0	2.0
Dried distillers solubles, corn	1.25	1.25
Corn fermentation solubles #3	.75	.75
Whey, dried	1.39	1.39
Skim milk, dried	.61	.61
Vitamin-trace mineral mix, Nopcosol M-4	.25	.25
Salt, iodized	.50	.50
Limestone, ground	1.20	1.20
Dicalcium phosphate, ground	.60	.60

Calculated analyses for both rations:

Protein	%	21.44
Fat	%	9.08
Fiber	%	3.61
Ash	%	5.98
Calcium	%	.93
Phosphorus	%	.60
Arginine	%	1.25
Glycine	%	1.16
Methionine	%	.42
Cystine	%	.32
Lysine	%	1.16
Tryptophan	%	.25
Productive energy, Cal/lb		1026
Choline	mg/lb	690
Folacin	mg/lb	.32
B_{12}	mcg/lb	6.1

Table 2-2. Results of Experiments Using Leghorn Cockerels With Rations Containing Raw Soybeans With Variables of Vitamins and Methionine

Diet	Composition	Average wts. (gms)			Feed cons./bird (gms)		Feed/gain	
		1 wk.	2 wks.	4 wks.*	0-2 wks.	2-4 wks.	0-2 wks.	2-4 wks.
2-5	Control - raw soybeans	70	120	223 C	210	310	3.01	2.84
2-8	Diet #5 + .16% methionine	79	138	300 A	195	401	2.04	2.47
2-9	Diet #5 + .68 mg/lb. folacin	67	111	225 C	187	322	2.72	2.83
2-10	Diet #5 + 6 mcg/lb B_{12}	70	118	248 BC	201	365	2.65	2.79
2-11	Diet #5 + 510 mg/lb choline	69	114	220 C	210	303	2.91	2.88
2-12	Diet #5 + methionine + folacin	80	138	301 A	210	397	2.19	2.44
2-13	Diet #5 + methionine + B_{12}	78	131	280 AB	205	350	2.29	2.36
2-14	Diet #5 + methionine + choline	79	137	311 A	204	367	2.16	2.11
2-15	Diet #5 + folacin + B_{12}	72	121	251 BC	212	349	2.72	2.75
2-16	Diet #5 + folacin + choline	71	117	233 C	206	327	3.00	2.71
2-17	Diet #5 + B_{12} + choline	70	120	247 BC	200	376	2.64	2.97
2-18	Diet #5 + methionine + folacin + B_{12}	74	131	300 A	198	423	2.23	2.51
2-19	Diet #5 + methionine + folacin + choline	78	138	282 AB	221	373	2.31	2.58
2-20	Diet #5 + methionine + B_{12} + + choline	77	135	303 A	214	369	2.32	2.27
2-21	Diet #5 + folacin + B_{12} + choline	72	123	248 BC	202	344	2.52	2.75
2-22	Diet #5 + methionine + folacin + B_{12} + choline	78	134	292 A	228	351	2.56	2.24

* Means having common capital or small letters are not significantly different while means not having common letters are significantly different at the .01 and .05 levels of probability, respectively.

Table 2-3. Comparisons of Pooled Results Due to Variables of Experiment Using Leghorn Cockerels With Rations Containing Raw Soybeans

No.		Average wts. (gms)			Feed cons./bird(gms)		Feed/gain	
birds	Variables of rations	1 wk.	2 wks.	4 wks.	0-2 wks.	2-4 wks.	0-2 wks.	2-4 wks.
187	No methionine added	70	118	237	204	337	2.77	2.82
187	With " " (.16%)	78	135	296	209	379	2.26	2.37
187	No folacin added	74	127	267	205	355	2.50	2.59
187	With " " (.68 mg/lb)	74	127	266	208	361	2.53	2.60
190	No B_{12} added	74	127	262	205	350	2.54	2.61
184	With B_{12} " (6 mcg/lb)	74	127	271	208	366	2.49	2.58
186	No choline added	74	126	266	202	365	2.48	2.62
188	With choline added (510 mg/lb)	74	127	267	211	351	2.55	2.56
(On experiment concurrently for comparison):								
	Positive control (commercial soy + oil, diet 2-1)	92	156	336	220	397	1.94	2.20
	Positive control, except using autoclaved soybeans, diet 2-2)	91	150	316	245	393	2.26	2.37

Table 2-4. Analysis of Variance of Four-week Body Weights of Leghorn Cockerels on Soybean Experiment

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	373	1,098,720		
Subclass	47	493,297	10,496	5.65**
Replications	2	5,069	2,535	.69
Treatments	15	378,788	25,253	6.92**
Methionine	1	330,924	330,924	90.71**
Folacin	1	30	30	.01
B_{12}	1	7,485	7,485	2.05
Choline	1	62	62	.02
M X F X B_{12} X C (Int.)	11	40,287	3,662	1.00
R X T (Int.)	30	109,440	3,648	1.96**
Error	326	605,423	1,857	

Standard error of mean = 12.5

** Significant at the 0.01 level of probability with additional vitamin B_{12} produced a slight response in growth but this only approached significance at the .05 level and was much less than to the response from methionine. Neither folic acid or choline supplementation, nor the interaction between all these components was of any benefit. It should be noted that these four components which were added to the various experimental rations were present in the basal ration at theoretically adequate amounts and thus any response from them would not really be expected, except for the fact that the rations contained raw soybeans.

The weights of birds that received the raw soybean ration plus the supplemental methionine were not greatly different from the weights produced by the cooked soybean rations with no supplemental methionine. Once again birds which received the unsupplemented raw soybean-containing ration consumed much less feed than the birds which received the cooked soybean ration. Methionine supplementation to the raw soybean ration improved feed consumption. In general, feed efficiency was associated with body weights, with the best feed efficiency with the birds which grew fastest. This was generally the case in all experimental treatments.

At various ages (6, 7 and 8 weeks) the pancreas of certain of these birds was removed and weighed; the weight of the pancreas as a percentage of body weight was determined (Table 2-5). The birds receiving the raw soybeans had greatly hypertrophied pancreata. The pancreata were larger than those of the control birds receiving the cooked soybeans, not only as to total weights but especially with respect to weights as percentages of body weight. Methionine supplementation did not alleviate this pancreatic hypertrophy, although growth was improved by the methionine supplementation.

Sections of the pancreas of birds from different experimental treatments were also taken for histological examinations and these sections were fixed, stained and slides were made of them for microscopic examination. In general, the pancreata from birds which had received the raw soybeans seemed to be enlarged or engorged but did not seem to be abnormal insofar as the cell makeup. The ascinar just seemed to be swollen or enlarged, thus increasing the overall size of the pancreas.

With a resting pancreas, many zymogen granules usually are present and they are large and packed rather closely together. In humans, the hormones secretin and pancreozymin affect the secretion from the pancreas. Pancreozymin increases the concentration of the enzymes secreted; whereas, secretin increases the water or the liquid component secretion. The hydrochloric acid which is produced in the stomach of humans goes into the intestine and there lowers the pH of the duodenum which causes a release of secretin which, in turn, causes pancreatic secretion. In addition to hydrochloric acid, the presence of water, soaps and peptones can cause the release of secretin and thus pancreozymin secretions (Guyton, 1958).

Since hydrochloric acid is produced in the produced in the proventriculus of the chicken, a study was conducted to determine the amount of acid present in the gizzard, which is just posterior to the proventriculus. The possibility existed that the presence of raw soybeans would either increase or decrease in some way the production of hydrochloric acid by the proventriculus and thus, in turn, affect the secretion of the proenzymes from the pancreas. In order to study the amount of acid produced, chicks on the different experimental treatments were fasted over night and then placed back on feed four hours before being sacrificed. This fast period followed by feeding was used to give all chicks the opportunity to eat at the same time so that all might be alike as far as feed consumption and the length of time the feed was present in the digestive tract were concerned. The total content of the gizzard was

was weighed and diluted with water to make a total of 100 milliliters. The pH of this mixture was then determined and the molarity of hydrogen ions(H+) was determined as a total per gizzard and also as an amount per gram of gizzard content. These data are shown in Table 2-6. There were no apparent differences nor trends due to the experimental treatments and the results varied widely from bird to bird.

Table 2-5. Body and Pancreata Weights of Chicks on Different Experimental Rations at Different Ages

Diet no.	Variables of ration	At 6 wks. Body wt.	At 6 wks. Panc. wt.	At 6 wks. %	At 7 wks. Body wt.	At 7 wks. Panc. wt.	At 7 wks. %	At 8 wks. Body wt.	At 8 wks. Panc. wt.	At 8 wks. %
		gms	gms		gms	gms		gms	gms	
2-1	Commercial soy + oil	515	1.91	.37				650	1.74	.27
	--------------------	505	1.30	.26				750	2.07	.28
2-2	Autoclaved soybeans	425	1.27	.30	600	1.51	.25	545	1.40	.27
	------------------	420	1.02	.24	570	1.70	.30	750	1.39	.27
2-5	Raw soybeans				370	2.73	.74	590	3.63	.62
	------------				407	2.78	.68	355	1.67	.47
2-8	Raw soybeans + methionine	450	3.25	.72	542	3.15	.58	780	4.26	.55
	-------------------------	535	4.23	.79	590	3.64	.62	845	3.49	.41
2-9	Raw soybeans + folacin	345	2.83	.82						
	----------------------	380	2.42	.64						
2-10	Raw soybeans + B_{12}	385	2.85	.74						
	----------------	310	2.79	.90						
2-11	Raw soybeans + choline	325	1.68	.52				480	2.31	.48
	----------------------	330	1.65	.50				540	2.61	.48
2-12	Raw soybeans + meth.+folic	515	2.98	.58				700	3.54	.51
2-14	Raw soybeans + meth.+ chol.	385	2.98	.77				785	3.76	.48
2-16	Raw soybeans + folic + chol.	370	2.70	.73						
	-------------------------	300	2.02	.67						
2-20	Raw soybeans +meth,chol,B_{12}							625	3.20	.51
2-21	Raw soybeans + folacin + chol. + B_{12}							585	3.48	.59

Table 2-6. Results Obtained From pH Determination of Gizzard Contents of Leghorn Cockerels at 5.5 Weeks of Age

Diet no.	Weight	Wt. of gizzard contents (gms)	Molar conc. of H^+ (10^{-7}) Per gm content	Molar conc. of H^+ (10^{-7}) Total
2-1	515	5.53	57	316
2-1	505	5.78	35	200
2-2	425	5.75	55	316
2-2	420	4.94	81	398
2-8	450	7.76	129	1000
2-8	535	6.91	58	398
2-9	345	4.04	99	398
2-9	380	4.24	24	100
2-10	385	4.84	26	125
2-10	310	4.16	120	501
2-11	325	6.19	102	631
2-11	330	7.76	81	631
2-12	515	8.22	61	501
2-14	385	8.10	62	501
2-16	370	2.64	95	251
2-16	300	3.80	83	316

Experiment III -- <u>An Evaluation of the Effectiveness of Several Antibiotics, as Well as Supplemental Methionine and Glycine in Raw Soybean Rations</u>

The report by Borchers *et al.* (1957) indicated that quite high levels of penicillin and streptomycin in combination (0.1 percent of each) were effective in eliminating the growth-depressing effect of raw soybeans when fed to rats. Since the exact mechanism by which antibiotics promote growth is not known, the possibility exists that rations containing raw soybeans could be routinely used to evaluate different antibiotics for their growth promoting activities. In certain experiments under certain environmental conditions, antibiotics do not give a growth response; thus, if a procedure could be developed for standardizing growth responses to antibiotics, this would be a valuable technique.

For this experiment, both raw and cooked soybeans were used with and without antibiotics. The antibiotics which were used were Pro-strep, Lincomycin, Terramycin and Gallimycin, all at the 50-grams per ton level. The 100-grams per ton level of Lincomycin was also used. These antibiotics represent a wide spectra of activity, from being effective against gram-positive to being effective against gram-negative bacteria; Oxytetracycline is considered a broad spectrum antibiotic. The antibiotic combination of procaine penicillin and streptomycin (1:3), Pro-strep, was used in rations containing both raw soybeans as well as the rations containing cooked soybeans. Also, the value of supplementing the raw soybean ration with .16 percent methionine and .10 percent glycine, both singly and in combination was determined. The rations receiving the amino acids supplementations were also repeated with Lincomycin supplementation.

Day-old Cobbs' White Rock cockerels were weighed and placed into 3-gram weight groups. Individuals from these groups were proportionately distributed at random into the various experimental pens in chick starter brooders. Each diet was fed to three pens of ten birds each. The experimental basal rations, as well as the experimental outline and results are shown in Tables 3-1 and 3-2.

Most supplements improved the performance of the raw-soy basal ration. The greatest single improvement was made with methionine. The differences in mean weights between the birds receiving the 50-grams per ton level of Lincomycin and the negative control ration was not significant at the .05 level; whereas, the differences due to this level of the other antibiotics were significant. The 100-grams per ton level of Lincomycin produced weights similar to those produced by the 50-grams per ton level of other antibiotics. The addition of 50-grams of Pro-strep per ton of ration improved performance in the soybean oil meal positive control ration as well as in the cooked whole soybean meal ration. Increasing the Lincomycin level did not improve feed efficiency. Glycine supplementation produced a somewhat lesser response in growth than did methionine. However, response from the combination of methionine plus glycine was not significantly greater than that produced by methionine supplementation alone.

The response to the Pro-strep supplementation was no greater in the raw soybean rations than it was in the cooked soybean rations. This is contrary to what normally would have been expected. The response due to Pro-strep supplementation was highly significant in all cases regardless of whether the rations contained cooked or raw soybeans. The analysis of variance of the 4-week body weights is shown in Table 3-3.

Certain birds -- a random sample -- were continued on to eight weeks of age at which time the body weights were obtained. Also, at nine weeks of age, the micro-hematocrit determination was made on birds from most of the experimental treatments to ascertain if an anemia was present. These results are shown in Table 3-4 and the analysis of variance of the eight-week body weights are shown in Table 3-5. The differences in body weights due to treatments were significant but the micro-hematocrits revealed no anemia.

In order to detect any cellular abnormality which might have been induced by raw soybeans, sections were taken for histological examinations at different periods of time from birds which were consuming cooked soybeans as well as from those fed the raw soybeans. At one week of age, birds from diets 3-1 and the raw soybean-containing ration (diet 3-5) were sacrificed and sections were taken from the proventriculus, duodenal loop (next to the gizzard), the pancreas (sections from the center and both ends), the center of the small intestine, the small intestine near the ceca and the mid part of the large intestine, between the ceca and the cloaca. Also the thyroid glands were removed from these birds for study. At three weeks of age, histological sections were taken from these same areas. In addition, sections of the gall bladder and liver were taken. Bouin's fixative was used to fix the different tissues and different stains including hematin and eosin were used to prepare the slides for examination. Upon microscopic examination, no abnormalities were observed in any of the tissues from the two groups of birds, except for the pancreata which were engorged and enlarged in the birds which had received the raw soybean rations.

Table 3-1. Composition of Basal Ration for Chicks in Experiment III

Ingredients	Percent of rations	
	Diet 3-1	Diet 3-5
Soybeans, raw or autoclaved, ground	--	37.0
Soybean oil meal, 44% protein	30.4	--
Soybean oil, crude	6.6	--
Corn, ground yellow	51.5	51.5
Fish meal and solubles, 57% protein	3.0	3.0
Alfalfa leaf meal, 20% protein	2.0	2.0
Dried distillers solubles, corn	2.0	2.0
Whey, dried	2.0	2.0
Vitamin-trace mineral mix, Nopcosol M-4	.25	.25
Salt, iodized	.50	.50
Limestone, ground	1.20	1.20
Dicalcium phosphate, ground	.60	.60

Calculated analyses for both rations:

Protein	%	21.44
Fat	%	9.08
Fiber	%	3.61
Ash	%	5.98
Calcium	%	.93
Phosphorus	%	.60
Arginine	%	1.25
Glycine	%	1.16
Methionine	%	.42
Cystine	%	.32
Lysine	%	1.16
Tryptophan	%	.25
Productive energy, Cal/lb		1026

Table 3-2. Summary of Experiment Using Amino Acids and Antibiotic Supplements in Raw Soybean Rations

Diet	Composition	Average weights (gms) 1 wk.	2 wks.	4 wks.*	Feed consumption per bird (gms) 0-2 wks.	2-4 wks.	Feed/gain 0-2 wks.	2-4 wks.
3-1	44% soybean meal + soybean oil	103.5	208	553 Bb	269	580	1.57	1.68
3-2	Diet #1 + Pro-strep, 50gms/ton	110.5	230	602 Aa	288	628	1.52	1.69
3-3	Cooked (autoclaved) whole soybean meal	87.8	180	466 Cc	262	570	1.88	1.97
3-4	Diet #3 + Pro-strep, 50 gms/ton	96.1	195	520 Bb	265	615	1.73	1.89
3-5	Raw whole soybean meal	76.1	127	332 Gg	225	409	2.60	2.01
3-6	Diet #5 + Lincomycin, 50 gms/ton	84.7	145	364 FGfg	248	434	2.40	1.98
3-7	Diet #5 + Lincomycin, 100 gms/ton	86.3	164	396 DEFef	283	517	2.30	2.23
3-8	Diet #5 + Terramycin, 50 gms/ton	82.9	152	382 EFf	250	466	2.24	2.02
3-9	Diet #5 + Gallimycin, 50 gms/ton	85.1	153	380 EFf	265	484	2.37	2.13
3-10	Diet #5 + Prostrep, 50 gms/ton	84.6	149	395 DEFef	274	535	2.40	2.22
3-11	Diet #5 + .16 percent methionine	82.9	158	426 CDEde	269	499	2.29	1.86
3-12	Diet #5 + .10 percent glycine	85.3	155	380 EFf	253	471	2.22	2.09
3-13	Diet #5 + .16 percent methionine + .10% glycine	84.2	162	436 CDcd	262	549	2.17	2.00
3-14	Diet #11 + Lincomycin, 50 gms/ton	88.7	169	462 Ccd	276	545	2.17	1.86
3-15	Diet #12 + Lincomycin, 50 gms/ton	82.9	152	384 EFf	242	478	2.20	2.06
3-16	Diet #13 + Lincomycin, 50 gms/ton	89.5	173	465 Cc	268	545	2.03	1.87

* Means having common capital or small letters are not significantly different while means not having common letters are significantly different at the .01 and .05 levels of probability, respectively.

Table 3-3. Analysis of Variance of Four-week Weights of Chick Experiment With Raw Soybeans Treated With Heat, Amino Acids, and Antibiotics

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	467	4,484,402		
Subclass	47	2,620,389	55,753	12.56**
Treatments	15	2,397,009	159,801	36.00**
Replications	2	16,906	8,453	1.90
T X R (Int.)	30	206,474	6,882	1.55*
Error	420	1,864,013	4,438	

Standard error of mean = 12.32

* Significant at the .05 level of probability

** Significant at the .01 level of probability

Table 3-4. Average Body Weights and Microhematocrit Values at 8 and 9 Weeks of Age, Respectively

Diet	Weight (gms)*	Hematocrit (%)
3-1	1455 ABCDbc	28.9
3-2	1665 Aa	31.0
3-3	1462 ABCDbc	31.0
3-4	1513 ABab	30.4
3-5	1022 Fg	29.0
3-6	1258 CDEFdef	--
3-7	1274 BCDEcdef	31.9
3-8	1182 EFefg	--
3-9	1246 CDEFdef	--
3-10	1218 DEFdef	30.4
3-11	1400 BCDbcd	29.5
3-12	1292 BCDEcdef	29.0
3-13	1365 BCDEbcde	29.5
3-14	1482 ABCb	28.4
3-15	1164 EFfg	29.1
3-16	1497 ABCab	29.9

* Means having common capital or small letters are not significantly different while means not having common letters are significantly different at the .01 and .05 levels of probability, respectively.

Table 3-5. Analysis of Variance of Eight-week Body Weights

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	98	4,457,020		
Treatments	15	2,677,040	178,469	8.32**
Error	83	1,779,980	21,446	

Standard error of mean = 59.2

** Significant at the .01 level of probability

Experiment IV -- Supplementation of Raw Soybean Rations With Iodinated Protein, Tolbutamide, Methionine and Feces

Studies by Beck (1958) and also Van Middlesworth (1957), indicated that increased fecal loss of thyroxin occurred in animals that were fed raw soybean protein. Many plants have an effect on the thyroid gland. In cabbage and similar plants, cyanogen compounds are present which can cause goiter in animals. Also, methyl cyanide is very active in this respect. Also known are several anti-thyroid drugs. Included among these are thiourea and thiouracil, as well as some other compounds such as PABA and sulfonamides. Anti-thyroid drugs act possibly by inhibiting the peroxidase enzyme systems of the thyroid (Guyton, 1958). In general, a thyroid deficiency slows growth and this is, of course, one of the symptoms from the feeding of raw soybeans. Iodinated proteins such as casein and serum protein have a thyroxin-like activity; therefore, the compound Protamone, an iodinated protein having one percent of thyroxin activity, was used in this study.

With the observation that the raw soybean rations caused an enlarged pancreas and the postulation that this was affecting the enzyme secretion, the question also arose as to whether the secretion of insulin was being affected. If an insulin deficiency, or diabetes, was being produced by the feeding of raw soybeans, this could account for many of the problems which were found to exist. It has been reported that the administration of insulin improves the appetite in that hypoglycemia causes hyperactivity of the stomach and insulin thus increases the gastric activity (Guyton, 1958). The compound aloxan (mesoxalyl urea) does not affect the beta cells of the pancreata of birds; whereas, in mammals this compound is used to destroy the insulin-producing cells.

In humans having diabetes, the compound tolbutamide, more commonly known as orinase, is widely used. The drug is an oral hypoglycemic and is very effective in the control of certain types of diabetes. However, its effect in the fowl has not been too widely studied and where it has been studied, it has been done mostly with intravenous injections. Studies by Mirsky and Gitelson (1957) indicated that the fowl may have a source of insulin other than the pancreas. Work by Hazelwood and Lorenz (1957) indicated that there was a response from the use of orinase in poultry but that younger birds were resistant to its effect. Mirsky and Gitelson (1958) later reported that diabetes could be produced in a goose but not in a chick or a duck. In order to study the effects of orinase, it was added to rations containing raw soybeans in this experiment.

In addition, with the indication that the protein might be very poorly utilized in rations containing the raw soybeans, it was thought desirable to feed the droppings from chicks back to these same chicks. Droppings from other groups of chicks of an older age which were receiving raw ground soybeans were also fed to younger chicks in order to supply the protein which might not be utilized in the first passage through the intestinal tract.

Since the corn which was incorporated into most of our experimental rations could have had an effect on the results which were previously obtained, two additional rations containing raw ground soybeans and cerelose (glucose) were mixed. One of these rations was supplemented with .32 percent DL-methionine. A positive control ration containing autoclaved soybeans was also included in the experimental design.

For this experiment 396 chicks which were four days old were randomly distributed into each of 36 pens in six starting batteries. These chicks were produced from DeKalb pullets crossed with White Leghorn males and were straight-run. The basal ration was made up of corn, ground soybeans, alfalfa, vitamins and minerals but did not contain any fish meal as the previous experimental rations had contained. In addition, two rations having protein from raw soybeans and alfalfa only were used. Cerelose was used as the energy source in these rations. The composition of these basal rations is shown in Table 4-1. The several variables in these experimental rations were produced by addition of the different experimental compounds to the ration. The experimental design of this trial is shown in Table 4-2.

In diet 4-3, the chicks were fed rations containing the raw soybean meal and each day their droppings and wasted feed were removed from the dropping pan and added back to the feed hopper trough. In diet 4-4, the droppings from a pen of eight Leghorn cockerels which were four weeks older were collected daily and added to feed troughs of the younger chicks. These four-week old cockerels were receiving a ration which contained raw soybeans (diet 4-2).

The body weights and feed consumption are shown in the same table as the experimental design while the analysis of variance of body weights is shown in Table 4-3. Also, from these birds the blood sugar levels were determined and pancreata, proventriculi, and livers weighed. These results are shown in Tables 4-4 and 4-5.

It may be observed that there was little or no effect on the blood glucose level due to the addition of the oral hypoglycemic or to the addition of protamone into the feed. However, there was a trend for the supplementation of 0.32 percent methionine to increase the glucose level of the birds receiving the raw soybean-corn ration. This was not so in birds receiving the ration containing no corn. Of course, the increased blood glucose level with the added methionine might be expected since methionine has been classified as a sugar-producing (glycogenic) amino acid. The weights of the livers as a percentage of body weights were not greatly affected by the different experimental treatments. However, the pancreas weights were tremendously affected, both as a total weight and as a percentage of the body weight. Methionine supplementation greatly increased the body weights of the birds receiving raw soybean rations. However, the weight of the pancreas in these methionine supplemented birds was greater than that of the birds which did not receive the methionine supplementation. As compared to the weights of birds receiving raw soybeans, only methionine supplementation increased the body weights significantly in this experiment. This improvement was noted both with the ration containing corn and especially the one using glucose as the source of energy. The raw soy-glucose control ration produced significantly ($P < .01$) smaller birds than did the raw soy-corn control ration while the weights of birds produced by these two rations were not different when the rations were supplemented with methionine. The addition of the droppings back to the feeds did not significantly affect the rates of growth of birds receiving rations containing raw soybeans.

Table 4-1. Composition of Rations Used in Experiment IV

Ingredients	Percent of rations: Diets 4-1 & 4-2	Percent of rations: Diets 4-11 & 4-12
Soybeans, raw or autoclaved, ground	41.00	54.00
Corn, ground yellow	53.85	--
Cerelose (glucose)	--	40.35
Alfalfa leaf meal, 20% protein	2.00	2.00
Vitamin-trace mineral mix , Nopcosol M-4	.25	.25
Salt, iodized	.50	.50
Limestone, ground	1.20	.80
Dicalcium phosphate, ground	1.20	2.10

Calculated analyses for rations:

Protein	%	20.83	20.92
Fat	%	9.47	9.75
Fiber	%	3.75	3.06
Calcium	%	.91	1.02
Phosphorus	%	.61	.71
Arginine	%	1.20	1.29
Glycine	%	.98	1.09
Methionine	%	.39	.37
Cystine	%	.30	.29
Lysine	%	1.06	1.25
Tryptophan	%	.23	.25
Productive energy, Cal/lb		1031	982

Table 4-2. Body Weights of Birds on Experiment Using Orinase, Protamone and Methionine in Raw Soybean Rations

Diet	Composition	Av. body wt. (gms) 1 wk.	2 wk.	Av. 4-wk. body wt. (gms) Male	Female	Sex Av.*
4-1	Positive control (autoclaved soy with corn)	78	135	266	256	261 Aa
4-2	Raw soybean-corn control	56	85	178	136	157 Ccd
4-3	" " " " + own droppings fed back daily	57	84	157	133	145 CDde
4-4	" " " " + other droppings	56	81	153	142	147 CDd
4-5	" " " " + protamone, 50 mg/Kg feed	58	84	157	143	150 CDcd
4-6	" " " " + orinase, 50 mg/Kg feed	59	87	173	143	158 Ccd
4-7	" " " " + protamone, 150 mg/Kg feed	58	86	173	132	153 CDcd
4-8	" " " " + orinase, 500 mg/Kg feed	59	88	181	148	165 Cc
4-9	" " " " + orinase (500) + protamone (150)	58	87	170	144	157 Ccd
4-10	" " " " + .32% methionine	65	107	226	238	232 Bb
4-11	Raw soy-cerelose control	54	75	148	117	133 De
4-12	Raw soy-cerelose control + .32% methionine	69	112	240	222	231 Bb

* Means having common capital or small letters are not significantly different while means not having common letters are significantly different at the .01 and .05 levels of probability, respectively.

Table 4-3. Analysis of Variance of Final Body Weights in Experiment Using Hormone-like Compounds, Methionine and Feces

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	357	925,042		
Subclass	23	660,390	28,713	39.33**
Treatments	11	600,462	54,578	74.76**
Sex	1	41,527	41,527	56.89**
T X S (Int.)	11	18,401	1,673	2.29*
Error	333	243,950	730	

Standard error of mean = 4.95

* Significant at the .05 level of probability

** Significant at the .01 level of probability

Table 4-4. Weights of Proventriculi, Livers and Pancreata of Birds After Different Lengths of Time on Experiment

	After 10 days						After 31 days					After 63 days		
	Proventriculi			Pancreata			Pancreata and livers					Pancreata		
								Av. wt. (gms)		% of body				
Diet	No. samples	Wt. (gms)	As. % of body	No. samples	Wt. (gms)	As % of body	No. samples	Panc.	Liver	Panc.	Liver	No. Samples	Wt. (gms)	As % of body
4-1	1	.81	.80	3	.52	.52	11	1.27	7.91	.37	2.32	3	1.70	.25
4-2	1	.77	1.08	2	.60	.93	8	1.60	7.12	.66	2.91	4	2.64	.61
4-3	1	.57	.88	2	.58	.85	2	1.82	--	.85	--	-	--	--
4-4	-	--	--	-	--	--	4	2.02	6.28	.93	2.89	-	--	--
4-5	-	--	--	-	--	--	-	--	--	--	--	4	2.35	.59
4-7	1	.74	1.05	2	.71	.92	4	1.95	6.33	.86	2.79	-	--	--
4-8	1	.75	1.05	2	.66	.91	4	1.49	5.05	.76	2.57	4	2.50	.64
4-9	-	--	--	-	--	--	2	2.03	--	.76	--	4	4.08	.60
4-10	1	.73	.84	2	.84	1.06	8	2.01	8.09	.62	2.48	-	--	--
4-11	1	.56	.94	2	.59	.88	4	1.83	4.90	1.15	3.07	4	2.80	.70
4-12	1	.77	1.01	2	.79	1.00	4	2.43	7.88	.74	2.41	3	4.41	.70

Table 4-5. Summary of Blood Glucose Levels in Six-week-old Birds Receiving Different Rations

Diet	No. samples	Av. blood glucose (mg%)
4-1	3	177
4-2	4	171
4-6	4	165
4-7	3	160
4-8	3	180
4-10	3	214
4-11	4	188
4-12	3	154

Experiment V -- Supplementation of a Raw Soybean Ration With a Surface-active Agent

The research by Nesheim *et al*. (1962) indicated that the fat from raw soybeans was very poorly absorbed in young chicks. Fat breakdown and absorption was dependent not only on the emulsifying action of the bile but also upon the digestive enzymes which are produced by the pancreas. The previous experiments had shown that the size of the pancreas was greatly affected when raw soybeans were fed. It was possible that the presence of this fat which was not absorbed slowed the passage of the feed through the intestinal tract and thus the birds were unable to eat enough feed for their protein, energy and other needs. Thus, this could bring about a greatly reduced rate of growth due to the lack of feed consumption or the loss of appetite. This theory cannot be disproven from the data as shown since in almost all cases where growth depressions occurred, a great reduction in feed consumption occurred. Whether the decreased feed consumption is a cause or effect cannot be determined from the data secured. Therefore, for this study an emulsifying or surface-active agent was added to certain of the rations. The detergent had been previously used in other experiments in preliminary trials and had shown a slight benefit in rations containing soybean oil meal. This detergent was supplemented at the .05 percent level to rations containing soybean oil meal and oil, autoclaved ground soybeans, raw ground soybeans and raw ground soybeans plus supplemental methionine. The basal rations were the same as used in experiment II and are shown in Table 2-1.

Single Comb White Leghorn cockerels which were five days of age were distributed into 3-gram weight groups and from these groupings were randomly distributed into the various experimental pens in chick starting batteries. Three different pens of chicks with eight birds per pen received each of the experimental rations. The experimental design, body weights, feed consumption and feed efficiency data are shown in Table 5-1 while the analysis of variance of the 4-week body weights is shown in Table 5-2.

The analysis of variance revealed that most of the differences in the experimental treatments were due to the four rations used and not to the supplemental detergent which was used with each of these four rations. In fact, very little differences were noted due to the supplemental detergent. The interaction between rations and detergents was not significant. When the effect due to detergent was disregarded and the experimental treatments were pooled so that a total of four treatments were present, the weights of the birds receiving soybean oil meal plus the oil were significantly higher than weights of any other groups of birds. Next the weights of the birds receiving the autoclaved soybeans were significantly lower at the .01 level of probability than the weights of the birds which had received the heated soybean oil meal plus the oil. The weights of the birds which received the raw ground soybeans plus the supplemental methionine were not significantly different from the weights of the birds which had received the autoclaved ground soybeans. The weights of the birds fed raw ground soybeans were significantly lower than the weights of the other three groups of birds. Although the weights were lower for the birds receiving the autoclaved ground soybeans as compared to the commercially produced soybean oil meal with oil, feed consumption was not depressed. However, when the raw soybean rations were used, feed consumption was depressed. The supplemental methionine combined with the raw ground soybean ration greatly improved feed consumption as well as body weight. Feed efficiency, in general, was affected by the rate of gain as in previous experiments.

Since great improvements in growth were produced by methionine supplemenetation of the ration containing raw soybeans, it was thought desirable to determine if the additional methionine was in some way affecting fat metabolism. Fat in raw soybeans has been found to be poorly utilized. Therefore, silicic acid columns and gas-liquid chromotography were used to study the lipid components from two groups of chicken livers. Livers were taken from five-week-old birds which had received the rations containing raw soybean -- both with and without supplemental methionine.

The fresh livers from each group were pooled, homogenized, shell frozen and lyophilized. The fats were then extracted and separated into components by chromotography in a silicic acid column with the use of a series of solvents. Also, samples of the fats were methylated and the methyl esters of the fatty acids were separated and the percentages of each determined with an Aerograph gas-liquid chromatograph.

The results of these studies are shown in Tables 5-3 and 5-4. In general, the composition of the lipids from the two groups of chicks was similar. The livers from the birds which had received additional methionine appeared to contain less sterol esters and lecithins but more cephalins. However, additional observations would have to be made to decide if these were significant differences.

Results from the gas-liquid chromatographic procedures revealed that the fatty acid compositions of the two groups of livers were almost identical when the averages were considered. There were considerable variations between the individual runs or replications from the same sample.

These data indicated that the lipid components of the livers of chicks receiving rations containing raw soybeans were not influenced greatly by supplemental methionine.

Table 5-1. Results of Experiment With Leghorn Cockerels Receiving Different Soybean Rations and a Detergent

Diet	Composition	Average wts. (gms) 1 wk.	2 wks.	4 wks.*	Feed cons./bird 0-2 wks.	2-4 wks.	Feed/gain 0-2 wks.	2-4 wks.
5-1	Soybean oil meal + oil	92	156	336 ABab)	220	397	1.94	2.20
				345 Aa				
5-2	Diet 1 + .05% detergent (Solar F-221)	92	161	354 Aa)	208	411	1.74	2.13
5-3	Autoclaved ground soybeans	91	150	316 BCbc)	245	393	2.26	2.37
				309 Bb				
5-4	Diet 3 + .05% detergent	88	146	303 BCc)	223	421	2.16	2.68
5-5	Raw ground soybeans	70	120	223 Dd)	210	310	3.01	2.84
				231 Cc				
5-6	Diet 5 + .05% detergent	72	114	239 Dd)	202	284	2.84	2.27
5-7	Diet 5 + .16% methionine	79	138	300 BCc)	195	401	2.04	2.47
				295 Bb				
5-8	Diet 7 + .05% detergent	78	137	290 Cc)	227	371	2.41	2.61

* Means having common capital or small letters are not significantly different while means not having common letters are significantly different at the .01 and .05 levels of probability, respectively.

Table 5-2. Analysis of Variance of Four-week Body Weights of Leghorn Cockerels on Detergent and Soybean Experiment

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	186	731,772		
Subclass	23	372,847	16,211	7.36**
Treatments (8)	7	326,693	46,670	21.19**
Rations (4)	3	316,820	105,606	47.96**
Detergent vs none	1	31	31	.01
R X D (Int.)	3	9,842	3,281	1.49
T X R (Int.)	14	42,537	3,038	1.38
Replications	2	3,617	1,809	.82
Error	163	358,925	2,202	

Standard error of means for eight treatments = 9.71;
for four treatments = 6.86

** Significant at the .01 level of probability

Table 5-3. Results of Separation of Components of Chicken-liver Lipids by Silicic Acid Columns

Component	Weight (gm) and Percent of Fractions			
	Group 5-5		Group 5-7	
Hydrocarbons	.008 gm	4.1%	.008 gm	4.3%
Sterol esters	.015	7.7	.008	4.3
Triglycerides	.020	10.3	.020	10.7
Sterols	.012	6.2	.013	7.1
Mono- and di-glycerides	.005	2.6	.004	2.2
Cephalins	.080	41.2	.089	48.4
Lecithins	.054	27.8	.042	22.8

Table 5-4. Results of Gas Chromatographic Analyses of Fats From the Livers of Two Groups of Chickens

Group	Run	Fatty acid methyl esters as percentages of totals			
		Linoleic	Oleic	Stearic	Palmitic
5-5	1	34.9	11.9	27.8	25.4
	2	31.2	14.1	28.6	26.0
	3	28.1	14.3	32.2	25.2
	4	32.5	14.7	29.2	23.7
	5	40.7	12.2	24.5	22.5
Average		33.5	13.4	28.4	24.6
5-7	1	32.3	12.8	29.4	25.5
	2	37.1	12.2	26.3	24.5
	3	26.8	17.2	33.1	22.9
	4	35.9	12.7	25.8	25.4
	5	25.7	21.9(omit)	27.8	24.6
Average		31.5	13.7	28.5	24.6

PART II - EXPERIMENT WITH RATIONS FOR TURKEY POULTS

Experiment VI -- Antibiotic and Methionine Supplementation of Raw Soybean Rations for Turkey Poults

This experiment was conducted in order to determine if the effects observed with chickens receiving raw soybeans would apply also to young turkey poults. Since the protein requirement for young turkeys is much higher than for chicks, their response to the supplementation of the raw soybean diet with antibiotics or methionine could be more dramatic than that of chicks. Since the antibiotic, Neomycin, is sometimes used to sterilize the intestinal tract of human as well as that of animal patients, this antibiotic at the .05 percent level of the ration was used with both raw soybean and autoclaved soybean rations. A feed grade mixture known as Neomix[1] (50% Neomycin) was used. It was also used in combination with methionine when added to the raw soybean ration. Penicillin[2] was incorporated into one ration -- also at the .05 percent level.

Broad Breasted Bronze and Beltsville Small White turkey poults which were one week of age were randomly distributed into 16 pens in starting

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[1] Neomix: Upjohn Company, Kalamazoo, Michigan
[2] Penicillin, P-50, Chas. Pfizer and Co., Terre Haute, Indiana

batteries. Both Bronze and White poults were placed in each of the pens. With one replication, each pen contained five Bronze and five White poults; whereas, in the second replication, six Bronze and four White poults were placed in each pen. For these 16 pens of birds, eight different experimental diets were prepared; thus, each diet was fed to two pens of birds. The basal ration is shown in Table 6-1 and the experimental outline and the body weights for both varieties of turkeys as well as feed efficiency are shown in Table 6-2.

It was interesting to note that at the .05 percent level of probability, the Neomix supplementation improved the rate of growth of the positive control ration containing soybeans; whereas, it significantly depressed the body weights of the birds which received the ration containing raw soybeans. However, when methionine supplemented the raw soybean ration, a significant improvement in the body weights was obtained. When the Neomix was combined with methionine, the Neomix significantly improved the rate of growth. Penicillin was not effective in improving the rate of growth at the level used. The supplementation of .32 percent of methionine did not produce weights which were significantly different from those produced by the supplementation of .16 percent methionine. The body weights produced by the birds receiving the raw soybean rations supplemented with both methionine and Neomix were not significantly different from the weights produced by the unsupplemented positive control ration (diet 6-1). The analysis of variance of the body weights is shown in Table 6-3.

Pancreata weights were taken both in the wet form as taken from the bird and also after drying. These weights are shown in Table 6-4. Once again it was observed that the feeding of the raw soybean rations greatly increased the size of the pancreas -- both in total weight and also in weight as a percentage of body weight. These pancreata were dried in an oven at 70° C. for 24 hours in order to determine the moisture content and thus decide if the additional weight was from excess water absorbed into these pancreata or from non-water products such as protein. The tables show the weights of the pancreata in both the as-is (wet) compared to the dry form and also the pancreata as percentages of body weights. Also shown are the weights of the dry pancreata as percentages of the wet pancreata weights. These dry weights as a percentage of the wet pancreata weights were very consistent, ranging around 23 to 25 percent with little or no variation. This indicated that the enlarged pancreata in some treatments were not due to excess water retention but probably to a protein component.

Table 6-1. Composition of Basal Ration Used for Poults in Experiment VI

Ingredients	Percent of ration Diet 6-1 and Diet 6-3
Soybeans, raw or autoclaved, ground	66.0
Corn, ground yellow	26.7
Alfalfa leaf meal, 20% protein	2.0
Vitamin-trace mineral mix, Nopcosol M-4	.4
Salt, iodized	.5
Limestone, ground	.5
Dicalcium phosphate, ground	2.4
Animal fat	1.0
Chromium oxide	.5

Calculated analyses for rations:

Protein	%	28.00
Fat	%	13.89
Fiber	%	4.36
Calcium	%	1.00
Phosphorus	%	.91
Arginine	%	1.67
Glycine	%	1.40
Methionine	%	.50
Cystine	%	.40
Lysine	%	1.56
Tryptophan	%	.32
Productive energy, Cal/lb		1018

Table 6-2. Results of Experiment Using Two Varieties of Turkey Poults With Different Experimental Rations

Diet	Composition	25-day body wts. Whites (gms)	25-day body wts. Bronze (gms)	25-day body wts. Var. Av. (gms)	43-day body wts. Whites (gms)	43-day body wts. Bronze (gms)	43-day body wts. Var. Av.* (gms)	Feed/gain 9-43 days
6-1	Positive control (autoclaved ground soybeans)	331	453	392	657	898	778 ABb	1.91
6-2	Positive control + neomix .1% (.05% neomycin base)	379	482	431	741	1003	872 Aa	1.83
6-3	Raw soybeans control	196	272	234	440	660	550 DEde	2.51
6-4	" " " + neomix .1%	199	256	228	383	533	458 Ef	2.47
6-5	" " " + methionine .16%	218	356	287	488	783	636 CDc	2.45
6-6	" " " + methionine .16% + neomix .1%	274	407	341	578	890	734 BCb	2.16
6-7	Raw soybeans control + .05% penicillin base (.165% P-50)	193	262	228	396	550	473 Eef	2.36
6-8	Raw soybeans control + methionine .32%	258	365	312	539	726	633 CDcd	2.25

* Means having common capital or small letters are not significantly different while means not having common letters are significantly different at the .01 and .05 levels of probability, respectively.

Table 6-3. Analysis of Variance of White and Bronze Poult Weights in Experiment Using Antibiotics and Methionine

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	140	6,363,814		
Subclass	15	4,607,948	307,197	21.87**
Treatment	7	2,734,174	290,596	27.81**
Variety	1	1,766,292	1,766,292	125.74**
T X V (Int.)	7	107,482	15,355	1.09
Error	125	1,755,866	14,047	

Standard error of a treatment mean = 28.3

** Significant at the .01 level of probability

Table 6-4. Pancreata Weights of Poults After Four Weeks on Experimental Feeds

Diet	Body wts. (gms)	Pancreas wt. (gms) As is	Pancreas wt. (gms) Dry	Pancreas as % of body wt. As is	Pancreas as % of body wt. Dry	Dry as % of as is
6-1	590	1.67	.43	.28	.07	25.7
6-2	640	1.66	.42	.26	.07	25.3
6-3	360	3.51	.88	.98	.24	25.1
6-4	345	4.25	1.00	1.23	.29	23.5
6-5	520	6.19	1.47	1.19	.28	23.7
6-6	725	8.19	1.96	1.13	.27	23.9
6-7	535	4.21	1.00	.79	.19	23.8
6-8	570	6.19	1.48	1.09	.26	23.9

PART III - EXPERIMENT WITH RATIONS FOR LEGHORN-TYPE PULLETS

Experiment VII -- Feeding Raw Soybean Rations to Pullets Starting at Various Ages From 9 to 19 Weeks

Since the previous experiments with younger chicks had shown a severe growth depression when raw soybeans were contained in the ration, this raw soybean product was incorporated into the rations of older chicks. For years, it has been recommended that the sexual maturity and the rate of growth and development of pullets which are to be used for laying stock be restricted in order to obtain better results once the hen reaches the laying house. This has been accomplished in a variety of ways, either by reducing the caloric or protein content of the feed or by manually restricting the amount of feed which the birds are fed. Also, decreasing light has been used to delay the onset of sexual maturity of pullets. Therefore, raw ground soybeans were combined into the feed of developing pullets to determine if a delay in sexual maturity would be obtained. If this delay of sexual maturity were obtained, the further effects during the early laying life of the bird would be studied.

For this experiment, pullets produced from a cross of DeKalb pullets mated with Single Comb White Leghorn males were used. These birds were nine weeks of age when some of the experimental treatments were started. The birds were randomized into weight groups and distributed into thirty experimental pens in chick finishing batteries with ten birds in each pen. Each of the experimental feeds was fed to three groups of birds. A practical type ration (appendix Table 2) was used as a control. This ration was a fairly low-energy ration and was of a type that was commonly used in growing pullets to be used for egg production. In addition to this ration, a higher-energy ration containing soybean oil meal and soybean oil was used and this ration was isocaloric and isonitrogenous to the rations which contained raw ground soybeans. The rations containing raw soybeans were started at various times from when the birds were 9 to 19 weeks of age. A different group of birds was started every two weeks during this period. One group of birds was fed the raw soybean ration starting at nine weeks of age with supplemental methionine at the .16 percent level. Until the time when the birds were started on the experimental rations, they received the practical control ration (the low-energy ration). Thus, some birds received this ration only to nine weeks of age and some received it to as late as 19 weeks of age. Feed and water were supplied *ad libitum* to all groups of birds. The birds were also placed on a restricted light program during which the length of day was gradually decreased over the period from 9 to 21 weeks of age. The birds were weighed at two-week intervals and feed consumption for these periods was also determined. In addition, individual body weights were obtained at 21 weeks of age.

The composition of the experimental rations are shown in Table 7-1 while the body weights and feed efficiencies of the birds for the different

periods of time are presented in Table 7-2. The analysis of variance for body weights revealed no significance due to treatments (Table 7-3).

When the birds were 21 weeks of age and the individual body weights had been obtained, birds from groups 1, 2, 3, 4, 7 and 9 were transferred to laying cages with one bird in each individual cage. A total of 22 birds for each of the above six groups of birds were continued into egg production. These groups were placed on a standard laying ration for three weeks in order to determine the age at which egg production started. After this three-week experimental period the birds were switched to three experimental laying rations -- one a positive control ration, another ration containing raw soybeans and still a third ration containing raw soybeans plus DL-methionine and an antibiotic, virginiamycin. (See phase II of experiment IX for the compositions of these rations.) These three rations were used to determine if there was a difference in the response of the birds to the raw and cooked soybeans due to the rations which they had been fed during their developing period. The possibility existed that the birds which had received the raw soybeans during the growing period had been sensitized or desensitized to the raw soybeans and thus the difference in the response would be observed when the birds were switched to the raw soybean rations after egg production had commenced.

The age at onset of egg production is shown in Table 7-2 and the differences were non-significant (Table 7-4). The summary of the egg production of the birds after switching to the three experimental laying rations for two months is shown in Table 7-5. The analysis of variance of these data is shown in Table 7-6 and reveals no significant differences due to any of the variables compared. However, with the small number of birds in this evaluation, rather large differences between treatments or very little variance within treatments would be required to reveal significance. There was a trend toward lowered production, however, with the birds which had received the raw soybean rations -- starting at 9, 15, and 19 weeks of age (groups 7-3, 7-7, and 7-9). Also, the birds that received laying ration 9-A obviously produced more eggs than did the birds receiving the raw soybean laying rations (diets 9-B and 9-C).

Table 7-1. Composition of Rations for Pullet Experiment

Ingredients	Percent of rations Diet 7-2	Diet 7-3
Soybeans, raw, ground	--	22.7
Soybean oil meal, 44% protein (cooked)	18.7	--
Corn, ground yellow	67.7	67.7
Animal fat	4.0	--
Alfalfa meal	2.5	2.5
Meat and bone meal, 50% protein	2.5	2.5
Whey, dried	1.0	1.0
Limestone, ground	1.0	1.0
Dicalcium phosphate	2.0	2.0
Salt, iodized	.4	.4
Vitamin-trace mineral mix, Nopcosol M-5	.25	.25

Calculated analyses for rations:

Protein	%	16.20
Fat	%	6.95
Fiber	%	3.51
Calcium	%	1.27
Phosphorus	%	.82
Arginine	%	.91
Glycine	%	.82
Methionine	%	.32
Cystine	%	.24
Lysine	%	.76
Tryptophan	%	.17
Productive energy, Cal/lb		1035

Table 7-2. Results of Pullet Experiment Using Raw Soybean Rations at Various Ages

Treatment no.	Composition of diet	Age started (wks)	Av. body weights at given ages 11 wk.	13 wk.	15 wk.	19 wk.	21 wk.	Age at first egg (days)
7-1	Practical control	9	2.03	2.48	2.88	3.26	3.64	163.5
7-2	High energy with cooked soy	9	2.05	2.53	2.97	3.42	3.74	162.6
7-3	As 2, except raw soybeans	9	1.98	2.42	2.84	3.29	3.65	167.9
7-4	As 3 + .16% methionine	9	2.03	2.43	2.86	3.28	3.79	160.3
7-5	As 3 (raw soybeans)	11	2.03	2.45	2.82	3.23	3.59	--
7-6	As 3 " "	13		2.45	2.88	3.32	3.64	--
7-7	As 3 " "	15			2.87	3.31	3.68	166.7
7-8	As 3 " "	17			2.95	3.25	3.57	--
7-9	As 3 " "	19				3.23	3.67	166.1
7-10	Practical control (G-62)	9				3.27	3.68	--

Table 7-3. Analysis of Variance of 21-week Body Weights of Pullets Started on Raw Soybean Rations at Different Ages

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	265	28.40		
Subclass	29	4.77	1.64	16.4**
Treatments	9	.85	.09	.9
Replications	2	1.09	.55	5.5**
T X R (Int.)	18	2.83	.16	1.6
Error	236	23.63	.10	

** Significant at .01 level of probability

Table 7-4. Analysis of Variance of Age in Days at Which Pullets on Six Developer Rations Started Egg Production

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	128	16,049		
Treatment	5	885	177	1.44
Error	123	15,164	123	

Table 7-5. Percent Egg Production on Hen-day Basis for Two Months and Egg Size From One Day's Production

Pullet group no.	Laying ration number 9-A	9-B	9-C	Mean
7-1	81.6	73.8	80.7	78.7
7-2	75.8	77.0	69.3	74.0
7-3	63.1	69.0	66.4	66.2
7-4	79.1	82.8	67.2	76.4
7-7	76.6	58.2	59.8	64.9
7-9	75.4	59.0	68.4	67.6
Mean	75.3	70.0	68.7	
Egg wt. (gms)	50.8	50.9	51.5	
No. eggs	31	22	24	

Table 7-6. Analysis of Variance of Egg production for Two Months by 72 Pullets

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	71	6,042		
Subclass	17	1,596	93.9	1.14
Groups	5	756	151.2	1.84
Diets	2	220	110.0	1.34
G X D (Int.)	10	620	62.0	.75
Error	54	4,446	82.3	

PART IV - EXPERIMENTS WITH RATIONS FOR LAYING HENS

Experiment VIII -- The Effects of Autoclaving, Pelleting and Supplementation With Zinc, Phosphorus, Methionine and Antibacterial Agents on Hen Rations Containing Raw Soybeans

Phase I

For this experiment, DeKalb pullets were hatched early in March, 1961. They were brooded in confinement for ten weeks and were then range-reared until egg production commenced late in July. The birds were moved into 36 experimental pens containing wood shavings for litter. A pre-experimental period of egg production was continued until October 31 at which time an attempt was made to equalize the 36 pens as to egg production and body weights with 24 birds in each pen.

The experimental rations were started on November 1, 1961 with the 12 original experimental variables. The experimental rations are shown in Table 8-1. Raw ground soybeans were incorporated into simplified rations (corn, soy, vitamins, and minerals) and also more conventional rations containing animal proteins. To determine the effects of methionine

Table 8-1. Composition of Original 12 Experimental Rations for Experiment VIII

Ration no.	Composition[1]
8-1	Corn, raw ground soybeans simplified ration
8-2	Corn, autoclaved ground soybeans simplified ration
8-3	Positive control ration using 44% protein soybean meal and added fat
8-4	As 8-3, except using autoclaved ground soybeans
8-5	" " " " ½ " and ½ raw ground soybeans
8-6	As 8-3, except using only raw ground soybeans
8-7	As 8-6 plus pelleting
8-8	As 8-6 plus .08% methionine
8-9	As 8-8 plus pelleting
8-10	As 8-6 plus arsanilic acid (1 lb. Progen/ton)
8-11	As 8-6 plus nitrofuran (1 lb. nf-180/ton)
8-12	As 8-6 plus virginiamycin (50 gms/ton)

[1] See Table 8-2 for the exact amounts of ingredients used.

Table 8-2. Composition of Layer-breeder Rations for Experiment VIII

Ingredients	Percent of ration no. 8-1	8-2	8-3	8-4	8-5	8-6
Corn, ground yellow	66.6	66.6	67.0	67.0	67.0	67.0
Raw soybeans, ground	27.0	--	--	--	11.5	23.0
Cooked soybeans, ground	--	27.0	--	23.0	11.5	--
Soybean oil meal, 44% protein	--	--	19.0	--	--	--
Fish meal & solubles, 60% protein	--	--	2.0	2.0	2.0	2.0
Alfalfa leaf meal, 20% protein	--	--	2.0	2.0	2.0	2.0
Limestone, ground	5.3	5.3	5.2	5.2	5.2	5.2
Dicalcium phosphate, ground	1.5	1.5	1.2	1.2	1.2	1.2
Salt, iodized	.25	.25	.25	.25	.25	.25
Animal fat	--	--	4.0	--	--	--

Calculated analyses for all rations:

Protein	%	16.1
Calcium	%	2.48
Phosphorus	%	.60
Arginine	%	.90
Glycine	%	.75
Methionine	%	.31
Cystine	%	.24
Lysine	%	.74
Tryptophan	%	.17
Productive energy, Cal/lb		1035

and three anti-bacterial compounds, supplements of methionine, arsanilic acid, nf-180 and virginiamycin were used with the rations containing raw ground soybeans. Part of the rations were pelleted to determine if the heat and pressure of the pelleting process were effective in improving the utilization of the raw soybean protein.

The different rations used were calculated to be isocaloric and isonitrogenous. This was accomplished in ration 3 by adding animal fat with the 44-percent-protein soybean meal to bring the energy level up to that of the rations containing the ground soybeans, either raw or autoclaved. The ether extract of the whole beans used was 17.7 percent and the protein content was 38.5 percent. The exact compositions of the basal rations used are shown in Table 8-2.

The criteria for measuring the relative values of the different rations were rate of egg production, efficiency of feed utilization, rate of mortality, egg size, fertility, and hatchability of eggs from the hens receiving the experimental rations. Initial and final body weights of the experimental birds were also determined.

On November 1, when the experimental rations were fed, all groups of birds were producing eggs at a high rate, approximately 70 percent. Soon after starting the experimental feeds, the rate of egg production of the birds receiving the raw soybean diets started dropping. The average egg production for each month is shown in Table 8-3. Only birds receiving diets 8-2, 8-3, 8-4, and 8-5 maintained egg production at about 70 percent or greater during this first month on experiment. Rations 8-1 and 8-9 allowed the most severe drops in egg production.

The rate of egg production by birds from all treatments was further lowered in the second month of this experiment (December). This drop in egg production was perhaps due to the severe winter weather at that time. Average egg production leveled off in January to that observed in December. Counter to this trend were birds receiving diets 8-9 and 8-12 which improved in production.

From observing the production through January, it is obvious that diet 8-4 containing the autoclaved whole soybean meal was about equal to diet 8-3 containing the commercially-produced soybean oil meal and animal fat. Diet 8-5 containing half autoclaved and half raw whole soybean meal was much better than diet 8-6 which contained only the raw product; perhaps the protein or amino acid needs of the birds were nearly met before the addition of the raw bean meal. The addition of virginiamycin at the level of 50 grams per ton to the ration containing the raw soybean meal dramatically improved the response to the feed. This ration (diet 8-12) produced the least drop in egg production in November and December of any ration containing raw ground soybeans. Then in January, egg production improved with diet 8-12 while it leveled off or was still becoming poorer with the birds receiving most of the other diets. For January, Diet 8-12 was only slightly less valuable than diet 8-4 as measured by egg production -- 57.3 and 59.1 percent production, respectively.

Pelleting of the rations containing the raw soybeans seemed to depress the performance of birds on these feeds. These birds had never eaten pellets before these were given. The addition of .08 percent methionine seemed to produce a small benefit to the birds receiving the all-mash but not the pelleted feeds.

Arsanilic acid was of no apparent value; nf-180 was apparently of some value as determined by egg production in December and January; while virginiamycin was of considerable benefit.

Feed efficiency for the November-December period was poor for the birds producing at a low rate. Diets 8-3 and 8-4 permitted good feed efficiency (4.35 and 4.37 pounds of feed per dozen eggs, respectively).

Phase II

Since the birds receiving many of the experimental rations were producing very poorly in January, certain changes were made in the experimental rations as shown in Table 8-7. The egg production and feed efficiency data obtained after these changes are also presented in Table 8-3. The supplemental methionine improved the performance in all cases where it was used. In fact, diet 8-12 produced egg production as good as that produced by diet 8-3. Thus, the combination of methionine and virginiamycin supplementation to a raw soybean ration improved production to a point equal to that produced by the positive control ration. Feed efficiency was equal for diets 8-3 and 8-12 during this period.

Body weights were taken at the start and end of the overall experiment. These averages are presented in Table 8-6. The birds receiving diets 8-1, 8-2, 8-5, 8-9, and 8-10 lost weight. Birds receiving diet 8-12 gained more than any other group. The differences in final body weight apparently reflected to an extent the adequacy of the rations being fed.

Eggs laid during the five-day periods in December, February, March and May were incubated and the percentage of fertile eggs and the percentage hatched of fertile eggs determined. These results are shown in Table 8-4. In general, satisfactory fertility and hatchability were produced by most of the experimental rations. However, the percentage hatch of fertile eggs produced by birds on diets 8-1 and 8-2 (the raw and autoclaved simplified rations) in February was very low. No explanation can be given for these results. Perhaps the vitamin mix may have been impotent or omitted in these feeds. Satisfactory performance was produced by these rations the following months.

Egg size was determined at two different times and these weights are shown in Table 8-5. Only small differences in mean egg size were noted.

Table 8-3. Percent Egg Production on Hen-day Basis and Feed Efficiency From Birds in Experiment VIII

Diet no.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
8-1	50.9	27.5	27.8	38.0	32.7	48.2	51.3[1]
8-2	70.5	55.4	43.0	45.5	37.8	49.5	49.0[1]
8-3	73.6	65.9	63.0	65.6	67.8	59.0[1]	56.6[1]
8-4	73.5	64.9	59.1	64.3	62.8	57.1[1]	50.2[1]
8-5	69.8	53.2	53.8	67.5	65.8	64.0[1]	55.2[1]
8-6	59.8	38.2	39.9	59.6	58.9	59.7	60.4[1]
8-7	58.3	37.3	37.4	52.3	58.9	56.8	60.1[1]
8-8	62.3	41.5	41.7	54.4	60.0	62.7	61.1[1]
8-9	46.0	30.2	34.0	53.9	52.9	56.8	63.3[1]
8-10	59.2	41.5	38.3	55.4	57.0	65.4	61.0
8-11	61.9	48.5	43.9	56.9	59.3	59.5	63.2
8-12	66.6	51.9	57.3	69.5	68.7	67.9	66.9

[1] These diets had been changed and re-allotted at this time and are listed here to show any overall carry-over effect.

Diet	Lbs. feed/doz. eggs		Diet	Lab. feed/doz. eggs	
no.	Nov.-Dec.	Jan.-May	no.	Nov.-Dec.	Jan.-May
8-1	6.89	8.49	8-7	5.88	5.57
8-2	4.68	6.12	8-8	5.04	5.66
8-3	4.35	5.13	8-9	6.66	5.82
8-4	4.37	4.82	8-10	4.72	5.44
8-5	4.80	5.20	8-11	4.74	5.84
8-6	5.13	5.79	8-12	4.77	5.13

Table 8-4. Percent Fertile and Percent Hatch of Fertile Eggs Set on Various Dates

Diet	Dec. 13 1961		Feb. 23 1962		Mar. 27 1962		May 14 1962	
8-1	80.7	84.0	92.0	35.0	82.0	90.0	95.6	90.0
8-2	96.3	93.5	85.4	35.9	78.4	69.0	93.0	95.0
8-3	94.3	96.1	84.7	73.4	91.1	94.2	--	--
8-4	95.6	96.7	94.3	89.5	93.7	95.0	--	--
8-5	93.2	97.8	95.2	85.7	94.2	97.3	--	--
8-6	91.4	95.6	92.9	88.4	96.7	93.4	91.5	96.1
8-7	92.9	95.5	95.3	92.5	96.5	95.2	94.9	96.5
8-8	91.7	97.3	91.1	89.9	95.1	96.0	92.1	96.3
8-9	89.6	92.4	91.0	85.2	91.3	91.9	99.0	94.8
8-10	87.6	99.5	90.8	93.8	95.1	99.3	95.2	94.4
8-11	93.3	98.1	96.2	95.6	94.5	91.7	90.8	95.3
8-12	91.6	96.6	94.2	84.5	92.5	92.9	90.0	90.9

Table 8-5. Average Egg Weights From Birds Receiving the Various Soybean Treatments

Diet	Dec. 4, 1961		May 7 - 11*, 1962	
	No. eggs	Av. wt. (gms)	No. eggs	Av. wt. (gms)
8-1	23	55.2	86	58.4
8-2	41	56.8	128	59.9
8-3	47	57.6	155	59.5
8-4	40	58.0	136	60.8
8-5	39	57.3	139	60.5
8-6	29	55.8	155	61.4
8-7	28	56.9	112	60.3
8-8	32	56.6	157	60.0
8-9	24	55.6	97	60.6
8-10	27	55.4	147	60.7
8-11	31	56.0	165	60.1
8-12	37	56.8	163	61.3

* The eggs weighed here were those being incubated. Eggs from some pens were not incubated due to absence of males.

Table 8-6. Average Body Weights (lbs.) of Experimental Hens

Diet	Initial	Final	Change
8-1	3.81	3.65	- .16
8-2	3.81	3.79	- .02
8-3	3.72	3.82	+ .10
8-4	3.84	3.86	+ .02
8-5	3.84	3.81	- .03
8-6	3.84	3.99	+ .15
8-7	3.85	3.91	+ .06
8-8	3.79	3.88	+ .09
8-9	3.92	3.80	- .12
8-10	3.96	3.81	- .15
8-11	3.76	4.00	+ .24
8-12	3.85	4.16	+ .31

Table 8-7. Ration Adjustments at End of January, 1962

Diet	Changes
8-1	Add 300 ppm Zn., 1.5% Dical PO_4, .1% methionine + 50 gm/ton nf-180
8-2	Add same as to Diet 8-1
8-3	No change
8-4	Add 300 ppm Zn., 1.5% Dical PO_4, .1% methionine
8-5	Add " " " " " " " "
8-6	Add a total of .16% methionine
8-7	" " " " " " + 300 ppm Zn
8-8	" " " " " " + 1.5% Dicalcium PO_4
8-9	" " " " " " + 300 ppm Zn + 1.5% Dical PO_4
8-10	" " " " " "
8-11	" " " " " "
8-12	" " " " " "

None of the above rations were pelleted

Experiment IX -- Supplementation of Raw Soybean Hen Rations With Methionine and a Detergent and the Use of Whole-grain Corn and Soybeans With a Pelleted Concentrate

Phase I

Since methionine supplementation in the previous year had proven beneficial, for this experiment, methionine was used at two levels -- .16 and .24 percent -- both with and without a surface-active agent (detergent) at the .05 percent level in the ration. Reports by several workers had indicated that the fat in raw soybeans was very poorly utilized. Therefore, the surface-active agent might help in the emulsification process of the fat globules in order to speed their absorption and thus utilization by the hen.

For this experiment, DeKalb pullets which had been hatched early in June were housed when 25 weeks of age. At this time, the birds were weighed and birds weighing less than 3.5 pounds or more than 4.5 pounds were removed. Of the remaining birds, about two-thirds of them had commenced egg production while about one-third had not yet laid. Thus, the 32 experimental pens were filled with 24 pullets each, adjusted so that in each pen 16 pullets were laying and 8 not laying. Adjustments were made also on weight so that there would be approximately the same average weight for each pen of birds.

Pens of birds which were to receive the whole grains and pellet rations were started on an adjustment period a week prior to the giving of the actual pelleted concentrate, whole grain corn and whole raw or cooked soybeans. During this period they were given an all-mash feed as well as pellets and concentrates.

On December 5th, 1962 the several different experimental feeds were offered *ad libitum*. The compositions of the basal rations are shown in Tables 9-1 and 9-2. All rations were balanced so that they were isocaloric and isonitrogenous to each other.

There was a drastic drop in egg production in the pens which received the pelleted concentrate and whole grain feeding. This occurred whether the beans were cooked or raw. The drop in egg production due to the all-mash ration containing raw soybeans, but without methionine supplementation, was not as severe as that caused by the whole grain ration. The amino acid supplementation in combination with the surface-active agent appeared to be beneficial in regard to egg production. The egg production was recorded for six months with trapping for 134 days during this period (December through May). During this six-month period of time, egg production was significantly decreased at the .01 level of probability by rations 9-3 through 9-6 and at the .05 level of probability by rations 9-3 through 9-8. Egg production of birds receiving rations 9-9, 9-10, and 9-11 which contained .16 percent methionine plus .05 percent surface-active agent, .24 percent methionine and .24 percent methionine plus .05 percent surface-active agent, respectively, was not significantly different from that of birds receiving diet 9-1 (positive control ration). Diets 9-3, 9-4 and 9-5 were significantly poorer than any other diet in this experiment.

The .16 percent level of methionine appeared to be of little or no benefit when added singly to the ration. This was also true of the .05 percent level of the surface-active agent. However, when these two components were added in combination to the ration containing raw soybeans, significantly improved egg production resulted. The rate of egg production on diet 9-9 was not different from that produced by the higher level of methionine with and without the surface-active agents (diets 9-10 and 9-11) nor from the positive-control ration, diet 9-1. These egg production results are presented in Table 9-3 and the analysis of variance in Table 9-4.

Determinations of blood pressure and of pancreas size were made on birds which were receiving diets 9-1, 9-6 and 9-7. The blood pressure was determined by the direct method in which the carotid artery was cannulated and pressure measured by the use of a strain-gauge and a polygraph. Both systolic and diastolic blood pressure were determined. There were no significant nor apparent differences in the blood pressures of the birds receiving these three rations (Table 9-5). Also, pancreas size was determined for these three groups of birds. Once, again, enlarged pancreata occurred with the feeding of the raw soybeans (Table 9-6). This increased pancreata size was very obvious, both in the actual weight of the pancreas and also in the weight of the pancreata as percentages of body weight. The methionine supplementation did not decrease the enlarged pancreas in birds receiving the raw soybean-containing ration.

Phase II

Since very good egg production was obtained from the hens on the last experiment (VIII) in which the raw soybean ration was supplemented with .16 percent methionine in combination with 50 grams per ton virginiamycin, this phase of the experiment was repeated. Three of the same rations of experiment VIII were used -- the positive control ration, the raw soybean control ration and the raw soybean control ration plus supplemental methionine and virginiamycin.

Hens from 12 pens which had been on experiment IX as just outlined were reallocated with the birds not being disturbed within a pen but with different pens being allotted to the three different rations in such a way that the production in each of the pens was approximately equal at the start of this phase of the experiment. These three ratinns were continued for four weeks and egg production was determined for this period of time. These egg production data are shown in Table 9-7 and the analysis of variance in Table 9-8.

The positive control ration produced the best results but this rate of production was not significantly different from that produced by the raw soybean-containing ration in which supplemental methionine and virginiamycin was used. The raw soybean-control ration (diet 9-B) produced significantly poorer results than did either diet A or C. This was true at the .01 level of probability. The rate of production by diet C was not different from that produced by diet A at the .01 level of probability but was significantly less at the .05 level. It appeared that switching from a cooked to a raw soybean ration at this stage of egg production was detrimental to the hens in this experiment.

Eggs were broken out for a period of 12 days toward the end of this stage of the experiment in order to determine the number of bloodspots produced by the birds receiving the various experimental treatments. In contrast to the report of workers from Washington State (Saxena et al., 1963), the percentage of bloodspots in these eggs was very low and there was no significant nor apparent increase in the incidence of bloodspots due to inclusion of raw soybeans in the ration. These results are shown in Table 9-7.

In addition, weights, Haugh scores, and shell thickness of eggs produced on four days were determined. These data are shown in Table 9-7. The birds receiving diet 9-B produced slightly smaller eggs than did birds receiving diets 9-A and 9-C. Shell thickness was slightly better in eggs produced by the birds receiving diet 9-A. The egg weights and shell thicknesses were quite variable within treatments and thus the small differences were non-significant.

Table 9-1. Composition of 1962-63 Layer Mash Used in Experiment IX

Ingredients	Percent of ration Diet 9-1
Corn, ground yellow	59.90
Soybean meal, 44% protein	21.40
Meat and bone meal, 50% protein	2.00
Fish meal and solubles, 57% protein	2.00
Alfalfa leaf meal, 20% protein	2.50
Limestone, ground	6.00
Dicalcium phosphate, ground	1.20
Salt, iodized	.25
Animal fat	4.50
Vitamin-trace mineral mix, Nopcosol M-4	.25

Calculated analyses for rations:

Protein	%	17.67
Fat	%	7.27
Fiber	%	3.42
Calcium	%	3.00
Phosphorus	%	.68
Arginine	%	1.02
Glycine	%	.90
Methionine	%	.35
Cystine	%	.25
Lysine	%	.90
Tryptophan	%	.19
Vitamins:		Amt./lb.
A	I.U.	4021
D_3	I.C.U.	750
E	mg	5.04
B_{12}	mcg	6.26
Riboflavin	mg	2.37
Niacin	mg	19.89
Pantothenic acid	mg	5.79
Choline	mg	537.00
Folic acid	mg	.23

Productive energy, Cal/lb 983

Table 9-2. Composition of Rations Using Whole Soybeans, Whole corn and Pellets in Experiment IX

Ingredients	Pounds
Corn, whole yellow	59.80
Soybeans, whole (raw, cooked, or both)	25.90
Oyster shell, hen size	4.36
Granite grit	1.00
Pelleted concentrate No. 63	10.00
	101.06

Pelleted concentrate No. 63

Ingredients	Lbs./10 lbs.
Fish meal and solubles, 57% protein	2.00
Meat and bone scraps, 50% protein	2.00
Alfalfa leaf meal, 20% protein	2.50
Limestone, ground	1.64
Dicalcium phosphate, ground	1.20
Salt, iodized	.25
Vitamin-trace mineral mix, Nopcosol M-4	.25
DL-methionine	.16
Antibiotic (Lincomycin) 50 gms/ton	+
	10.00

Table 9-3. Summary of Production and Quality of Eggs and Feed Efficiency of Hens Receiving Different Rations (Individual trap records for 134 days, Dec.-May, 1962-63)

				Egg size and quality[2]			Feed per dozen eggs for various periods		
Diet	No. pens	Composition	% Prod.*	Egg wt.	Haugh score	Shell thickness	Dec.-Feb.	Mar.-Apr.	May
9-1	4	Positive control	70.8 ABa	55.4	88.4	13.7	5.17	5.37	5.57
9-3	4	Pellets + whole grain (raw soybeans)	51.4 Dd	54.1	89.9	13.4	7.02 5.79	4.98	5.50
9-4	4	Pellets + whole grain (½ raw and ½ cooked) (soybeans)	51.3 Dd	53.4	88.8	13.3	6.20 5.23	5.89	4.77
9-5	4	Pellets + whole grain (cooked soybeans)	51.0 Dd	54.9	88.8	13.3	6.45 7.63	6.33	7.00
9-6	3	All-mash control using raw ground soybeans	63.5 Cc	54.2	89.7	13.5	5.33	5.26	5.35
9-7	2	Diet 9-6 + 0.16% methionine	64.7 BCbc	55.9	87.0	13.6	4.96	4.87	4.91
9-8	2	Diet 9-6 + 0.05% surface active agent[1]	65.4 BCbc	54.5	90.8	13.6	4.90	4.98	5.21
9-9	2	Diet 9-6 + 0.16% methionine + 0.05% surface active agent	71.3 ABa	55.9	90.2	13.5	4.50	4.76	4.73
9-10	2	Diet 9-6 + 0.24% methionine	69.4 ABCab	55.9	89.4	13.5	4.68	5.37	5.02
9-11	2	Diet 9-6 + 0.24% methionine + 0.05% surface active agent[1]	71.7 ABa	57.0	88.5	13.8	4.93	5.01	5.70

1 Solar F-221, Swift and Co.
2 From sample of eggs laid on one day in January - an average of about 8 eggs from each pen.
* Means having the common capital and small letters are not significantly different; means not having the common capital and small letters are significantly different at the .01 and .05 level of probability, respectively.

Table 9-4. Analysis of Variance of Egg Production for 134 Days Trap Period

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	551	233,012		
Treatments	10	74,280	7428.0	25.3**
Error	541	158,732	293.4	

Standard error of the mean = 2.42

** Significant at the .01 level of probability

Table 9-5. Results of Blood Pressure Determinations on Hens After Receiving Three Experimental Rations for Six Months

Diet	Pen	Badge		Systolic	Diastolic
9-1 Cooked soybeans	3	72		195	160
		81		164	141
	7	1056		192	163
		254		170	138
	41	804		181	152
		813		172	138
	45	932		140	118
		942		198	153
			Av.	176.5	145.4
9-6 Raw soybeans	12	344		160	138
		349		153	131
		368		175	141
	33	553		183	145
		555		187	155
		1071		178	145
	37	51		153	132
		680		186	152
			Av.	171.9	142.2
9-7 Raw soybeans + .16% methionine	13	370		180	148
		371		192	152
		383		182	150
		1089		157	132
	34	45		180	144
		584		165	147
		587		184	146
		751		170	136
			Av.	176.3	144.4

Table 9-6. Pancreata Weights of Hens After Receiving Experimental Feeds for Six Months

Diet		Body Wt. (gms)	Pancreas wt. (gms)	Pancreas % of body
9-1		1895	3.7	.195
		1860	3.6	.194
		1835	3.1	.169
	Mean	1863	3.5	.186
9-6		1495	3.8	.254
		1745	5.3	.304
	Mean	1620	4.6	.279
9-7		1635	5.1	.312
		1290	3.9	.302
		2410	6.7	.278
		2110	4.9	.232
	Mean	1861	5.2	.281

Table 9-7. Summary of Production and Quality of Eggs From Hens Receiving Three Different Rations

Diet	No. hens	Composition	% Prod. / hen day*	Size, quality and shell-/				Blood spots	
				No. eggs	Av. wt. (gms)	Haugh score	Shell thickness (.001 in.)	No. eggs	Percent spots
9-A	58	Positive control (diet 8-3)	65.9 Aa	142	63.3	74.9	13.2	421	5.5
9-B	61	Raw ground soybeans control (diet 8-6)	56.5 Bc	144	60.4	74.5	12.9	409	3.2
9-C	66	Diet 9-B + .16% methionine + virginiamycin (50 gms/ton)	62.0 Ab	175	62.0	74.5	12.7	490	5.5

* Means having common capital or small letters are not significantly different while means not having common letters are significantly different at the .01 and .05 levels of probability, respectively.

Table 9-8. Analysis of Variance of Individual Trap Records of Egg Production for 185 Hens

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Total	184	528		
Treatments	2	21	10.50	3.76
Error	182	507	2.79	

Standard error of mean = .123

* Significant at the .05 level of probability

SUMMARY AND DISCUSSION

From the review of literature and results reported herein, it is obvious that the composition of raw soybeans is such that poor results are usually obtained when fed to poultry. Several workers have reported benefits from amino acid and also antibiotic supplementation of chick rations containing raw soybeans. Other conditions such as age of birds were also found to have effects. Therefore, these studies were conducted primarily to find ways to put these results into use in practical type rations for chicks, poults and hens and to delve further into the exact causes for the poor performance when raw soybeans are fed. It was desirable to find a combination or combinations of feedstuffs, additives and conditions whereby it would be economically feasible to incorporate raw soybeans into poultry rations and to obtain from them satisfactory results in production and other economic factors. It would be advantageous if farmers could use products from their farms without the additional costs of treatments.

Of the many raw soybean-containing experimental rations used, some were so supplemented that results produced when fed were not significantly different from the results produced by feeding positive control rations. Not only was this true, but the supplements were reasonably priced and used at relatively low levels so that their use under certain conditions would be economically feasible.

In an attempt to determine the source of the inconsistencies reported by different workers in the use of raw soybeans, experiment I was conducted with the use of four different varieties of soybeans, both in the cooked and the raw form. In the raw form there were significant differences in the weights produced by the four different varieties of soybeans. In the autoclaved form none of the four varieties were significantly different at the

.01 level of probability; however, there were differences produced which were significant at the .05 level.

The variety, Chippewa, which was the best performer in the raw form was the poorest performer in the autoclaved form as determined by growth rate of chicks. All varieties studied were significantly improved by autoclaving. Methionine supplementation also significantly improved the growth rate of chicks receiving the raw soybean ration; both the glycine and lysine used singly were without benefit. The use of rolled oats as a substitute for corn did not significantly change the results obtained. The protein of corn as compared to that of oats did not seem to be an important factor in these studies. From this study, it would seem that plant geneticists and breeders would profit by selecting soybean breeding stock on the basis of the growth rate of chicks receiving raw or heat-treated soybeans in addition to the conventional bases of selection such as gross yield per acre, oil content, and disease resistance.

In experiment II, it was shown that the benefit from methionine was probably from its use in protein formation and not as a source of labile methyl groups. When used as a supplement in raw soybean meal rations for chicks, methionine significantly improved growth; whereas, growth was not affected by supplemental choline, folic acid and vitamin B_{12}. All of these components were added at levels greater than is normally thought to be the requirement for chicks of this age. Also, in this study, when pancreata from different birds on the different experimental rations were taken at different ages from six to eight weeks, it was observed that the feeding of raw soybeans caused greatly enlarged pancreata, both in total weight and also in pancreas weight as a percentage of body weight.

Determinations of the amount of acids produced by the proventriculus were made to see if there was a difference in the acid production due to the feeding of raw soybeans. There were no consistent trends in acid content of the gizzard due to the different experimental treatments. Thus, from this, one would conclude that excess or decreased acid production would not be caused by the change in stimulation of pancreata or release of proenzymes.

Experiment III was conducted to determine the effect of four different antibiotics at the 50 grams per ton level in chick rations containing raw soybean meal. All of the antibiotics used improved the growth rate of the chicks. In addition, supplemental methionine and glycine, singly and in combination, significantly increased rates of growth. The birds receiving the ration containing autoclaved ground soybeans grew significantly slower than the birds which received the positive control ration containing 44 percent protein soybean meal and soybean oil. This was true when Pro-strep was added to these two rations also. The weights of the birds receiving the combination of Lincomycin and methionine were not significantly different from the weights of the birds which received the autoclaved ground soybean ration without the amino acid supplementation. However, feed efficiency with the raw supplemented ration was not as good as that produced by the autoclaved soybean meal ration.

Benefits from iodinated protein, tolbutamide and feces were not apparent in experiment IV. Once again, supplemental methionine was beneficial in two types of basal rations containing raw soybean meal. This study indicated that neither lack of insulin nor thyroxin production was a factor in the growth inhibitions due to raw soybeans since the addition of either tolbutamide (orinase) or iodinated protein (protamone) had no effect. The possibility exists that enough of the inhibitor or the component was present in the soybeans to reach a threshhold (maximum or minimum) so that additional supplementation of these products would not influence the results.

Further studies into the size of the pancreata as well as the proventriculi and livers of chicks receiving these different experimental rations were made. The weights of the proventriculi and the livers did not seem to be influenced as were the weights of the pancreata. Histological examination did not reveal differences in these tissues as were revealed in the pancreata.

In experiment V, no growth response in young chicks could be observed from supplementing a raw soybean ration with a detergent. This detergent was also added to rations containing cooked soybean meal and no benefits were obtained in any of the rations. Supplemental methionine apparently did not greatly influence the lipid composition of the livers of chicks receiving raw soybeans. Separation of lipid components by use of silicic acid columns and solvents, as well as fatty acid analyses by gas-liquid chromatography, were performed but these techniques revealed no major differences in fat metabolism.

Some of the growth results obtained with turkey poults in experiment VI were somewhat puzzling. The antibiotic, Neomycin, improved the growth rate of birds receiving the positive control ration but depressed growth produced by the raw soybean ration. However, Neomix was beneficial when added to raw soybean rations containing supplemental methionine. Penicillin at the level used was of no apparent benefit.

Experiment VII with Leghorn-type pullets revealed that the birds were not particularly affected by the feeding of raw soybean rations between the ages of 9 and 19 weeks. Thus, the feeding of raw soybeans during these ages was not an effective way to delay the sexual maturity and growth of pullets. Body weights and the age at sexual maturity were not significantly influenced by the treatments used. Apparently, raw soybeans can be safely utilized in rations for growing pullets.

Experiment VIII with laying hens showed that changing to rations containing raw ground soybeans after egg production had started caused a severe drop in egg production. This reduced egg production was largely prevented by supplementing the ration with methionine and an antibiotic. Pelleting and supplementation with other anti-bacterial agents were without benefit.

The hens in experiment IX responded favorably to supplementation of methionine and a detergent in the rations containing ground raw soybeans. The birds receiving their ration in the form of a pelleted concentrate and whole grains of corn and raw or autoclaved soybeans produced eggs at a much lower rate than birds receiving the same ration in the all-mash form. The digestive system was apparently unable to adequately utilize the whole soybeans although grit was provided. The seed coat and large particle size of the whole soybeans may have prevented the digestive enzymes from being in contact with the nutrients long enough for digestion.

In all experiments, autoclaving of the raw soybeans improved the rate of growth and feed efficiency and decreased pancreatic hypertrophy. However, in general, the rates of growth produced by the rations which contained autoclaved soybeans were not as great as those produced by rations which contained a commercially produced soybean oil meal with added fat or soybean oil. Whether this was due to poor availability of fat in the whole autoclaved soybeans was not determined.

When included in raw soybean rations, methionine supplementation was consistent in almost always giving significant improvements in rates of growth, egg production, and other factors. Ithas long been known that in chick rations based upon corn and soybean oil meal with other supplements, methionine is the first limiting amino acid. Therefore, it is reasoned that the most benefit from amino acid supplementation would be from this amino acid, methionine. This does not necessarily indicate that methionine is any less digested or absorbed than the other amino acids but that, of the total protein components, methionine is the amino acid which is most deficient and thus, its supplementation would enhance growth. Supplementation of another amino acid might not improve growth at all because methionine would be the first limiting amino acid and supplementation of another amino acid might cause a more severe imbalance.

Either poor digestion or poor absorption of the protein from the intestinal tract could cause protein or amino acid deficiency or, if certain amino acids were absorbed and others not, this could bring about an amino acid imbalance. If the feces from chickens could be collected without being contaminated or before being attacked by bacteria, they could be analyzed for amino acids and thus it could be determined if either poor absorption or poor digestion occurred. The feces could be extracted to remove the amino acids which were already broken down from protein. Then the remaining intact protein could be separated and these two fractions -- the free amino acids and the complex protein containing amino acids -- could be analyzed and thus it could be determined if amino acids were not being absorbed or were not being broken down first and then, of course, not being absorbed.

Certain antibiotics were effective in promoting growth and also egg production but other antibacterial agents such as arsanilic acid and a nitrofuran produced little or no beneficial effects in these respects. Since the exact methods whereby the growth-depressing characteristics of raw soybeans are active have not yet been elucidated, the possibility exists that there is a substance or a toxin in raw soybeans which kills or destroys the enzyme system which brings about absorption of amino acids or other nutrients from the intestinal tract. The poor results from feeding raw soybeans could be due either to poor absorption or poor digestion of the nutrients. If the results are due to poor absorption, the antibiotics benefit may be by the prevention of the amino acids tie-up or use by bacteria; thereby allowing absorption to take place over a longer time and more completely from both the small and large intestines. If antibiotics were not used, the bacteria could utilize the amino acids in the large intestine before absorption could occur.

Feeding raw soybeans produced enlarged pancreata in all cases where determined. Although satisfactory performance was produced with these rations when properly supplemented with methionine and antibiotics, pancreatic hypertrophy was not corrected. The recent report by Nesheim (1963) indicated two or more inhibiting factors in raw soybeans. One factor could cause pancreatic enlargement without affecting growth but usually an abnormality such as this would be expected to be associated with reduced growth; while the second factor in some way could affect growth and also the pancreas. It seems more likely, however, that the loss of some metabolic function prevents digestion and thus growth due to the loss of normal pancreatic function.

The work by Magee and Hong (1956) and also by Nishioka (1959) indicated that the rate of secretion of the pancreatic enzymes was possibly influenced by the protein and amino acid content of the digestive tract affecting secretin release. In the hydrolysis of raw soybean protein, perhaps the order of release of the amino acids is such that certain of them that stimulate secretin and pancreozymin release are present or absent in certain areas. Also, the ratio between certain of the amino acids may be a factor in the hormone release. The physical structure of the raw soybean protein may be altered due to denaturing in heating so the amino acids are released faster in the digestive

tract. With the raw beans, complete hydrolysis may not occur until much further down the digestive tract, thus causing abnormal amino acid proportions in certain areas of the tract.

The poor results obtained with both raw and autoclaved whole soybeans in experiment IX may have been due to factors such as these. The whole beans were probably providing intact protein to be digested much further down the digestive tract than would have been true if ground or crushed beans had been used. The preliminary study of the acid production of the proventriculus indicated no effect of raw soybean on the pH of the gizzard.

Since several reports have shown that fat from raw soybeans is poorly utilized, it is possible that the presence of this fat slows the passage of the food through the intestinal tract and thus the birds are unable to eat enough feed for their protein and other needs. This could bring about the greatly reduced rate of growth due to lack of feed consumption or loss of appetite. This cannot be disproven from the data shown since in almost all cases where a growth depression occurred a great reduction in feed consumption occurred. Whether the decreased feed consumption is cause or effect cannot be determined from the data presented. However, when a detergent was added to rations of chicks or hens (experiment V and IX), different results were obtained. With chicks, no apparent benefit was obtained; whereas, in laying hens a significant increase in the rate of egg production resulted, especially when the detergent was used in combination with supplemental methionine.

It seems likely that the protein utilization and the fat utilization problem concerning raw soybeans may be two different problems -- a physiological and/or biochemical problem for the protein utilization versus a physical problem for the fat utilization. This may be true if the fat is not utilized due to the protein covering or protecting it from the digestive enzymes and

emulsifier. Thus, the fat would not be acted upon until the protein had been digested from around it.

Apparently, there is quite a difference in the response of birds from the feeding of raw soybeans which can be attributed to the age and also the state of production of the birds. When birds had commenced laying and were switched to raw soybean rations, they went out of production. However, when birds that were just starting to lay were fed rations containing raw soybeans, they were able to continue egg production at a satisfactory level with no apparent detrimental effects.

In all cases, young birds -- chicks and poults -- were severely affected by the raw soybeans and retarded growth, poor feed consumption and utilization, and enlarged pancreata were general symptoms. When raw soybeans were used in the rations of pullets from 9 to 19 weeks of age, no significant depressions in growth occurred.

There were indications that birds were able to adapt to the use of raw soybeans. If pullets were started on them before egg production reached a high level, they were able to continue to produce. However, switching to raw soybean with egg production at a high level was detrimental. Also, with young birds, feed efficiency of raw soybean rations was better in relation to controls after two weeks on the experimental feeds than during the first two weeks. This may, of course, have been due to the lowered protein requirement resulting from advancing age.

CONCLUSIONS

Raw soybeans can be used in poultry feeds with satisfactory results with proper supplementation. The best performance in these experiments occurred with supplements of methionine and certain antibiotics.

Sensitivity of poultry to raw soybeans apparently decreases with age up to the onset of egg production. Then, an adjustment period is apparently needed in order to allow pullets to effectively use raw soybeans for egg production. If raw soybeans are to be used, they should be started before egg production so as not to cause excessive stress. A protein deficiency may occur when switching to raw soybean rations after egg production commences.

Different varieties of soybeans were tested and differences in rates of growth were produced by the different varieties.

Overcoming the growth depression of raw soybeans by amino acid and antibiotic supplementation does not prevent pancreatic hypertrophy; this may indicate that at least two factors are affecting body growth and pancreas size.

Although supplemental methionine was beneficial, supplementation of raw soybean chick rations with vitamin B_{12}, folic acid and choline at relatively high levels did not prove beneficial. Likewise, the supplementation with hormone-like compounds having activity to increase insulin and thyroxin-like effects did not improve the performance of chicks. The recycling of feces from chicks receiving the raw soybeans also did not affect the rate of growth.

Pelleting was not beneficial to raw soybean-rations fed to laying hens. Likewise, the feeding of whole grain soybeans, either cooked or autoclaved, did not produce satisfactory results in laying hens when used in combination with a pelleted concentrate. The selection by the hens of the pellets and the upsetting of the nutritive balance was thought to be part of the cause for these poor results.

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Table 1. Compositions of Vitamin-trace Mineral Mixes*

	Per 5 lb.		
	Nopcosol M-4	Nopcosol M-5	Nopcosol M-7
Vitamin A, USP Units	4,000,000	3,500,000	5,000,000
Vitamin D_3, IC Units	1,500,000	1,000,000	1,500,000
Vitamin E, I Units	7,500	1,000	5,000
Riboflavin, gm	3	3	3
d-Pantothenic acid, gm	5	5	5
Niacin, gm	20	20	30
Choline chloride, gm	200	200	300
Menadione sodium bisulfite, gm	--	0.5	--
Vitamin B_{12}, gm	10	5	6
Zinc bacitracin, gm	--	4	4
Butylated hydroxy toluene, gm	113.4	113.4	113.4
Manganese, %	2.4	2.4	2.4
Zinc, %	1.1	1.1	2.2
Iron, %	0.8	0.8	0.8
Copper, %	0.08	0.08	0.08
Iodine, %	0.048	0.048	0.048
Cobalt, %	0.008	0.008	0.008

* Produced by Nopco Chemical Company, Harrison, New Jersey

Table 2. Composition of Practical Control Rations Used in Experiment VII

Ingredients	Percent of ration
Corn, ground yellow, No. 2	38.0
Soybean oil meal, 44% protein	12.5
Alfalfa meal, dehyd., 17% protein	5.0
Oats, ground, heavy	22.25
Meat and bone meal, 50% protein	2.5
Whey, dried	.5
Wheat flour middlings, stand.	15.0
Limestone, ground	1.25
Dicalcium phosphate, feeding grade	1.5
Animal grease, choice	1.0
Salt, iodized	.25
Vitamin-trace mineral mix Nopcosol M-5	.25

Calculated analyses:

Protein	%	16.1
Fiber	%	6.4
Fat	%	4.9
Calcium	%	1.4
Phosphorus	%	.83
Arginine	%	.88
Glycine	%	.67
Methionine	%	.28
Cystine	%	.22
Lysine	%	.73
Tryptophan	%	.16
Productive energy, Cal/lb		805