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Effects of Various Factors on the Rachitogenic Activity of Isolated Soy Protein

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EFFECTS OF VARIOUS FACTORS ON THE RACHITOGENIC ACTIVITY OF ISOLATED SOY PROTEIN

BY

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EFFECTS OF VARIOUS FACTORS ON THE RACHITOGENIC ACTIVITY OF ISOLATED SOY PROTEIN

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor Date

Head, Animal Science Department Date

EFFECTS OF VARIOUS FACTORS ON THE RACHITOGENIC ACTIVITY OF ISOLATED SOY PROTEIN

Abstract

DONALD JAMES ARSHEM

Under the supervision of Dr. C. W. Carlson

The nature of the rachitogenic activity of isolated soy protein was investigated in fifteen studies. A RP-100-glucose purified diet was fed to day-old turkey poults housed in stainless steel batteries. Feed and water were provided ad libitum.

Corn, oat hulls, solvent-extracted soybean meal, full-fat extruded soybeans, C-1 protein, casein and texturized forms of soy protein were added to the basal diet at various levels. All feedstuffs were added at two particle sizes: natural form of the feedstuff ($>$ 250 u); and ground in a ball mill ($<$ 250 u). The effects of autoclaving for 60 minutes at 120°C were also investigated.

Calcium, phosphorus and Vitamin D_3 levels were maintained at or above the National Research Council recommendations in all diets. Parameters used for evaluation of the rachitogenic activity were body weight, percent bone ash to indicate extent of calcification and percent mortality at the end of the four-week trials.

The following conclusions were derived from these investigations:

1. Substitution of corn, oat hulls or solvent-extracted soybean meal at 5 percent of the basal diet improves the poults' body weight and bone ash. Grinding these natural feedstuffs to less than 250 microns eliminates these beneficial effects.

2. The addition of 3 percent feed grade dicalcium phosphate to the isolated soy-glucose basal diets improves the poults' body weight and percent bone ash regardless of the particle size of the product.
3. Grinding a practical turkey starter to less than 250 microns greatly depresses body weight and bone ash of the poults.
4. Autoclaving the isolated soy protein of the basal diet improves the poults' body weight and bone ash. Grinding the autoclaved diet depresses the poults' body weight and bone ash. These responses are different and separable since the order can be reversed. For example, grinding first or autoclaving first followed by the other produces similar results.
5. Soaking and drying either the protein portion or the sugar portion of the diet is not a means of increasing the effective particle size of the diet.
6. Texturized forms of isolated soy protein were not effective means of overcoming the rachitogenic effect of small particle size.
7. Levels of phytic acid comparable to that present in soy protein did not produce any inhibitory effects when added to casein-glucose diets.

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INTRODUCTION

The soybean, often called the golden bean, has a long history as a food source for man and animals. The first written record referring to the soybean was in about 3,000 B.C. when a Chinese emperor described more than 300 remedies for the cure of human ills which could be prepared from soybeans (Hanson, 1969). The crop was repeatedly mentioned in later records of the Chinese culture and was considered its most important cultivated legume. The soybean was considered one of the five sacred grains essential for the existence of Chinese culture.

The soybean was thought to have been brought to the Atlantic Coast of America in the 18th Century as ballast in the hold of a sailing vessel. According to Ewing (1947), the first published account of the soybean plant in the United States appeared in 1804. In 1850, the U.S. Department of Agriculture started research designed to study the use of the crop as forage, green manure, silage or hay. Domestically-produced soybeans were first extracted in a North Carolina cottonseed mill in 1915 (Ewing, 1947), although oils were previously imported from the Orient for many years.

Today over a billion bushels of soybeans are grown annually in the United States. The bulk of this crop is extracted, yielding about 11 pounds of oil and 47 pounds of cake or meal from each bushel. Over 90 percent of the oil is used in edible products such as margarine, shortening, salad oils, cooking oils and mayonnaise. The remaining oil is used for industrial purposes in such diverse products as paints, varnishes, linoleum, rubber fabrics, soap stock and glycerine.

The soybean meal is used primarily by the livestock and poultry industry as a protein supplement, although about 1 percent of the meal is utilized as edible protein in such products as bread and bakery goods, macaroni, spaghetti, baby foods, high protein beverages, meat extenders and dietary specialties. The bulk of these latter products utilize only the highly purified, acid-precipitated protein portion of the meal.

Soybeans have long been recognized for their nutritional value. N. A. Ferri, author of The Wonder Food, Soybeans, says: "The soybean is richer than beef in proteins, richer than milk in calcium, richer than egg in lecithin and almost richer than any single source of the protective and essential food substances such as vitamins, mineral salts, amino acids, lecithin, and the unsaturated fatty acids." Unfortunately soybeans are not nearly as perfect for rations as Ferri states. Osborn and Mendel (1917) reported impaired growth in rats fed raw soybean meal, thus setting off a chain of research which has uncovered many unidentified growth and antinutritional factor(s) in soybean meal.

The purpose of this work was to attempt to characterize one of these antinutritional properties of isolated soy protein, namely its rachitogenic activity. Carlson, McGinnis and Jensen (1964a) and Carlson et al. (1964b) partially characterized such a factor(s) in isolated soy protein which was heat labile and resulted in rickets in turkey poults. This rachitogenic activity was readily offset by small additions of toasted soybean meal. Attempts to isolate this

factor by fractionation procedures were unsuccessful thus leading to the necessity for a study of the physical nature of the product.

The original intent of this study was to examine the effects of various particle sizes on the rachitogenic activity of isolated soy protein. After the publications of Griffith (1968, 1969, 1970), concerning the effects of particle size on phosphorus utilization, this study evolved towards the characterization of the rachitogenic activity of various forms of isolated protein.

LITERATURE REVIEW

Nature of Rickets

Rickets or poor bone calcification may occur in young animals during active growth. According to Bechtel (1962), rickets stems from malnutrition, which may be of varied origin. In its more advanced and florid forms, deformity-producing changes are typical as the result of softening, enlarging, curving and twisting of certain skeletal tissues. Hence the origin of the term "rickets" from the English "wrikken" (meaning to twist).

During growth, long bones undergo constant remodeling in which the major sequence of events is: growth of epiphseal cartilage, mineral deposition by means of osteoblasts, and mineral resorption by means of osteoclasts resulting in a gradual lengthening and thickening of the bone shafts. Primarily, the resorption occurs medially thus forming a hollow shaft which provides adequate strength with a minimum of weight. In a rachitic condition, mineral deposition in the cartilaginus matrix is subnormal. The resulting abnormal proliferation of uncalcified cartilage cells leads to enlargements in the metaphysical areas at the epiphysis of the long bones. Histologically, the metaphysical tissues show irregular capillary penetration along with osteoid-coated islands of cartilage matrix which require extensive repair during convalesence, as described by Reith and Ross (1965).

Normal bone formation in poultry is dependent upon an adequate supply of the structural elements of bones: calcium, phosphorus and

magnesium. The chief mineral compound of mature compact bone is hydroxyapatite $3\ Ca_3(PO_4)_2 \cdot Ca(OH)_2$. Hydroxyapatite is embedded in an organic matrix composed mainly of collagen, a protein, and mucopolysaccharides. Magnesium frequently replaces one of the calcium atoms and thus tends to add qualities of resilience to the bone. The trace minerals (manganese, zinc, copper and iodine), the vitamins (choline, niacin, biotin, folic acid, Vitamin A and Vitamin D_3) and, under certain conditions, Vitamin B_{12} have all been shown essential for normal bone formation.

The first evidence that turkey poults required manganese was reported by Ringrose, Martin and Insko (1939). Poults fed a manganese-deficient diet developed perosis. In later studies on the manganese requirements of turkey poults, Kealy and Sullivan (1966) observed that a deficiency of manganese caused slipped tendon, a twisting and bending of the distal end of the tibia or proximal end of the metatarsus and enlargement of the hock.

In studies on the histopathology of manganese deficiency in chicks, Wolbach and Hegsted (1953) and Leach (1968) concluded that the epiphyseal cartilage was the site of the malformity commonly called perosis or slipped tendon. Their studies showed in particular that endochondrial bone growth was retarded or suppressed.

Young, Edwards and Gillis (1958) and O'Dell et al. (1958) reported the failure of cartilage cell development at the epiphyseal plate in the leg bones of chicks on zinc-deficient diets. Dermatitis and thickening of the skin on the wings and legs were also observed.

Savage, Bird and O'Dell (1963) observed leg deformities in 66 percent of a group of four-week-old turkey poults fed a copper-deficient diet. The histopathology was not studied but it may have been similar to that reported by Gallagher (1957) and Carlton and Henderson (1964) in copper-deficient chicks. They found the epiphyseal cartilage thickened due to an increase in immature noncalcified cartilage cells and the normal process of bone formation was retarded.

Iodine deficiency has been found to result in hock enlargement, shortened leg bones and thickening and bending of the metatarsus in chicks by Briggs and Lillie (1946). This condition was brought about by the additions of theouracil, a thyroxine antimetabolite, to the chick's diets.

Vitamin A deficiency was found to result in retardation of epiphyseal cartilage cell formation in the young chick by Wolbach and Hegsted (1952a). In this condition all sequences of cartilage formation were retarded thus resulting in retardation of tunneling and ossification processes resulting in failure of endochondrial bone growth. Wolbach and Hegsted (1952b) also studied hypervitaminosis A in chicks and observed that all histological sequences concerned in bone growth were accelerated in comparison to normal growth patterns. On the other hand, Thayer and Nelson (1968) reported that leg bone deformities developed in turkey poults given a daily intake of excessive quantities of Vitamin A palmitate.

Jukes (1940) reported that choline could prevent a form of perosis in chicks which is revealed by slipping of the Achilles tendon from its

condyles. Histopathologic studied by Wolbach and Hegsted (1953) found this condition resembled that observed with manganese deficiency.

Schaefer et al. (1950), using slipped tendon as the only measure of perosis in chicks, fed insufficient folic acid and showed that folic acid was required in addition to choline for the prevention of perosis. No histopathological studies have been reported on perosis due to folic acid deficiency.

Vitamin D Metabolism

The role of Vitamin D in the prevention of rickets has been studied by many investigators. Scott, Hughes and Loy (1932) were the first to study rickets in turkey poults. Since that time much evidence has accumulated establishing that Vitamin D enhances calcium absorption. The primary mechanism of action of Vitamin D is not fully understood, but two laboratories have made great strides toward that end within the last decade.

Wasserman and Taylor (1963) reported that treatment of rachitic chicks with Vitamin D_3 increased the ability of a supernatant fraction, obtained by centrifuging an homogenate of intestinal mucosa, to bind calcium. They later isolated a calcium-binding protein (CaBP) from this fraction which increased in rachitic animals after Vitamin D_3 was administered. In a later study, Wasserman et al. (1968) reported that this protein would combine with 1 mole of calcium per mole of protein.

CaBP has also been found in other tissues, including chick kidney and the uterus of laying hens as well as in intestinal homogenates

from rats, dogs and monkeys. Wasserman et al. (1968) concluded that CaBP is a primary factor in calcium transport, and that the level of duodenal CaBP is directly correlated with an animal's ability to absorb calcium.

According to DeLuca (1969) the physiological essence of Vitamin D action is the elevation of plasma calcium and phosphate which is in turn responsible for normal bone calcification. First, Vitamin D induces a calcium transport system in the intestine (Wasserman et al., 1968). This is an active cation-oriented transport system in which phosphate is transferred secondarily to the calcium. On the other hand, Vitamin D induces the mobilization of old or deep bone by inducing osteoclasts thus releasing calcium and phosphorus. Parathyroid hormone augments this absolutely Vitamin D-dependent process (DeLuca, 1967). A combination of these two actions by Vitamin D brings about an elevation of plasma Ca^{++} and HPO_4^{--} which, in turn, brings about normal mineralization of bone (DeLuca, 1969).

DeLuca (1967) observed a time lag between the administration of oral or intraveneous doses of Vitamin D and a resulting increase in serum calcium. No time lag was observed, however, in the tissue uptake of tritium (H^3) labeled Vitamin D. Therefore, it was concluded that Vitamin D must have been transformed into some active metabolite before it could function to increase serum calcium levels. Studies of Zull, Czarnowska-Misztal and DeLuca (1966) showed that administration of actinomycin D, which blocks protein synthesis, would inhibit the function of Vitamin D.

The administration of Actinomycin D must be prior to the administration of Vitamin D in order to observe this effect.

These studies prompted more extensive investigations with labeled Vitamin D. It was found by silicic acid column chromatography, that the radioactive metabolites of Vitamin D could be partitioned into four specific bands (DeLuca, 1969). These bands were analyzed for Vitamin D activity with chicks. One band, later identified as 25-hydroxy cholecalciferol (25-HCC), was found to be 1.5 times as active as Vitamin D in curing rickets, and there was no time lag between the administration of this metabolite and the initiation of calcium absorption.

The results of these experiments and others related to them were presented in a summary by DeLuca (1969). It appears that Vitamin D is carried to the liver by an α_2-globulin where it is converted to 25-HCC. The 25-HCC is, in turn, carried from the liver by another α 2-globulin to the bone and intestine. At the intestinal mucosal cell 25-HCC finds its way into the nuclear membrane by some unknown mechanism where it unmasks a specific DNA. Complementary RNA is formed which codes for a component(s) of the calcium transport system of the brush borders which is manifested as calcium dependent ATPase. Figure 1 illustrates this mechanism in more detail while pointing out some of the questionable areas. A mechanism of this type may function in ostoid tissue but this has not been studied.

Recent evidence on the administration of 1,25 dihydroxy cholecalciferol (1,25-DHCC) was presented by Omdahl, Tanaka and DeLuca (1971). It was found that 1,25-DHCC was very active in stimulating

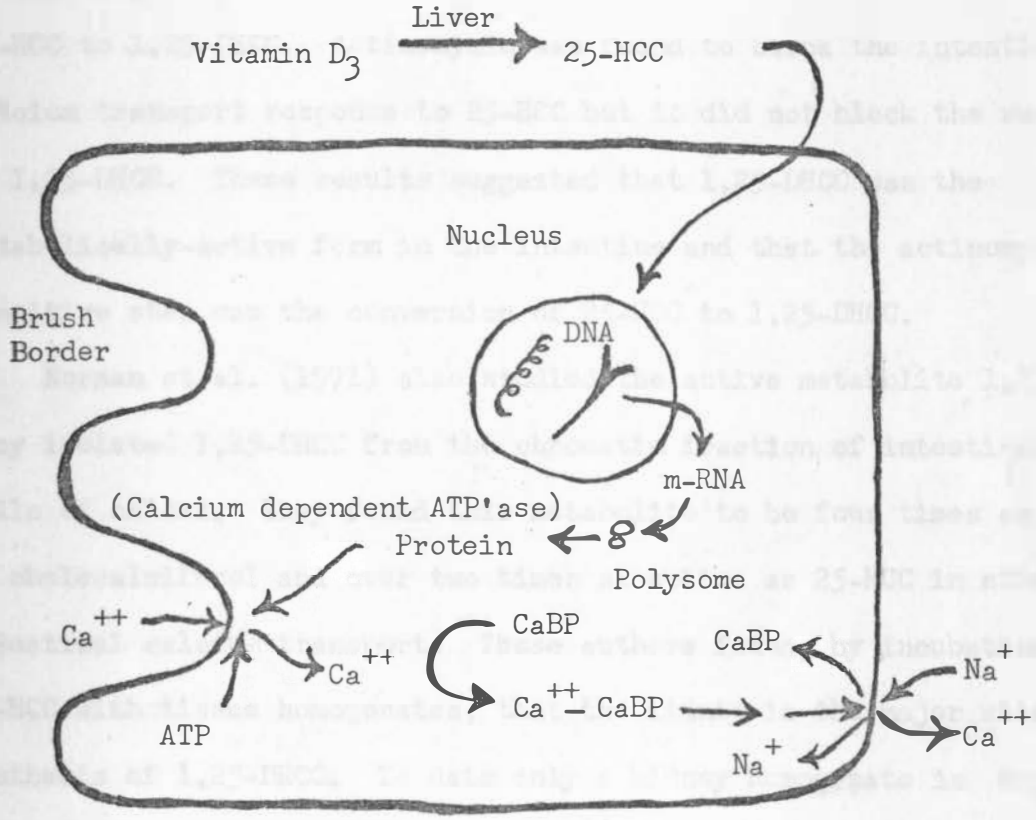

Figure 1. Schematic representation of the proposed mechanism of action of vitamin D in the intestine (DeLuca, 1969).

calcium absorption in the chick since a shorter time lag was required than found with 25-HCC. The maximal response of 1,25-DHCC was shorter lived but its magnitude exceeded that of 25-HCC by a factor of two. These authors found that prior administration of either cycloheximide or actinomycin D to Vitamin D-deficient rats blocked the conversion of 25-HCC to 1,25-DHCC. Actinomycin was found to block the intestinal calcium transport response to 25-HCC but it did not block the response to 1,25-DHCC. These results suggested that 1,25-DHCC was the metabolically-active form in the intestine and that the actinomycin sensitive step was the conversion of 25-HCC to 1,25-DHCC.

Norman et al. (1971) also studied the active metabolite 1,25-DHCC. They isolated 1,25-DHCC from the chromatin fraction of intestinal mucosa cells of chicks. They found this metabolite to be four times as active as cholecalciferol and over two times as active as 25-HCC in stimulating intestinal calcium transport. These authors found, by incubation of 25-HCC with tissue homogenates, that the kidney is the major site of synthesis of 1,25-DHCC. To date only a kidney homogenate is capable of producing significant quantities of a polar metabolite which exactly co-migrates with <u>in vivo</u>-produced intestinal 1,25-DHCC on liquid-liquid partition chromatography. The data can best be summarized as follows:

$$\text{Cholecalciferol} \xrightarrow{\text{liver}} \text{25-HCC} \xrightarrow{\text{kidney}} \text{1,25-DHCC} \xrightarrow{\text{intestine}} Ca^{++}\ \text{transport}$$

These data suggest that the protein synthesis occurs in the kidney since calcium transport is not blocked when actinomycin is administered prior to administration of 1,25-DHCC but is blocked when actinomycin is administered prior to administration of 25-HCC. Since kidney homogenates were the only tissues which have been able to produce a polar metabolite which co-migrates with 1,25-DHCC, it seems apparent that another mechanism is involved rather than the one presented by DeLuca (1969). At this time no alternative mechanism has been proposed.

Extraction of Growth and Antirachitic Factors

Several researchers have attempted to isolate and concentrate factor(s) from soybeans which stimulate growth in turkey poults. Kratzer et al. (1959) demonstrated the presence of a growth-promoting and an anti-perotic factor(s) which could be extracted with methanol but was insoluble in acetone. The activity of the methanol extract disappeared upon ashing thus indicating the organic nature of the factor(s). In later work, Kratzer, Starcher and Martin (1964) demonstrated that a water-acetic acid insoluble fraction of the ether insoluble fraction of a crude methanol extract produced a growth response in turkey poults when added to isolated soy protein diets. The greatest response was obtained from a benzene soluble fraction after adding 200 ml of acetic acid. The response to benzene fractions was still obtained in the presence of high levels of Vitamin D_3.

Wilcox et al. (1961a) demonstrated that a water extract from soybean meal showed growth-stimulating properties. Subsequent dialysis of the water extract formed two fractions. These fractions were added to poult diets containing isolated soy protein at two levels (1 and 4 percent water extract or 5 and 20 percent soybean meal equivalents). In all experiments poults receiving the water extract of soybean meal and the dialyzable portion of the water extract were significantly heavier than poults fed the basal diets. In several experiments poults fed the nondialyzable fraction of the water extract at a 4 percent level were highly significantly ($P<0.01$) heavier than the poults fed the basal diets.

Water extracts of heated or raw soybean meal partially prevented rickets and markedly improved growth of turkey poults in studies by Carlson, McGinnis and Jensen (1964a). Two protein levels were used (30 and 39 percent protein from isolated soy protein). Water extract of soybean meal was added at 1, 2 and 4 percent of the diet, which was equivalent to 5, 10 and 20 percent soybean meal. Rickets observed in the basal groups were partially prevented by adding the water extract at 2 and 4 percent of the diet. No statistically significant improvement in growth or bone ash was obtained when the water extract was added to the lower protein diets. However, marked responses were obtained by adding the water extract at 2 and 4 percent to the 39 percent isolated soy protein diets. The factor(s) that prevented rickets was either more concentrated in or more easily extracted from raw soybean meal. The factor(s) was not specifically associated with

protein because an extract made at pH 4.7, where the protein is insoluble, was as effective as an extract made at pH 6.8 where more of the soybean protein is soluble. The water extract, however, was not nearly as active as soybean meal in the prevention of rickets.

Griffith and Young (1964) made water extracts of unheated soybean flakes. The extracts were dried and heated prior to feeding to turkey poults. The growth factor(s) was not precipitated at pH 4.7 with the principal proteins but remained in the water soluble fraction. This fraction gave a growth response but did not improve the utilization of phosphorus from calcium phosphate. The factor(s) responsible for improving phosphorus utilization and calcification were not extracted by water from either soybean meal or unheated soybean flakes, but instead remained in the insoluble residue. This indicated that soybean meal had a water soluble factor(s) which improved growth and a water insoluble factor(s) which improved phosphorus availability.

Jensen and Mraz (1966) also studied the effects of a water extract of soybean meal which was added to isolated soy diets of chicks. They found that the water extract stimulated growth but did not improve bone ash. This further substantiated the concept that the growth factor(s) and antirachitic activity of soybean meal are different substances.

A water extract of raw soybean meal increased growth of turkey poults when added to purified diets based on isolated soybean protein or casein-gelatin (Griffith and Young, 1966), although previous attempts by these investigators to extract the growth factor(s) from heated soybean meal failed. Earlier Griffith, Young and Scott (1966) were unable to

remove the growth factor(s) from heated soybean meal but could remove a large part of the factor(s) in unheated flakes by water extraction. The factor(s) did not appear to be associated with the protein in these studies which would agree with findings by Carlson, McGinnis and Jensen (1964a).

Results of extraction with ethanol also have been varied. Wilcox et al. (1961b) found that an active growth-promoting material was not extracted from soybean meal by 100 percent acetone or 95 percent ethanol. Later Westerfeld and Hermans (1962) showed that an extract made of isolated soybean protein with sulfuric acid and ethanol could stimulate turkey poult growth. When added at a level equivalent to 20 percent soybean meal, the extract stimulated growth by 15 percent.

Effects of Autoclaving

Carlson et al. (1964b) reported on a heat labile factor in isolated soy protein which exhibited rachitogenic properties. Autoclaving raw soybean meal or C-1 protein for 30 minutes at 120°C overcame the growth-depressing and rachitogenic effects of both raw soybean meal and C-1 protein. This antirachitogenic effect of autoclaving could be only partially duplicated by a ten-fold increase in the Vitamin D_3 content of the diets. This response demonstrated a rachitogenic activity in isolated soy protein when used at high levels in the diets of turkey poults.

More recently, Thompson (1968) and Thompson et al. (1968) studied the effects of autoclaving times on the rachitogenic activity of isolated soy protein. The isolated soy protein was autoclaved for

various times increasing from 0 to 240 minutes. The rachitogenic activity of the isolated soy protein was progressively destroyed as time increased from 0 minutes to the optimum of 60 minutes. When the protein was autoclaved longer than 80 minutes, deleterious effects were observed which were assumed to be a result of protein destruction.

Another study by Thompson et al. (1970) showed that Vitamin D_3 was somewhat effective in overcoming the rachitogenic effect of isolated soy protein, as at a ten-fold increased level it improved both growth and calcification. Autoclaving the protein, however, resulted in a still greater growth rate and higher bone ash when fed to turkey poults. This study also demonstrated interrelationships between source of calcium and phosphorus and autoclaving, turkey strain and autoclaving and Vitamin D_3 level and strain. Autoclaving proved to be the most effective means of overcoming the rachitogenic effect of isolated soy protein.

Raw soybean meal and isolated soy protein both exhibit rachitogenic properties which can be destroyed by autoclaving. The exact mechanism has not yet been explained but it is the belief of this author that heat denaturation of a proteinacious antimetabolite may be possible.

Effects of Particle Size

Griffith (1966) demonstrated a response in growth, percent bone ash and phosphorus availability which was obtained by adding soybean hulls to an isolated soy protein diet for chicks. These results,

however, could not be duplicated by replacing the hulls with cellulose, suggesting that the property of improving phosphorus utilization exhibited by soybean meal was not due to its cellulose components. Griffith and Young (1967) showed that the ash of soybean hulls did not affect phosphorus availability. Increasing the cellulose content of the diet from 3 percent to 8 percent while keeping the diets isocaloric resulted in a small improvement in phosphorus availability.

Studies of the physical form of the diet have been undertaken by several investigators. Hardin and Milligan (1963) demonstrated a significant growth response by feeding coarse corn in place of finely-ground corn in broiler rations. The coarse corn was passed through a #4 Tyler screen while the fine was passed through a #8 Tyler screen.

Pelleting has resulted in growth responses in chicks as shown by Hinds and Scott (1958). A consistent response was noted by pelleting the corn fraction of a practical-type diet but no growth response was observed when a semi-purified diet was pelleted. The growth response disappeared when the pelleted corn was soaked in water.

Allred et al. (1957) noted that pelleting the diet resulted in a growth response in both poults and chicks even after grinding the pellets to a mash consistency. They concluded that the physical form does not explain all of the effects of pelleting but that some chemical change was taking place during the pelleting process. Perhaps either the heat or the moisture was inactivating a growth inhibitor which was present in the diets.

Griffith (1968) studied the effects of soybean products on the bone ash of chicks fed phosphorus-deficient purified diets. The experiments with chicks showed that replacing purified cellulose with ground soybean hulls in a purified, phosphorus-deficient diet resulted in an increase in bone ash. This response could not be duplicated by using sand instead of soybean hulls, nor by adding antibiotics, ammonium chloride or lignin to the diet. The response to soybean hulls disappeared when the hulls were ground to a powder before use in the diet. He also found that grinding soybean meal to a powder caused the loss of its antirachitic properties. Restricting intake of the coarse-hull diet to the same level as that of the powdered-hull diet did not reduce the bone ash. Griffith concluded that the antirachitic effect of soybean meal and hulls was due to physical properties imparted to the diet rather than to a chemical compound.

Another study by Griffith (1969) compared various coarse products and their effects on bone ash and growth rate when fed to chicks. These studies showed that phosphorus utilization by chicks was decreased when diets based on isolated soy protein or blood fibrin, or when diets based on soybean meal or cottonseed meal were fed in finely-powdered form. The bone ash of chicks was not affected by powdered diets containing casein or corn gluten meal. When added to a purified diet in small amounts (1 to 5 percent), soybean hulls, ground bagasse, ground wood or cellophane spangles were equally effective in improving bone ash. Kaolin, excess salt, lactose or inositol did not affect bone ash of chicks when fed in purified diets containing either coarse or

powdered soybean hulls. It was concluded from this study that some coarse material is necessary in most of the chick diets studied in order to achieve adequate phosphorus utilization, but that phosphorus utilization from diets containing casein or corn gluten meal was relatively unaffected by the presence or absence of coarse material.

Griffith (1970), while studying phosphorus availability with purified diets, attempted to establish the size of particle necessary to alter the phosphorus availability. Broiler chicks were fed five diets containing 7 percent soybean hulls. It was found that particles larger than 420 microns accumulated in the gizzard and significantly increased the bone ash of the chicks. Unfortunately, the hulls were ground and then passed through a series of screens sifting out the various particle sizes. It is not known if the various sizes represented a uniform sample of soybean hulls or if some portion was more readily cracked thus altering the constituent ratios.

Another experiment was reported by Griffith (1970) in which blood agar was used to stimulate a coarse particle in the feed. This was found to have little or no effect on the phosphorus utilization by the chick. This would indicate that the presence of a rough particle in the feed consumed was not sufficient to improve utilization unless it remained in a hard and rough state in the digestive tract.

The objectives of the present study were to further characterize the nature of the effects of particle size which were observed by Griffith.

EXPERIMENTAL PROCEDURE

Wrolstad Small White turkey poults, obtained from either Sunshine State Hatchery at Watertown, South Dakota; Willmar Poultry Company at Willmar, Minnesota; or Northern Turkey Hatchery Inc. at Frazee, Minnesota, were used in all studies except Experiments 70-14 and 70-15 in which Orlop poults from Jerome Hatchery at Barron, Wisconsin, were used. Nine or ten poults were randomly assigned to replicate pens in a stainless steel battery brooder at one day of age. Three or four replicate groups were assigned to each experimental diet. Body weights and percent tibia ash at four weeks were determined to measure the poults' responses to treatment effects. Experiments 67-01 through 69-9 and Experiment 70-16 were designed in a complete random block design and the means were separated by Duncan's multiple range test according to Steel and Torrie (1960). Experiments 69-10 through 70-15 were arranged in a complete factorial design and the interactions tested according to Steel and Torrie (1960).

The basal diet was a glucose-isolated soy protein semi-purified diet (Table 1). The treatments consisted of separate additions at a level of 5 percent of the diet, of corn, oat mill by-product (oat hulls) or soybean meal of different particle sizes for Experiments 67-01 through 68-04. Experiment 68-05 utilized a practical diet (Carlson and Bonzer, 1962) in both a coarse and powder form. The remaining experiments utilized various sources of isolated soy protein to test the effects of different particle sizes. All known vitamins and minerals were added in excess of National Research Council recommendations (NRC, 1966) (Table 2).

Table 1. Composition of Basal Purified Diet

Ingredient	Percent of Diet
Isolated soybean protein[1]	44.0
Cerelose[2]	42.3
Solka floc[3]	3.0
Corn oil	2.0
Dicalcium phosphate	4.0
Limestone	1.5
Minerals[4]	1.4
Vitamins and additives[5]	1.8

[1] RP-100, Ralston Purina Co., St. Louis, Mo. (See Appendix).

[2] A form of glucose, Corn Products Company, New York, New York.

[3] A cellulose product, Brown Company, New York, New York.

[4] Minerals amt./kg. of diet:

NaCl, gm.	4.97	KCl, gm.	4.97
$MgSO_4 \cdot 7\ H_2O$, mg.	3.00	$MnSO_4 \cdot H_2O$, mg.	197.00
$ZnSO_4 \cdot H_2O$, mg.	307.00	$FeSO_4$, mg.	307.00
$CuSO_4 \cdot 5\ H_2O$, mg.	88.00	$CoCl_2 \cdot 6\ H_2O$, mg.	22.00
KI, mg.	11.00	$Na_2MoO_4 \cdot 2\ H_2O$, mg.	22.00
Na_2SeO_4, mg.	44.00	H_3BO_4, mg.	11.00
$AlK(SO_4)_2 \cdot 12\ H_2O$, mg.	123.00	$Na_2SiO_3 \cdot 9\ H_2O$, mg.	50.00
NaBr, mg.	22.00		

[5] Vitamins and additives amt./kg. of diet:

Vitamin A, U.S.P.U.	15,408.00	Vitamin D_3, I.C.U.*	900.00
Vitamin E, I.U.	12.05	Menadione ($NaHSO_3$), mg.	17.00
Vitamin B_{12}, ug.	33.00	Folic acid, mg.	9.00
Pyridoxine, mg.	22.00	Riboflavin, mg.	22.00
Thiamine, mg.	22.00	Calcium pantothenate, mg.	44.00
Niacin, mg.	99.00	D-L methionine, gm.	7.07
Choline chloride, gm.	3.52	P-aminobenzoic acid, mg.	110.00
Glycine, gm.	4.97	Biotin, ug.	438.00
Ascorbic acid, mg.	22.00		
Oxytetracycline HCl, mg.	4.00		
Ethoxyquin**, mg.	146.00		

* Vitamin D_3 at National Research Council recommendations.

** Santoquin, an antioxidant, Monsanto Chemical Company, St. Louis, Mo.

Table 2. National Research Council Nutrient Requirements of Starting Poults (0 to 8 weeks)[1]

Ingredient	Amt./kg.
Total protein (%)	28.0
Vitamins	
Vitamin A activity, U.S.P. units	4,000.0
Vitamin D, I.C.U.	900.0[2]
Vitamin E	Varies
Vitamin K_1, mg.	0.7
Thiamine, mg.	2.0
Riboflavin, mg.	3.6
Pantothenic acid, mg.	11.0
Niacin, mg.	70.0
Pyridoxine, mg.	3.0
Choline, mg.	1,900.0
Folacin, mg.	0.9
Vitamin B_{12}, mg.	0.003
Minerals	
Calcium, %	1.2
Phosphorus[3], %	0.8
Sodium[4], %	0.15
Potassium, %	0.4
Manganese, mg.	55.0
Iron, mg.	60.0
Copper, mg.	6.0
Zinc, mg.	70.0
Amino acids	
Arginine	1.6
Lysine	1.5
Methionine + Cystine	0.87
Tryptophan	0.26
Glycine[5]	1.0
Isoleucine	0.84

[1]These figures are estimates of requirements and include no margins of safety, (N.R.C., 1966).

[2]The 1960 N.R.C. recommendations are 880 I.C.U. of Vitamin D_3 per kilogram of diet.

[3]At least 0.5 percent of the total feed should be inorganic phosphorus.

[4]Equivalent to 0.37 percent of sodium chloride.

[5]The poult can synthesize glycine but the synthesis does not proceed at a rate sufficient for maximum growth.

Tibia ash was determined by the following method (A.O.A.C., 1965): The poults were sacrificed and the left leg was removed. After excising the tibia, all adhering tissue was removed with a scissors and the tibia was immersed momentarily in boiling water, removed and rubbed clean with a cheesecloth. The bones were extracted for 24 hours in ethanol to remove the water and 24 hours in ether to remove the fat, following by air-drying for 12 hours to remove traces of ether. The extracted bones were dried in an oven at 100°C for 20 hours. They were then weighed, broken into small pieces and placed into pre-weighed crucibles which were placed into a muffle furnace for ashing. The temperature was increased 100°C at hourly intervals to a maximum of 600°C which was maintained for 15 hours. The crucibles were cooled to room temperature, weighed, and the percent bone ash was calculated as that portion remaining.

The finely-ground corn, oat mill by-product and soybean meal which were used in Experiments 67-01 and 67-02 were prepared by grinding in a Wiley mill using a 500 micron screen. The finely-ground form of diets for the other experiments were prepared by grinding the ingredients in a ball mill for 8 to 12 hours, or until they would pass through a 250 micron screen. The coarse-textured products were fed in their unprocessed state, largely in particle size in excess of 1,000 microns.

In Experiment 68-05, a practical corn-soy diet (Table 3) was fed to turkey poults in both its natural form and in a finely-ground form ($<$250 u). The practical diet (Carlson and Bonzer, 1962) was ground in the ball mill for 24 hours before sifting through a 250 micron screen.

Table 3. Composition of Practical Turkey Starter Diet

Ingredient	Percent of Diet
Ground yellow corn	39.4
Soybean meal (50%)	43.0
Alfalfa meal (17%)	2.0
Dried buttermilk	2.0
Fish meal	2.0
Trace mineral salt[1]	0.5
Dicalcium phosphate	2.0
Limestone	2.0
Methionine	0.1
Lysine	1.0
Vitamin supplement[2]	1.0
Corn oil	5.0

[1]Trace mineral salt:

Minerals	Percent	Provides/kg. of diet
NaCl	97.00	4.85 gm.
Mn	0.45	22.50 mg.
Zn	0.50	25.00 mg.
Fe	0.17	8.50 mg.
Cu	0.05	2.50 mg.
Co	0.01	500.00 ug.
I	0.01	500.00 ug.
S	0.30	15.00 mg.

[2]Vitamin supplement:

Vitamins and Additives	Amt./kg. of diet
Vitamin A, U.S.P.U.	10,560.00
Vitamin D_3, I.C.U.	880.00
Vitamin E, I.U.	44.00
Menadione sodium bisulfite, mg.	2.20
Vitamin B_{12}, ug.	17.60
Riboflavin, mg.	8.80
Pantothenic acid, mg.	17.60
Niacin, mg.	88.00
Choline Chloride, mg.	880.00
Oxytetracycline HCl, mg.	9.70
Ethoxiquin, mg.	220.00

Any particles which would not pass through the screen were reground, thus preventing any change in composition of the feed.

In Experiments 68-06 and 68-07, the cerelose and isolated soy protein were soaked in tap water overnight, placed in enamel pans to a depth of 3 cm and dried in an incubator with the humidstat removed. The temperature was maintained at 38°C for 36 hours while air was constantly passing over the mixture. The dried product was ground in a Wiley mill with a 2 mm screen to form the coarse particle size. Another sample was ground for 24 hours in the ball mill. A positive control was used in these experiments which consisted of replacing 3 percent of the cerelose with feed grade dicalcium phosphate to provide high levels of calcium and phosphorus in the diet. Magnesium carbonate also replaced magnesium sulfate in one diet to test the effect of the magnesium source.

Experiment 68-08 was conducted to test the effect of reautoclaving and regrinding of the protein source. The autoclaving was done in enamel trays in an electrically-operated steam autoclave. The protein source was spread to a depth of 3 cm and autoclaved at 120°C and 15 pounds of pressure for 60 minutes.

Edi-Pro, a textured form of isolated soy protein, (see Appendix) replaced all or part of the RP-100 in Experiment 68-09. This product is shipped in tows of filaments in an acid state containing 68 percent moisture. It was first necessary to wash it overnight in running tap water to remove the acid and salts. The product was then dried for 24 hours at 38°C in an incubator. The resulting dry fibers were ground

in a Wiley mill with 2 mm screen for the coarse-textured product and in a ball mill for 24 hours for the finely-textured product.

Experiment 69-11 utilized full-fat soybean meal obtained from Triple "F" Feeds*. The beans were processed by the extrusion method developed by that company. The high level of fat in the extruded beans caused the soybeans to cake while attempting to grind them in the ball mill. It was necessary to add 5 percent solka floc to the sample to soak up the excess oil. Since this would still cake in the ball mill, the product was ground in the Wiley mill with a 500 micron screen to be used as the finely-ground form. Commercially available solvent-extracted soybean meal was also used in this experiment (Table 4). This was prepared by grinding in the ball mill for 24 hours.

Experiment 70-12 utilized RP-100, C-1 assay protein and Fibrotein, a textured form of isolated soy protein (see Appendix). Fibrotein is shipped in an acid state with 68 percent moisture similar to Edi-Pro. This product was washed and dried in the manner described above for Edi-Pro.

Experiment 70-14 was conducted to study the effects of casein as a protein source. Casein was substituted isonitrogenously and supplemented with amino acids to meet the N.R.C. requirements (Table 2).

An anti-foaming agent** or surfactant, obtained from General Electric Corporation, was used in Experiment 70-15. The emulsion was

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* Triple "F" Feeds, P. O. Box 3600, Des Moines, Iowa 50322.

** AF-71 emulsion, approved as direct food additives up to 100 ppm. General Electric Silicone Products Department, Waterford, New York 12188.

Table 4. Composition of Diets Used in Experiment 69-11 (Percent)

Ingredient	Diets 1	2	3
Full-fat soybean meal[1]	25.0	--	--
Solvent-extracted soybean meal	--	25.0	--
Isolated soy protein[2]	34.0	30.5	44.0
Cerelose	30.1	27.6	42.3
Solka floc	2.2	2.2	3.0
Corn oil	--	6.0	2.2
Minerals, vitamins and additives[3]	8.7	8.7	8.7

[1]Extruded soybeans obtained from Triple "F" Feeds, Des Moines, Iowa.
[2]RP-100 obtained from Ralston Purina Co., St. Louis, Mo.
[3]Same as Table 1.

added to the isolated soy protein diets at a level of 100 ppm of silicone solids as permitted by the Food and Drug Administration. The experiment was designed to test the theory that decreased particle size may set up electrostatic forces which would decrease solubility of the diet. The surfactant would increase the solubility and thus increase nutrient absorption.

In an effort to determine if amino acid balance peculiar to soy protein was causing the effects observed with isolated soy protein diets, Experiment 70-16 was conducted. The experiment was designed to test the same amino acid balance from different sources. The diets used are presented in Table 5. Diet 1 is the isolated soy basal used throughout the previous experiments. Diet 2 has the same amino acid balance but casein is used as the major protein source with amino acids

supplemented to provide the same balance as Diet 1. Diet 3 is a 30 percent crude protein diet with isolated soy protein and Diet 4 is a basal diet with casein as the protein source. Diets 1, 2 and 4 were calculated to contain 38 percent crude protein.

Table 5. Composition of Diets Used in Experiment 70-16 (Percent)

Ingredient	Diets			
	1	2	3	4
RP-100[1]	44.06	--	34.54	--
Casein[2]	--	27.86	--	42.31
Cerelose	42.69	53.19	54.22	42.60
Solka floc	3.00	3.00	3.00	3.00
Lime	1.50	1.50	1.50	1.50
Dicalcium phosphate	4.00	4.00	4.00	4.00
Corn oil	2.00	--	--	2.00
Methionine hydroxy analogue	0.71	1.69	0.71	0.55
Glycine	0.50	2.17	0.50	1.48
Arginine	--	1.83	--	1.00
Histidine	--	0.27	--	--
Isoleucine	--	0.26	--	--
Leucine	--	0.86	--	--
Lysine	--	0.38	--	--
Phenylalanine	--	1.11	--	--
Tryptophane	--	0.03	--	--
Threonine	--	0.31	--	--
Minerals, vitamins, additives[3]	1.67	1.67	1.67	1.67
Crude protein (%)	38.28	38.28	30.00	38.28

[1]Isolated soy protein obtained from Ralston Purina Co., St. Louis, Mo.

[2]Vitamin-free casein obtained from Nutritional Biochemicals, Cleveland, Ohio.

[3]Same as Table 1.

RESULTS

Experiment 67-01

The results of this experiment are presented in Table 6. The analysis of variance is presented in Table 7. The addition of 3 percent dicalcium phosphate to the basal diet produced highly significant ($P<0.01$) increases in calcification as measured by bone ash, hereafter referred to as bone ash, per se. These poults did not, however, show any significant responses in weight gains. Mortality was highest in the dicalcium phosphate diet, but the difference from the basal control was not great enough to be statistically significant.

The addition of 5 percent corn to the basal diet resulted in highly significant ($P<0.01$) increases in bone ash and significant ($P<0.05$) increases in weight gains, irrespective of particle size. The poults on the diet of finely-ground corn, showed superior calcification as indicated by higher bone ash values than those on the coarse preparation. The mortality was higher with the coarse preparation and the feed was not utilized as efficiently. This seems to indicate either that there was better utilization of corn when ground to less than 500 microns, or that there was less wastage of feed due to the smaller particle size.

The addition of 5 percent coarse oat hulls to the diet resulted in highly significant ($P<0.01$) increases in the poults' bone ash but only significant ($P<0.05$) increases with the smaller particle size. Both preparations of oat hulls resulted in a significant response in

Table 6. Effects of Corn, Oat Hulls and Soybean Meal and Particle Size (Normal Grinding Versus 500 u) on Four-week-old Turkey Poults, Experiment 67-01

Diet	Body Weight (grams)	Bone Ash (percent)	Mortality (percent)	Feed Efficiency (feed/gain)
1. Basal (1760 Vitamin D_3)	201	34.8	61	2.4
2. Basal + 3% dicalcium phosphate	199	40.3**	67	2.2
3. Basal + 5% corn (>500 µ)	263*	40.4**	61	2.4
4. Basal + 5% corn (<500 µ)	262*	44.4**	58	1.8
5. Basal + 5% oat hulls (>500 µ)	263*	44.2**	53	1.7
6. Basal + 5% oat hulls (<500 µ)	255*	38.8*	33	2.1
7. Basal + 5% soybean meal (>500 µ)	224	40.0*	53	2.5
8. Basal + 5% soybean meal (<500 µ)	243	35.7	39	1.9

* Significantly different ($P<0.05$) from the basal.

** Highly significantly different ($P<0.01$) from the basal.

Table 7. Analysis of Variance, Experiment 67-01

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]		Feed Efficiency	
		Mean Square	F	Mean Square	F	Mean Square	F	Mean Square	F
Treatment	7	2,954.94	3.34*	47.58	9.33**	236.46	1.49	0.362	1.86
Residual	24	883.33		5.10		157.75		0.195	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$)

** Highly significantly different ($P < 0.01$).

weight gains. The poults on the diet of finely-ground oat hulls had lower mortality than those fed the coarse product. The poorer feed efficiency obtained with the finely-ground diet would indicate either less utilization or more wastage due to the birds attempting to select more appealing feedstuffs. With the 500 micron product, some of the oat fibers were still large enough to be distinguishable with the unaided eye.

Poults fed the coarse-ground soybean meal showed a significant bone ash response but no significant response in weight gains. The birds fed finely-ground soybean meal showed no significant changes in either percent bone ash or body weight. Mortality was lower for the birds fed the finely-ground diet.

Poults fed the finely-ground preparations of corn or soybean meal showed a tendency for improved feed efficiency values as compared to the values obtained with coarse-ground diets. This may show a tendency for better utilization of the smaller particle size. The fine preparation of oat hulls resulted in the opposite effect. The only statistically significant differences shown as a result of particle size or between the various feedstuffs was that of coarse oats and coarse soybeans, with the latter being inferior. There is no good explanation for this at this time.

Experiment 67-02

This study was conducted as a repeat of Experiment 67-01. The data from this experiment are presented in Table 8. The analysis of

Table 8. Effects of Corn, Oat Hulls and Soybean Meal and Particle Size (Normal Grinding Versus < 500 μ), Experiment 67-02

Diet	Body Weight (grams)	Bone Ash (percent)	Mortality (percent)	Feed Efficiency (feed/gain)
1. Basal	231	32.1	39	1.8
2. Basal + 3% dicalcium phosphate	288*	37.2*	11	1.7
3. Basal + 5% corn (> 500 μ)	296*	44.0**	14	1.8
4. Basal + 5% corn (< 500 μ)	256	32.6	19	1.7
5. Basal + 5% oat hulls (> 500 μ)	294*	46.3**	11	1.8
6. Basal + 5% oat hulls (< 500 μ)	327**	42.4**	17	1.6
7. Basal + 5% soybean meal (> 500 μ)	277	35.5	8	1.6
8. Basal + 5% soybean meal (< 500 μ)	258	31.7	25	1.8

* Significantly different ($P < 0.05$) from the basal.

** Highly significantly different ($P < 0.01$) from the basal.

Table 9. Analysis of Variance, Experiment 67-02

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]		Feed Efficiency	
		Mean Square	F	Mean Square	F	Mean Square	F	Mean Square	F
Block	3	35.07		11.12	1.86	10.27		0.016	
Treatment	7	3,552.66	4.42**	133.36	22.30**	396.03	1.74	0.031	
Residual	21	804.60		5.98		227.35		0.034	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

variance is presented in Table 9. The addition of dicalcium phosphate to the basal diet resulted in significant ($P < 0.05$) increases in both body weight and percent bone ash. The poults showed no corresponding responses in mortality or feed conversion. Dicalcium phosphate appeared to improve the feed conversion of the turkey poults as compared to the basal diet but this change was not statistically significant.

The addition of the coarse preparation of corn at the 5 percent level to the poults' diet resulted in a highly significant ($P < 0.01$) increase in percent bone ash and a significant increase in body weight. There were no significant responses obtained with the addition of the finely-ground corn. Feed conversion by the poults on the finely-ground diet was improved but not by a significant degree. Mortality was higher for the poults fed the fine preparation.

Poults fed finely-ground oat hulls evidenced a highly significant ($P < 0.01$) increase in both body weight and bone ash. The poults on the coarse preparation showed a highly significant increase in percent bone ash which was greater than with the fine preparation but only a significant ($P < 0.05$) increase in body weight. Feed conversion data were lower for the poults fed the finely-ground preparation, indicating either better utilization or less wastage from the birds picking over the feed. Mortality was somewhat lower for the poults fed the coarse preparation.

The addition of soybean meal at the 5 percent level resulted in no significant responses from the poults but the body weight and bone

ash values suggesting that the coarse preparation was superior to the fine product. Percent mortality and the feed conversion data support this observation.

Experiment 68-03

With the possibility that 500 microns was too large, this experiment was conducted to study the effects of grinding to less than 250 microns. The addition of 3 percent dicalcium phosphate to diets fed to poults again resulted in highly significant ($P < 0.01$) increases in body weight, percent bone ash and livability as indicated by the data in Table 10. The analysis of variance is presented in Table 11. The feed conversion data for this experiment were not available. The finely-ground preparations were ground in a ball mill until they would pass through a 250 micron screen.

Poults on the diets consisting of 5 percent coarse-textured corn produced highly significant increases in percent bone ash and body weight but did not show a significant difference in livability. Poults fed the finely-prepared corn showed a significant increase in body weight but no response was shown with percent bone ash or mortality.

The addition of ground oat hulls to the diet gave a highly significant ($P < 0.01$) increase in percent bone ash and body weight but only a significant ($P < 0.05$) decrease in mortality. Poults fed the finely-ground oat hulls did not show any significant responses.

The addition of coarse soybean meal to the poults' diet gave a highly significant ($P < 0.01$) increase in body weight but the responses

Table 10. Effect of Corn, Oat Hulls and Soybean Meal and Particle Size (Normal Grinding Versus < 250 μ), Experiment 68-03

Diet	Body Weight (grams)	Bone Ash (percent)	Mortality (percent)
1. Basal	137	24.0	96
2. Basal + 3% dicalcium phosphate	227**	37.1**	19**
3. Basal + 5% corn (> 250 μ)	251**	36.0**	60
4. Basal + 5% corn (< 250 μ)	212*	24.3	98
5. Basal + 5% oat hulls (>250 μ)	289**	40.6**	50
6. Basal + 5% oat hulls (< 250 μ)	131	26.6	98
7. Basal + 5% soybean meal (>250 μ)	267**	26.8	78
8. Basal + 5% soybean meal (< 250 μ)	151	23.8	99

* Significantly different ($P < 0.05$) from the basal.

** Highly significantly different ($P < 0.01$) from the basal.

Table 11. Analysis of Variance, Experiment 68-03

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Particle size	1	53,564.59	30.39**	298.30	90.04**	1,690.69	3.40*
Feedstuff	3	2,512.16	1.42	70.02	2.11	460.30	
Residual	19	1,762.39		33.13		497.90	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).
** Highly significantly different ($P < 0.01$).

noted for mortality or percent bone ash were not significant. The finely-ground soybean meal yielded no significant responses.

Statistical analysis of this experiment resulted in a highly significant F value when the effect of particle size was tested with all three parameters. In all cases the poults fed the finely-ground preparations had lower body weights and bone ash values and higher mortality values than the coarse preparations.

Experiment 68-04

The addition of 3 percent dicalcium phosphate to the diet resulted in highly significant ($P < 0.01$) improvement in percent bone ash, livability and feed conversion as shown by the data in Table 12. The analysis of variance is presented in Table 13. The poults showed only significant ($P < 0.05$) increases in body weight. The particle size of the finely-ground feeds used in this experiment was measured by a micrometer in a microscope using oil immersion. It was found that the particle size ranged as low as 3 microns. It was very difficult to determine the upper limit of the range due to aggregate particles. All of the finely-ground feed passed through a 250 micron screen.

Poults fed the diets containing 5 percent coarse yellow corn or oat hulls showed highly significant ($P < 0.01$) improvement in body weight, percent bone ash and feed conversion. The poults fed the finely-ground diets showed no significant responses.

Poults fed the coarse soybean meal did not respond as those fed the coarse corn and oat hulls, however, they did show a highly

Table 12. Effect of Corn, Oat Hulls and Soybean Meal and Particle Size (Normal Grinding Versus < 250 μ), Experiment 68-04

Diet	Body Weight (grams)	Bone Ash (percent)	Mortality (percent)	Feed Efficiency (feed/gain)
1. Basal	318	29.0	89	2.3
2. Basal + 3% dicalcium phosphate	428*	41.9**	21**	1.6**
3. Basal + 5% corn (> 250 μ)	488**	39.9**	47	1.6**
4. Basal + 5% corn (< 250 μ)	300	31.1	60	2.1
5. Basal + 5% oat hulls (> 250 μ)	475**	41.9**	50	1.6**
6. Basal + 5% oat hulls (< 250 μ)	333	33.1	53	1.9*
7. Basal + 5% soybean meal (> 250 μ)	404	32.8	59	1.7**
8. Basal + 5% soybean meal (< 250 μ)	436**	33.1	67	2.1

* Significantly different ($P < 0.05$) from the basal.

** Highly significantly different ($P < 0.01$) from the basal.

Table 13. Analysis of Variance, Experiment 68-04

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]		Feed Efficiency	
		Mean Square	F	Mean Square	F	Mean Square	F	Mean Square	F
Block	3	6,283.04	1.16	3.69		196.5		0.11	
Particle Size	1	64,549.13	11.89**	156.41	5.80**	108.7		1.03	7.36**
Feedstuff	3	3,114.24		27.78	1.03	69.2		0.80	5.71**
Residual	24	5,430.98		26.96		333.8		0.14	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

significant ($P<0.01$) reduction in feed conversion. The poults fed the finely-ground soybean meal showed a highly significant ($P<0.01$) increase in body weight which is in contrast to the results in Experiment 68-03.

Statistical analysis of this experiment again resulted in a highly significant F value when the effect of particle size was tested with three of the four parameters (Table 12). This would indicate that much of the growth inhibitory effect and rachitogenic activity of the isolated soy protein may be due to the small particle size involved.

Experiment 68-05

This experiment was conducted to test the hypothesis that growth inhibition and rachitogenic activity is not restricted to isolated soy diets. A practical corn-soy diet was fed to turkey poults in both its unprocessed form and ground in a ball mill to less than 250 microns. The results of this experiment are presented in Table 14. The analysis of variance is presented in Table 15.

Feeding the ground practical diet to poults resulted in highly significant ($P<0.01$) decrease in body weight and percent bone ash. Body weight of the poults on the finely-ground diets was comparable to that found when poults were fed isolated soy-glucose diets. Bone ash values of poults fed the finely-ground practical starter appeared slightly higher than those with poults fed the isolated soy-glucose diets. These results substantiate the hypothesis that the rachitogenic activity is largely due to physical properties rather than chemical properties of soy protein.

Table 14. Effect of Practical Turkey Starter Diet and Particle Size, Experiment 68-05

Diet	Body Weight (grams)	Bone Ash (percent)	Mortality (percent)
1. Practical turkey starter (> 250 μ)	539**	45.3**	0
2. Practical turkey starter (< 250 μ)	299	39.1	0

** Highly significantly different ($P < 0.01$)

Table 15. Analysis of Variance, Experiment 68-05

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality	
		Mean Square	F	Mean Square	F	Mean Square	F
Treatment	1	230,640.00	44.80**	153.76	24.37**	0	
Residual	14	5,149.50		6.31		0	

** Highly significantly different ($P < 0.01$).

Experiment 68-06

This experiment was designed to test the effects of soaking and/or autoclaving a portion of the diet in an effort to increase the particle size of the ration. The experiment was also designed to examine whether the response previously observed with 3 percent additions of feed-grade dicalcium phosphate to the diets, may have been partially due to the coarseness of the dicalcium phosphate. Therefore, a diet was formulated using dicalcium phosphate which had been ground in the ball mill to a fine powder. A practical starter was also fed in both a powder form and natural form to verify the results of the last experiment (68-05). Magnesium sulfate was added to the practical diet to test the response to added sulfate. A basal diet was also formulated using magnesium carbonate in place of magnesium sulfate to test the effects of magnesium sources. The results of this experiment are shown in Table 16. The analysis of variance is presented in Table 17.

Soaking the cerelose portion of the diet resulted in a somewhat detrimental effect on the poults' bone ash and body weight, although the differences were not significant (Diets 1 and 2). Soaking the protein resulted in little difference as shown by the results for Diets 1 and 4. Grinding the soaked protein, however, resulted in a significant ($P < 0.05$) decrease in the body weight of the poults but with little change in bone ash. Autoclaving the protein portion of the ration resulted in a slight depression in body weight but caused a significant ($P < 0.05$) increase in bone ash. Grinding the autoclaved protein resulted in a slight depression in both body weight and bone

Table 16. Effects of Soaking, Autoclaving, Grinding and Magnesium Source, Experiment 68-06

	Diet	Body Weight	Percent Bone Ash	Percent Mortality[3]
1.	Basal	387 bc[1]	34.3 ab	40 ef
2.	Basal with cerelose soaked	293 ab	32.4 a	27 cde
3.	Basal with protein[2] soaked and ground (< 250 μ)	249 a	33.2 ab	53 f
4.	Basal with protein soaked	393 bc	36.5 abc	20 bcd
5.	Basal with protein autoclaved and ground (< 250 μ)	256 a	38.9 bcd	33 de
6.	Basal with protein autoclaved	316 ab	40.8 cde	20 bcd
7.	Basal + 3% dicalcium phosphate ground (< 250 μ)	350 ab	41.5 de	30 de
8.	Basal + 3% dicalcium phosphate	375	41.5 de	23 cde
9.	Basal with $MgCO_3$	501 cd	34.9 abc	17 abcd
10.	Practical turkey starter	678 e	45.3 e	0 a
11.	Practical turkey starter with $MgSO_4$	611 de	46.3 e	3 ab
12.	Practical turkey starter ground (< 250 μ)	262 ab	41.4 de	10 abc

[1]Values with the same subscript do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

[2]RP-100, Ralston Purina Co., St. Louis, Mo.

[3]Analyzed by an arcsine transformation.

Table 17. Analysis of Variance, Experiment 68-06

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Block	2	6,383.46	1.49	8.30		279.63	3.01
Treatment	11	56,266.99	13.14**	64.29	7.00*	528.72	5.68*
Residual	22	4,283.45		9.18		93.01	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

ash, although the differences are not significant. Replacing the magnesium sulfate in the ration with magnesium carbonate, as shown by comparing Diets 1 and 9 resulted in an apparent, but nonsignificant increase in the body weight and bone ash.

The practical turkey starter produced poults with highly significantly ($P < 0.01$) greater bone ash and body weight. Grinding this diet depressed bone ash only slightly while the body weight was significantly ($P < 0.05$) decreased. Addition of magnesium sulfate to the practical turkey starter resulted in little or no effect upon body weight or bone ash as did grinding the phosphorus source as shown by comparing Diets 7 and 8.

Soaking a portion of the diet may alter the appearance of the diet but does not appear to alter the effective particle size. The coarse rough granules produced by this method are soluble and apparently disperse in the upper part of the gastrointestinal tract. This does not appear to alter the ability of the poult to absorb calcium or phosphorus.

Experiment 68-07

This experiment was designed to test the effects of magnesium source. The design was a random block design with treatments duplicated for each magnesium source. In this arrangement it was possible to separate the variance due to magnesium alone. The treatments included a basal diet, protein portion autoclaved, protein portion autoclaved and ground to less than 250 microns, protein portion soaked and the addition of 3 percent dicalcium phosphate to the basal diet. The results are

summarized in Table 18. The analysis of variance is presented in Table 19.

In this experiment the poults fed diets containing magnesium carbonate had lower body weights than those fed diets containing magnesium sulfate. The same was true with bone ash values except for the treatment in which the protein was autoclaved and ground. In this case the poults fed diets containing magnesium carbonate had slightly higher bone ash values than those fed diets containing magnesium sulfate. The F value obtained from comparing the magnesium sources was highly significant ($P < 0.01$) in the analysis of bone ash data, magnesium sulfate being the better of the two magnesium sources.

The results of this experiment also indicate that autoclaving the protein portion of the ration depressed body weight while increasing bone ash. This was contradictory to most previous experiments. Soaking the protein again proved detrimental to both body weight and bone ash. The addition of 3 percent feed grade dicalcium phosphate to the diets did not improve the poults' bone ash or body weight in this experiment. The high bone ash value obtained with the basal diet is not consistent with previous studies.

Experiment 68-08

This experiment was conducted to investigate the nature of the response previously observed on autoclaving the protein. The various treatments of the protein portion of the ration were as follows: autoclaved; autoclaved and ground; autoclaved, ground and reautoclaved;

Table 18. Effects of Magnesium Source, Experiment 68-07

	Diet	Body Weight	Percent Bone Ash	Percent Mortality[3]
1.	Basal with $MgSO_4$	390 c[1]	39.1 de	32 e[1]
2.	Basal with $MgSO_4$ with protein[2] autoclaved	348 c	43.5 f	30 e
3.	Basal with $MgSO_4$ with protein autoclaved and ground	320 abc	31.5 bc	50 cde
4.	Basal with $MgSO_4$ with protein soaked	241 ab	28.1 ab	92 a
5.	Basal with $MgSO_4$ + 3% dicalcium phosphate	360 c	38.3 de	44 dc
6.	Basal with $MgCO_3$	333 bc	28.3 ab	78 abc
7.	Basal with $MgCO_3$ with protein autoclaved	329 bc	42.1 ef	47 de
8.	Basal with $MgCO_3$ with protein autoclaved and ground	232 a	32.6 c	65 bcd
9.	Basal with $MgCO_3$ with protein soaked	262 abc	27.1 a	81 ab
10.	Basal with $MgCO_3$ + 3% dicalcium phosphate	349 c	36.8 d	33 c

[1]Values with the same subscript do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

[2]RP-100, Ralston Purina Co., St. Louis, Mo.

[3]Analyzed by an arcsine transformation.

Table 19. Analysis of Variance, Experiment 68-07

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Block	3	6,005.11	1.93	2.85		129.88	
Magnesium source	1	6,335.29	2.03	73.74	11.87**	380.01	2.83*
Treatment	4	22,192.31	7.13**	264.40	42.58**	1,232.53	9.18**
Magnesium x treatment	4	2,344.95		43.13	6.94**	435.59	3.24*
Residual	27	3,113.82		6.21		134.31	

[1] Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

and autoclaved, ground, reautoclaved and reground. The process was also reversed with the protein being ground first and then autoclaved. Diets 8 and 9 were formulated with magnesium carbonate replacing magnesium sulfate to again test magnesium source effects. A positive control diet containing an added 3 percent feed grade dicalcium phosphate was also included. The results of this experiment are summarized in Table 20. The analysis of variance is presented in Table 21.

It is apparent from this experiment that the response obtained by feeding protein which has been repeatedly autoclaved is not additive in terms of body weight, but it apparently is additive in terms of bone ash. Autoclaving the protein resulted in a significant ($P<0.05$) increase in body weight and bone ash. Grinding the autoclaved protein resulted in a slight but insignificant decrease in both body weight and bone ash. Reautoclaving the ground protein, however, stimulated bone mineralization while depressing even more the body weight. Regrinding resulted in depression of both body weight and bone ash. Reversing the order by grinding first and then autoclaving appeared to make little difference to the poults.

Replacement of magnesium sulfate with magnesium carbonate resulted in an insignificant increase in body weight but a slight depression in bone ash. The poults responded differently to the autoclaved protein in the magnesium carbonate diet (9) than to the autoclaved protein in the magnesium sulfate diet (2). This difference is unexplainable as it contradicts previous experiments. The addition of 3 percent feed grade

Table 20. Effects of Autoclaving, Grinding, Reautoclaving and Regrinding, Experiment 68-08

	Diet	Body Weight	Percent Bone Ash	Percent Mortality[1]
1.	Basal with $MgSO_4$	283 ab[2]	32.0 a	36 cde
2.	As 1 with protein[3] autoclaved	316 abc	40.7 bcd	16 ab
3.	As 2 with protein ground	305 ab	37.7 b	20 bcd
4.	As 3 with protein reautoclaved	297 ab	44.0 e	26 bcd
5.	As 4 with protein reground	274 ab	43.2 de	10 a
6.	As 1 with protein ground	261 a	31.8 a	50 e
7.	As 6 with protein autoclaved	292 ab	39.0 a	10 a
8.	Basal with $MgCO_3$	337 bcd	31.0 a	46 e
9.	As 8 with protein autoclaved	399 d	42.6 cd	16 ab
10.	As 1 + 3% dicalcium phosphate	382 cd	38.9 bc	22 abc

[1]Analyzed by an arcsine transformation.

[2]Values with the same subscripts do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

[3]RP-100, Ralston Purina Co., St. Louis, Mo.

Table 21. Analysis of Variance, Experiment 68-08

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Treatment	9	8,272.60	7.83**	96.14	23.70**	818.15	9.06**
Residual	30	1,056.39		4.06		90.34	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

dicalcium phosphate to the basal diet again resulted in significant increases in both body weight and bone ash.

Experiment 68-09

This experiment was designed to study the effects of adding a textured form of isolated soy protein to diets in an attempt to determine whether the antirachitic nature of soy protein diets was due to the texture of the diet. Unfortunately two problems occurred: (1) insufficient Edi-Pro was available to completely replace the RP-100 in Diets 3 and 6; and (2) the high salt content of the Edi-Pro was difficult to remove. Addition of Edi-Pro at 10 percent of the diet was justified on the basis that in previous experiments, a 5 percent addition of a coarse product was sufficient to reduce the rachitogenic activity of isolated soy protein. It was therefore assumed that 10 percent additions of the Edi-Pro would be adequate to show the antirachitic effect, if any was present.

Raw Edi-Pro was washed overnight in running tap water, but the salt was not completely removed. It was found by analysis after the experiment was underway that the salt content of Diets 4 and 5 was approximately 7 percent and Diets 3 and 6 approximately 1.5 percent. The results of this experiment are presented in Table 22. The analysis of variance is presented in Table 23. High salt content of Diets 4 and 5 may have caused the depressed growth observed in the poults. It did not, however, seem to affect the ability of the poults to mineralize bone.

Table 22. The Response of Poults to Edi-Pro, Experiment 68-09

	Diet	Body Weight	Percent Bone Ash	Percent Mortality[1]
1.	Basal with $MgSO_4$	345 c[4]	33.5 a	41 bcd
2.	As 1 with protein[2] autoclaved	328 c	44.7 d	30 ab
3.	As 1 with 10% Edi-Pro[3]	326 c	39.2 b	39 abc
4.	As 1 with 44% Edi-Pro ground (< 250 μ)	254 ab	37.2 ab	43 bcd
5.	As 1 with 44% Edi-Pro autoclaved	209 a	43.5 cd	52 d
6.	As 3 + 3% dicalcium phosphate	319 bc	44.3 cd	49 cd
7.	As 1 + 3% dicalcium phosphate	357 c	40.7 bc	27 a
8.	Basal with $MgCO_3$	377 c	33.5 a	33 ab

[1]Analyzed by an arcsine transformation.

[2]RP-100, Ralston Purina Co., St. Louis, Mo.

[3]Textured isolated soy protein, Ralston Purina Co., St. Louis, Mo.

[4]Values with the same subscript do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

Table 23. Analysis of Variance, Experiment 68-09

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Treatment	7	12,451.40	7.18**	81.07	14.05**	316.53	4.47**
Residual	24	1,734.00		5.77		70.75	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

Inclusion of autoclaved protein in the diets appeared to stimulate bone mineralization in the poults regardless of the texture of protein but made little change in body weight. When the Edi-Pro was ground prior to addition to the diets, a depression in the body weight was observed but only a slight decrease in bone ash.

The addition of 3 percent feed grade dicalcium phosphate to the basal diet resulted in a highly significant ($P < 0.01$) increase in the bone ash but no significant change in the body weight. The exchange of magnesium carbonate for magnesium sulfate resulted in no significant differences in the bone ash or body weight of the poults in this experiment.

Experiment 69-10

This experiment was conducted in a factorial design with autoclaving, grinding and protein source as the main effects. The two protein sources used, C-1 and RP-100, have different natural particle sizes, approximately 250 microns and 420 microns, respectively. The purpose of the experiment was to determine if both isolated soy proteins would have the same effect on poult growth and bone ash. Table 24 presents the means of the main effects and two-way interactions. The analysis of variance is presented in Table 25.

Autoclaving the protein source resulted in a significant ($P < 0.05$) decrease in body weight and a highly significant ($P < 0.01$) increase in the bone ash. Grinding the protein source did not significantly alter the variance in the poults' body weight and bone ash.

Table 24. Summary of Means of Main Effects and Two-way Interactions, Experiment 69-10

Treatment	Body Weight	Percent Bone Ash	Percent Mortality[1]
Main Effects			
Autoclaving	*	**	
Nonautoclaved	298	34.0	37
Autoclaved	270	41.1	35
Grinding			
< 250 μ	271	37.3	33
> 250 μ	296	37.9	40
Protein source		**	
C-1	294	39.9	31
RP-100	274	35.2	41
Two-way Interactions			
Protein x grinding			
C-1, > 250 μ	308	40.5	34
C-1, < 250 μ	281	39.4	27
RP-100, > 250 μ	285	35.2	45
RP-100, < 250 μ	262	35.2	38
Protein x autoclaving	*	*	
C-1, autoclaved	295	41.9	31
C-1, nonautoclaved	245	37.4	39
RP-100, autoclaved	294	35.0	31
RP-100, nonautoclaved	302	33.1	44
Autoclaving x grinding			
Nonautoclaved, > 250 μ	309	34.0	40
Nonautoclaved, < 250 μ	287	34.0	34
Autoclaved, > 250 μ	284	41.7	39
Autoclaved, < 250 μ	256	40.6	31

[1] Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$)

Table 25. Analysis of Variance, Experiment 69-10

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Replicates	3	200.30		6.70		8.14	
Autoclaving (A)	1	6,084.70	4.32*	405.99	34.91**	39.25	
Grinding (G)	1	5,006.50	3.56	2.72		388.79	1.35
A x G	1	94.19		2.82		1.39	
Protein (P)	1	3,514.15	2.50	178.13	15.32**	839.68	2.91
A x P	1	6,559.13	4.66*	63.11	5.43*	60.94	
G x P	1	52.38		2.76		0.18	
A x G x P	1	1,885.90	1.34	3.03		28.11	
Residual	21	1,407.56		11.63		288.46	

[1] Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

The source of protein did have a highly significant ($P < 0.01$) effect on the body weight. The C-1 protein which had the smaller initial particle size resulted in poults with the heaviest body weight and greatest percent bone ash. This appears to be contrary to previous observations in which diets with smaller particle sizes resulted in the lowest body weight and bone ash.

The only two-way interaction which proved significant ($P < 0.05$) was the protein by autoclaving interaction. Autoclaving the C-1 protein source resulted in an increase in body weight and bone ash. Autoclaving the RP-100 protein source, however, resulted in an increase in bone ash but a decrease in body weight. The increase in bone ash due to autoclaving the C-1 protein appeared to be of a much greater magnitude than autoclaving the RP-100 protein source. This would indicate a difference in the two protein sources in terms of a heat labile rachitogenic factor.

Experiment 69-11

This experiment was conducted to determine the response of turkey poults fed diets containing three different protein sources which had been autoclaved and ground. The experiment was designed in a factorial arrangement. The three protein sources were: full-fat extruded soybeans, obtained from Triple "F" Feeds; solvent-extracted dehulled soybean meal; and RP-100. The diets are shown in Table 4, page 27. These diets were calculated to be isonitrogenous but were not isocaloric. Table 26 presents the means of the main effects and the

two-way interactions. The complete analysis of variance is presented in Table 27.

The poults responded with highly significant ($P < 0.01$) differences in all three main effects for the parameters of body weight and bone ash. The poults fed the solvent-extracted soybean meal had the fastest growth, highest bone ash and lowest mortality although the latter difference was not significant. The poults fed the RP-100 diets had the lowest body weight, lowest bone ash and highest mortality.

Autoclaving the protein source resulted in poults with highly significantly ($P < 0.01$) increased body weight and bone ash in comparison with those fed nonautoclaved protein sources. Mortality, however, was not significantly altered by autoclaving the diets.

Modifying the particle size of the protein source resulted in highly significant ($P < 0.01$) differences in body weight, bone ash and percent mortality data. Poults fed the coarse feed produced the fastest gain, highest bone ash and lowest mortality.

None of the interactions were significantly different for either the parameters of body weight or mortality. With respect to the bone ash parameter, there was a highly significant ($P < 0.01$) protein by autoclaving interaction and protein by grinding interaction and a significant ($P < 0.05$) grinding by autoclaving interaction. In the protein by autoclaving interaction, the autoclaved solvent-extracted soybean meal diets resulted in poults with the highest percent bone ash while the non-autoclaved RP-100 diets resulted in poults with the lowest percent bone ash. All other combinations were not significantly different and clustered around the mean.

Table 26. Summary of Means of Main Effects and Two-way Interactions, Experiment 69-11

Treatment	Body Weight	Percent Bone Ash	Percent Mortality[1]
Main Effects			
Protein source	**	**	
Full-fat	407	33.5	31
SBM 50	483	36.5	21
RP-100	342	30.1	39
Autoclaved	**	**	
Autoclaved	453	35.3	26
Nonautoclaved	369	31.4	34
Grinding	**	**	**
< 250 μ	358	30.2	39
> 250 μ	464	36.5	22
Two-way Interactions			
Protein x autoclaving		**	
Autoclaved, full-fat	432	33.2	32
Autoclaved, SBM 50	531	39.9	13
Autoclaved, RP-100	395	32.9	34
Nonautoclaved, full-fat	381	33.9	29
Nonautoclaved, SBM 50	436	33.2	29
Nonautoclaved, RP-100	290	27.2	44
Protein x grinding		**	
Full-fat, > 250 μ	487	39.9	18
SBM 50, > 250 μ	538	38.7	13
RP-100, > 250 μ	367	31.0	34
Full-fat, < 250 μ	327	28.2	44
SBM 50, < 250 μ	429	34.4	28
RP-100, < 250 μ	318	29.2	44
Grinding x autoclaving		*	
Autoclaved, > 250 μ	502	37.5	21
Autoclaved, < 250 μ	403	33.2	32
Nonautoclaved, > 250 μ	425	35.6	23
Nonautoclaved, < 250 μ	313	27.3	45

[1] Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

Table 27. Analysis of Variance, Experiment 69-11

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Autoclaving (A)	1	41,844.36	20.66**	91.22	24.79**	330.93	1.98
Grinding (G)	1	67,317.45	33.24**	237.32	64.49**	1,741.82	10.39**
A x G	1	246.59		24.02	6.53*	156.78	
Protein (P)	2	39,820.74	19.66**	83.57	22.71**	615.46	3.67
A x P	2	1,704.30		31.94	8.68**	186.44	1.11
G x P	2	6,171.50	3.05	65.84	17.89**	129.37	
A x G x P	2	11,099.86	5.48*	9.60	2.61	254.64	1.52
Residual	12	2,025.28		3.68		167.59	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

The data in the protein by grinding interaction were similar, with poults fed the nonground full-fat soybean diets or the solvent-extracted soybean diets, having higher bone ash than those fed the ground full-fat soybean diets and the ground RP-100 diets. Grinding did not result in as large a decrease in the bone ash of the poults on the RP-100 diets as was observed with the other two protein sources. The full-fat soybean diets showed the greatest decrease in the bone ash as a result of grinding.

The grinding by autoclaving interaction was also significant ($P<0.05$). The autoclaved-nonground diets resulted in poults with higher percent bone ash than the nonautoclaved-ground diets. This indicates that both factors play a role in nutrient absorption. Body weight and percent mortality of the poults followed the same general trends throughout but were not significantly different.

Experiment 70-12

This experiment was designed to test the effects of different forms of isolated soy protein on growth, percent bone ash and mortality of turkey poults. Three forms of isolated soy protein were used, each having different initial particle sizes: RP-100, approximately 420 microns; C-1, approximately 250 microns; and Fibrotein, a textured form of isolated soy protein. The experiment was arranged in a factorial design with grinding in a ball mill and autoclaving as the other main effects. The means of the main effects and two-way interactions

are presented in Table 28. The analysis of variance is presented in Table 29.

The poults responded differently in terms of growth rate and mortality to the different protein sources. Poults fed the Fibrotein diets grew highly significantly ($P<0.01$) slower and had highly significantly ($P<0.01$) greater mortality. Bone ash was not significantly different. The RP-100 diets produced poults with the greatest body weight and lowest mortality.

Autoclaving the protein source resulted in poults with highly significantly ($P<0.01$) increased bone ash but a nonsignificant decrease in body weight and a nonsignificant increase in mortality.

Grinding the protein sources followed the same pattern as previous experiments. Poults fed diets with the protein ground to less than 250 microns had significantly ($P<0.05$) lower bone ash values while a nonsignificant decrease in body weight and a nonsignificant increase in mortality was observed.

The two-way interactions presented a similar picture. With respect to bone ash there was a highly significant ($P<0.01$) protein by autoclaving interaction. This appears to be due to the difference in response to the autoclaved and nonautoclaved forms within a protein type. As shown in Table 28, the poults fed the RP-100 autoclaved diets had the highest percent bone ash while those fed the RP-100 nonautoclaved diets had the lowest percent bone ash. The difference between the autoclaved and nonautoclaved diets was less within the C-1 protein group while the autoclaved diets actually resulted in

Table 28. Summary of Means of Main Effects and Two-way Interactions, Experiment 70-12

Treatment	Body Weight	Percent Bone Ash	Percent Mortality[1]
Main Effects			
Protein Source	**		**
RP-100	292	36.5	33
C-1	273	36.5	36
Fibrotein	189	36.5	55
Autoclaving		**	
Autoclaved	248	38.7	42
Nonautoclaved	255	34.3	41
Grinding		*	
< 250 μ	242	35.7	42
> 250 μ	261	37.3	41
Two-way Interactions			
Protein x autoclaving		**	
RP-100, autoclaved	287	40.6	30
RP-100, nonautoclaved	296	32.4	36
C-1, autoclaved	282	39.1	35
C-1, nonautoclaved	264	33.7	38
Fibrotein, autoclaved	174	36.4	59
Fibrotein, nonautoclaved	205	36.5	50
Protein x grinding			
RP-100, > 250 μ	300	37.8	30
RP-100, < 250 μ	284	35.2	36
C-1, > 250 μ	272	36.3	34
C-1, < 250 μ	274	36.6	38
Fibrotein, > 250 μ	210	37.7	57
Fibrotein, < 250 μ	169	35.2	52
Autoclaving x grinding		**	
Autoclaved, > 250 μ	259	40.6	41
Autoclaved, < 250 μ	236	36.8	42
Nonautoclaved, > 250 μ	262	34.0	41
Nonautoclaved, < 250 μ	248	34.6	42

[1] Analyzed by an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

Table 29. Analysis of Variance, Experiment 70-12

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Autoclaving (A)	1	675.22		233.15	33.50**	0.08	
Grinding (G)	1	4,070.63	2.88	30.99	4.45*	30.16	
A x G	1	199.39		56.88	8.17**	0.50	
Protein (P)	2	47,522.44	33.61**	0.005		2,155.29	9.01**
A x P	2	2,531.35	1.79	69.88	10.04**	264.95	1.11
G x P	2	1,804.98	1.28	10.44	1.50	124.41	
A x G x P	2	197.77		4.61		20.70	
Residual	36	1,414.08		6.96		239.24	

[1] Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

poults with lower bone ash values than those fed nonautoclaved diets in the Fibrotein group. It is interesting to note that the poults fed the nonautoclaved RP-100 diets had the highest body weight and the lowest percent bone ash.

The F ratio due to the protein by grinding interaction was not significant for any of the parameters evaluated. The autoclaving by grinding interaction was highly significantly ($P < 0.01$) different for bone ash. Poults fed the autoclaved nonground diets had the highest percent bone ash followed by the poults fed the autoclaved ground diets. The lowest bone ash values were observed for the poults fed the nonautoclaved diets. Percent mortality and body weight appeared to be influenced to a greater extent by the particle size as the nonground diets resulted in heavier poults with lower mortality than the poults fed the ground diets.

Experiment 70-14

Soy protein is known to contain phytic acid which is capable of forming an insoluble calcium phytate complex in physiological pH ranges. In an effort to determine if this complex was forming to tie up the calcium, a casein diet was produced which contained 1 percent phytic acid. This casein + phytic acid diet was compared to the basal casein diet and the basal isolated soy protein diet. A factorial arrangement of the diets was completed by including the factors of grinding and autoclaving as in previous experiments. Means of the

main effects and two-way interactions are presented in Table 30. The analysis of variance is presented in Table 31.

The poults responded similarly to the various protein sources with respect to body weight and bone ash, however, significant ($P < 0.05$) differences were observed in mortality. Poults fed the RP-100 diets had the lowest percent mortality while those fed the casein + phytic acid diets had the highest percent mortality.

Autoclaving the various diets resulted in a significant ($P < 0.05$) difference in the poults' bone ash but no difference in body weight or mortality. Poults fed the autoclaved protein diets had the highest percent bone ash. Grinding the various diets resulted in poults with highly significant ($P < 0.01$) lower bone ash values. No significant differences were observed in body weight or mortality due to the different particle sizes.

The only significant difference in the two-way interactions was found in the grinding by autoclaving interaction with respect to the body weight parameter. Poults fed the nonautoclaved, nonground diets had the greatest body weight while those fed the nonautoclaved ground diets had the lowest body weight. Poults fed the autoclaved ground diets had nearly the same body weight as those fed the nonautoclaved nonground diets. The same pattern was not observed in the bone ash values. The poults fed the autoclaved nonground diets had comparatively lower body weight but the highest percent bone ash. This response in body weight does not seem to follow the pattern set by most previous experiments.

Table 30. Summary of Means of Main Effects and Two-way Interactions, Experiment 70-14

Treatment	Body Weight	Percent Bone Ash	Percent Mortality[1]
Main Effects			
Protein source			*
RP-100	338	38.3	44
Casein	331	39.8	49
Casein + phytic acid	323	40.5	55
Autoclaving		*	
Autoclaved	333	40.5	50
Nonautoclaved	328	38.6	49
Grinding		**	
> 250 μ	333	40.7	49
< 250 μ	328	38.4	50
Protein x autoclaving			
RP-100, autoclaved	342	40.2	46
RP-100, nonautoclaved	334	36.5	43
Casein, autoclaved	326	40.4	47
Casein, nonautoclaved	335	39.2	51
Casein + phytic acid, autoclaved	330	40.9	57
Casein + phytic acid, nonautoclaved	316	40.1	53
Protein x grinding			
RP-100, > 250 μ	347	39.4	40
RP-100, < 250 μ	328	37.2	48
Casein, > 250 μ	339	40.7	53
Casein, < 250 μ	322	38.8	45
Casein + phytic acid, > 250 μ	314	41.8	54
Casein + phytic acid, < 250 μ	332	39.2	56
Grinding x autoclaving	**		
Autoclaved, > 250 μ	314	41.9	51
Autoclaved, < 250 μ	352	39.1	49
Nonautoclaved, > 250 μ	353	39.5	47
Nonautoclaved, < 250 μ	304	37.7	51

[1] Analyzed by an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

Table 31. Analysis of Variance, Experiment 70-14

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Autoclaving (A)	1	256.22		43.66	6.39*	9.85	
Grinding (G)	1	406.00		60.80	8.90**	10.72	
A x G	1	22,590.58	9.61**	3.18		95.08	
Protein (P)	2	867.82		19.89	2.91	463.20	4.79*
A x P	2	593.48		10.61	1.55	82.38	
G x P	2	1,775.08		0.62		261.84	2.71
A x G x P	2	3,171.64	1.35	12.82	1.88	153.26	1.58
Residual	36	2,350.79		6.83		96.64	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

Experiment 70-15

This experiment was designed to determine if a surfactant would aid in getting the finely-ground diets into solution thus improving nutrient utilization. Autoclaving and grinding were included in the factorial arrangement as in the previous experiments.

The summary of means of the main effects and two-way interactions is presented in Table 32. The analysis of variance is presented in Table 33.

The addition of the surfactant to the basal RP-100 diet resulted in an increase in body weight and percent bone ash. Percent mortality was also increased when the surfactant was added to the diets. Autoclaving the protein resulted in a highly significant ($P < 0.01$) increase in bone ash but no significant difference in body weight. Percent mortality of the poults on the autoclaved diets was not significantly altered.

Grinding the protein to less than 250 microns resulted in no significant change in body weight but a significant ($P < 0.05$) decrease in bone ash. Mortality was also slightly increased by feeding the finely-ground diets. There were no significant differences found in any of the two-way interactions.

Experiment 70-16

Since the rachitogenic activity of isolated soy protein appeared to be augmented by high protein levels in the diet, this study was conducted to test the response to lower protein levels and also to test

Table 32. Summary of Means of Main Effects and Two-way Interactions, Experiment 70-15

Treatment	Body Weight	Percent Bone Ash	Percent Mortality[1]
Main Effects			
Surfactant (S)			
+ Surfactant	367	39.0	45
- Surfactant	338	38.3	44
Autoclaving		**	
Autoclaved	358	40.6	46
Nonautoclaved	347	36.8	43
Grinding		*	
>250 μ	363	39.9	44
<250 μ	342	37.5	45
Two-way Interactions			
Surfactant x autoclaving			
+ S, autoclaved	374	40.9	47
+ S, nonautoclaved	360	37.1	44
- S, autoclaved	342	40.2	46
- S, nonautoclaved	334	36.5	43
Surfactant x grinding			
+ S, >250 μ	379	40.4	48
+ S, <250 μ	355	37.7	42
- S, >250 μ	347	39.4	40
- S, <250 μ	328	37.3	48
Autoclaving x grinding			
Autoclaved, >250 μ	358	42.0	48
Autoclaved, <250 μ	358	39.2	45
Nonautoclaved, >250 μ	368	37.8	41
Nonautoclaved, <250 μ	326	35.8	46

[1] Analyzed by an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

Table 33. Analysis of Variance, Experiment 70-15

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Autoclaving (A)	1	956.60		114.58	15.78**	79.86	
Grinding (G)	1	3,585.36		46.54	6.41*	0.08	
A x G	1	3,758.00		1.28		107.92	
Surfactant (S)	1	6,826.38	1.70	3.85		11.13	
A x S	1	450.96		0.01		7.83	
G x S	1	33.61		0.59		423.18	2.46
A x G x S	1	1,079.08		6.15		70.55	
Residual	24	4,019.95		7.26		171.96	

[1]Analyzed as an arcsine transformation.

* Significantly different ($P < 0.05$).

** Highly significantly different ($P < 0.01$).

the effects of amino acid balance. The composition of these diets is presented in Table 5, page 28. The experiment was conducted in a randomized design with the means separated by Duncan's multiple range test. The results are presented in Table 34. The analysis of variance is presented in Table 35.

No significant treatment effect was observed for the poults' bone ash values or percent mortality. Diets 1 and 2 had the same amino acid ratio but they were supplied by RP-100 soy protein and casein + amino acids, respectively. The poults fed Diet 1 had significantly ($P<0.05$) greater body weight than those fed Diet 2. When the RP-100 diet with lower protein level (Diet 3) was fed there was a slight increase in body weight as compared to the basal RP-100 diet. Although neither value was significant, the casein basal diet (Diet 4) resulted in poults with lower body weight than those fed the RP-100 basal (Diet 1) while the bone ash values were just reversed, being higher for the casein diet.

Table 34. Summary of Means, Experiment 70-16

	Treatment	Body Weight	Percent Bone Ash	Percent Mortality[1]
1.	RP-100 basal (38% protein)	263 ab[2]	31.8 a	43 a
2.	Casein + amino acids (38% protein)	202 c	33.0 a	48 a
3.	RP-100 (30% protein)	295 a	32.1 a	40 a
4.	Casein basal (38% protein)	211 bc	34.1 a	55 a

[1]Analyzed as an arcsine transformation.

[2]Values with the same subscript do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

Table 35. Analysis of Variance, Experiment 70-16

Source of Variation	Degrees of Freedom	Body Weight		Percent Bone Ash		Percent Mortality[1]	
		Mean Square	F	Mean Square	F	Mean Square	F
Treatment	3	7,768.75	7.20**	6.78		180.29	1.07
Residual	12	1,078.77		10.77		168.35	

[1]Analyzed as an arcsine transformation.

** Highly significantly different ($P < 0.01$).

DISCUSSION

The earlier investigations reported here were designed to test the hypothesis that the rachitogenic activity of isolated soy-glucose diets is due to the small particle size inherent in these diets.

In the first two experiments (67-01 and 67-02), 5 percent additions to the basal diet of corn, oat hulls and soybean meal were fed to turkey poults at two different particle sizes. The finely-ground diets had particle sizes up to 500 microns and resulted in variable responses in the poults in terms of bone ash and body weight. Very little difference was observed between the coarse and fine preparations. It appeared that this might have been due to the fact that there was still close similarity between the particle sizes of the two diets. The addition of 3 percent feed grade dicalcium phosphate to the basal diet did result in consistent responses in the bone ash but not in the body weight.

In the next two experiments (68-03 and 68-04), the fine particle size was reduced to less than 250 microns by grinding in a ball mill. In nearly every instance poults fed the finely-ground diets had lower body weight and percent bone ash than those fed the coarse diets. Growth on the finely-ground soybean meal preparation in Experiment 68-04 (Diet 8) and oat hulls in Experiment 68-02 (Diet 6) were exceptions which cannot be explained. Differences in feed efficiency observed in these experiments are due to differences in weight gain, not in feed consumed. It appears from these results that reducing the particle size reduces the ability of the poults to utilize the nutrients in the diet.

Experiment 68-05 was conducted to determine if this particle-size phenomenon was peculiar to isolated soy-glucose diets or if it was applicable to any soy-based diet. A practical turkey starter was fed in both a finely-ground form (particles less than 250 microns) and in its normal form. Poults on the finely-ground diet had highly significantly ($P < 0.01$) lower body weight and percent bone ash than those fed the practical diet. These data suggest that a typical soy protein diet fed in a finely-ground form to turkey poults would result in decreased body weight and percent bone ash. Again differences observed in feed efficiency were due to differences in weight gain and not to differences in feed consumption.

In Experiment 68-06, attempts were made to increase the particle size of the basal diet. The cerelose portion of the diet was soaked and dried resulting in large, rough-textured chunks of sugar. These chunks were crushed with a rolling pin to produce a particle size small enough to be consumed by the poults. Apparently the poults were able to reject this diet in terms of particle size as it proved slightly detrimental in both body weight and bone ash parameters.

Soaking and drying the protein portion of the diet resulted in no significant response in bone ash and body weight. Grinding this soaked RP-100 resulted in a significant ($P < 0.05$) decrease in body weight and bone ash. These data indicate that the effective particle size was not actually increased by soaking or else it returned to its natural particle size when consumed by the poult. Apparently the particles were dispersed in solution in the upper part of the gastrointestinal tract,

thus having no effect upon the lower GI tract where absorption of nutrients takes place. This concurs with results reported by Griffith (1970) using blood agar which dissolved readily in the upper part of the GI tract.

Previous experiments showed a response to 3 percent additions of feed grade dicalcium phosphate. Two diets were prepared in an effort to determine if this response was due to the particle size of the dicalcium phosphate or due to the additional calcium and phosphorus. One was prepared with the dicalcium phosphate ground to less than 250 microns and the other with the dicalcium phosphate in its normal state. A slight, but nonsignificant difference was observed in body weight and essentially no difference in bone ash by grinding. This indicates the response from dicalcium phosphate was due to the additional calcium and phosphorus available rather than the particle size of the dicalcium phosphate.

In an effort to improve the performance of the poults fed the basal diet, magnesium carbonate was used in place of magnesium sulfate in Experiments 68-06, 68-07, 68-08 and 68-09. In three out of four experiments magnesium carbonate improved the performance of the turkey poults. This indicates either a difference in availability of the magnesium sources or sulfate toxicity. Little is known as to the level of sulfate which could prove toxic but since most of the minerals were added as sulfate salts, the level in this diet was quite high. All subsequent experiments were conducted with magnesium carbonate as the magnesium source.

Autoclaving has been shown to result in improved body weight and percent bone ash, according to Thompson et al. (1970). Experiment 68-08 was designed to determine if the autoclaving was additive or if the effects of grinding and autoclaving are separate. Reautoclaving the ground diets slightly improved the body weight and bone ash. Grinding the autoclaved diets consistently lowered body weight and bone ash values. This indicates that two factors are involved. One appears to be inherent in soy protein and is heat labile while the other appears to be of a physical nature involving the particle size of the diet. It appears to make no difference whether the protein is autoclaved and then ground or if it is ground first and then autoclaved.

Efforts to increase the effective particle size of the diet by replacing the isolated soy protein with a textured form of isolated soy protein did not alter the rachitogenic properties of the diet. Apparently the textured forms were easily dissolved in the upper part of the GI tract similar to the soaked protein described earlier. Experiment 68-09 was further complicated by the high salt level of the Edi-Pro diets, thus resulting in poults with body weights and bone ash values similar to the basal RP-100 diets. Autoclaving the protein resulted in highly significant ($P<0.01$) increases in the bone ash value of the poults in this experiment.

The work reported by Thompson et al. (1970) was based primarily upon C-1 protein obtained from Skidmore Enterprises. Several differences were observed between these results and those obtained with RP-100 as the protein source. Experiment 69-10 was arranged in a

factorial arrangement to test the effects of autoclaving and grinding both protein sources. As noted earlier, the inherent particle sizes of these two proteins differ. The poults fed the C-1 protein diets were heavier and had higher bone ash values than those fed the RP-100 diets. It appears that the C-1 protein is improved by autoclaving whereas the RP-100 diets were not improved as much. The procedure used for autoclaving was that described by Thompson et al. (1970) and may not be optimal for RP-100 protein but more appropriate for C-1 protein. Grinding appeared to have a greater effect on the C-1 protein than the RP-100 although the differences were not significant.

Experiments 69-11 and 70-12 were conducted in an effort to further characterize the effects produced by grinding and/or autoclaving various types of soy protein. Experiment 69-11 compared full-fat extruded soybeans, solvent-extracted soybean meal and RP-100. Highly significant ($P < 0.01$) differences were observed in the poults' body weight and bone ash value due to differences in protein source. The solvent-extracted soybean diets resulted in poults with heavier weights and bone ash values than any other protein source. The full-fat extruded soybean diets resulted in poults with body weight and percent bone ash in the middle between that for the solvent-extracted soybean meal diets and the RP-100 isolated soy protein diets. Autoclaving the solvent-extracted soybean meal diets and the RP-100 isolated soy protein diets improved the bone ash while autoclaving the full-fat extruded soybean diets had a negative effect on the bone ash. This indicates that the

extrusion process may generate sufficient heat to destroy the rachitogenic factor(s) inherent in soy protein.

Grinding the various protein sources to less than 250 microns resulted in a decrease in body weight and bone ash in all instances. The decrease was much greater with full-fat extruded soybean diets than with the other protein sources.

RP-100, C-1 and Fibrotein were used in Experiment 70-12. Each protein source was both ground and autoclaved. Poults fed the different protein sources had highly significant differences ($P < 0.01$) in body weight. In this experiment, poults fed the RP-100 diets had the highest body weight and bone ash, while those fed the Fibrotein diets had the poorest performance. Salt was not a problem with the Fibrotein diets as it was with the Edi-Pro diets, however, the performance was similar. Apparently the process of texturizing the soy protein alters the availability of other nutrients.

Autoclaving the RP-100 diets resulted in poults with higher percent bone ash than those fed the autoclaved C-1 diets in contrast to Experiment 69-10. Autoclaving the Fibrotein diet did not affect bone ash in contrast to the significant response noted with autoclaved Edi-Pro. The process of texturizing may be different between the two sources thus explaining this difference in response.

Griffith (1969) reported that casein powdered in a ball mill did not alter the bone ash of chicks. Experiment 70-14 was designed to test this phenomenon along with the addition of phytic acid to a level comparable to that found in soy protein. Poults fed the casein diets

performed as well or better than poults fed the RP-100 diets. Autoclaving and/or grinding the casein and casein + phytic acid diets made little difference in body weight and bone ash, while the poults fed the RP-100 diets responded similarly to previous experiments.

The addition of a surfactant to increase the solubility of the finely-ground diets had little effect as shown in Experiment 70-15. Apparently the response observed with finely-ground diets is not due to decreased solubility of the isolated soy protein. Some physical factor other than solubility is involved. Autoclaving and/or grinding resulted in responses consistent with previous experiments.

In Experiment 70-16 a casein + amino acid diet was formulated which had the same amino acid balance as the RP-100 basal diet. This diet was thought to be similar to the isolated soy basal diet in every way except there would be no rachitogenic factor(s) present. The results show a significant ($P < 0.05$) decrease in the body weight and no difference in the bone ash. These results seem to indicate that the rachitogenic activity is not due to a chemical factor, but that rather, the poor performance of the turkey poults is due to the physical nature of the diet.

The nature of the response obtained by autoclaving is not understood. It may be a partial hydrolysis of some of the protein but there is no evidence to support this. The response observed by grinding either natural feedstuffs or the isolated soy protein may be a result of decreased physical stimulation of the gastrointestinal tract. Coarse particles of feed may increase the motility of the gastrointestinal

tract by a mechanism of physical irritation of the mucosa. This may increase the mixing and churning action of the intestinal tract thus bringing more nutrients in contact with the mucosa for absorption. The finely-ground diets may not stimulate this movement and therefore pass smoothly down the tract with little mixing, resulting in decreased absorption.

A basis for this theory resides in studies done with ruminant animals with all concentrate rations (Pearce and Moir, 1963; Weston and Hogan, 1967; Welch, 1967; Welch and Smith, 1969a, 1969b; and Church, 1969). Under these conditions it was found that rumen motility decreases and the rumen contents stratify thus causing decreased microbial action and absorption due to stagnation. Rumen motility can be greatly increased by adding a small amount of hay or plastic chips which irritate the rumen wall.

Little evidence of this effect is now available for monogastric animals. One recent study presented by Dziuk and Duke (1970), using fluoroscopy and cineradiography, showed that duodenal flow appeared to be dependent upon contractions of the gizzard. A reverse flow of duodenal contents was frequently observed during gizzard relaxation. No evidence is available as to the mechanism involved in gizzard stimulation or whether it is sensitive to the particle size of the feed. Both the ruminant and monogastric tracts, however, are composed of smooth muscle cells which are known to contract in response to physical stimulation, thus the possibility does exist (Guyton, 1966).

Unfortunately a means to accurately measure intestinal motility was not available to this author. An investigation of this phenomenon would seem highly desirable when techniques become available.

SUMMARY AND CONCLUSIONS

The nature of the rachitogenic activity of isolated soy protein was investigated in fifteen studies using day-old turkey poults. A RP-100-glucose purified diet was used as the basal diet throughout these experiments. Various substitutions such as corn, oat hulls, solvent-extracted soybean meal, full-fat extruded soybeans, C-1 protein, casein and texturized soy proteins, were investigated. The effect of autoclaving the protein for 60 minutes at 120°C and of grinding the protein to particle sizes less than 250 microns were also investigated. Calcium, phosphorus and Vitamin D_3 levels were maintained at or above the National Research Council recommendations in all diets. Parameters used for evaluation of the rachitogenic activity were body weight, percent bone ash and percent mortality at the end of the four-week studies.

The following conclusions were derived from these investigations:

1. Substitution of corn, oat hulls or solvent-extracted soybean meal at 5 percent of the basal diet improves the poults' body weight and bone ash. Grinding these natural feedstuffs to less than 250 microns eliminates these beneficial effects.
2. The addition of 3 percent feed grade dicalcium phosphate to the isolated soy-glucose basal diets improves the poults' body weight and percent bone ash regardless of the particle size of the product.

3. Grinding a practical turkey starter to less than 250 microns greatley depresses body weight and bone ash of the poults.
4. Autoclaving the isolated soy protein of the basal diet improves the poults' body weight and bone ash. Grinding the autoclaved diet depresses the poults' body weight and bone ash. These responses are different and separable since the order can be reversed. For example, grinding first or autoclaving first followed by the other produces similar results.
5. Soaking and drying either the protein portion or the sugar portion of the diet is not a means of increasing the effective particle size of the diet.
6. Texturized forms of isolated soy protein were not effective means of overcoming the rachitogenic effect of small particle size.
7. Levels of phytic acid comparable to that present in soy protein did not produce any inhibitory effects when added to casein-glucose diets.

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APPENDIX

SPECIFICATIONS AND USES OF C-1 ASSAY PROTEIN*

Assay Protein C-1 is a high purity chemically isolated soy protein, widely used in nutritional research. It is a complete protein requiring no supplementation with gelatin. Because Assay Protein C-1 is low in vitamins and minerals, it can be used for evaluating the vitamin interrelationships and nutrient requirements for various animals. Its economy has enabled nutritionists to expand research with experimental animals. It is used in diverse studies utilizing growing chicks, laying hens, turkey poults, weanling and growing pigs, sheep, calves, rabbits guinea pigs and rats.

Specifications

Protein	90% or more (moisture free, N X 6.25)
Moisture	9.0% ± 1.59
Ash	Less than 2.5% (moisture free)
Fat	Less than 0.5% (moisture free)
SO_2	Less than 0.05% (moisture free)

The remainder, about 6 percent, is composed of carbohydrates and phosphorus and sulfur compounds. A major portion of these are held in close association with the protein. The phosphorus compounds are largely organic, amounting to about 2 percent of the balance and include phytin and sugar phosphates. The sulfur portion approximating 1 percent consists largely of inorganic sulfate. Carbohydrates amounting to about 2.5 percent include simple sugars, hemicellulose and cellulose.

*Skidmore Enterprises, Cincinnati, Ohio.

Vitamin and Amino Acid Analysis of C-1 Assay Protein

The vitamin and amino acid composition of Assay Protein C-1 has been determined by microbiological assay, with the exception of thiamine for which the thiochrome method was used. The accuracy of microbiological assays is approximately ± 5 percent.

A large portion of the water soluble vitamins are removed during the isolation of Assay Protein C-1.

Vitamins - Values are expressed as micrograms per gram of moisture-free protein.

Amino Acids - The first 10 in the listing, arginine through valine, are the familiar "essential" amino acids. Glycine also is essential for chicks. All values are expressed as grams of amino acid per 100 grams of moisture-free protein.

Rations for experimental animals may require supplementation with methionine and/or glycine depending upon the animal's requirements and upon the level of C-1 used. Appropriate literature sources should be consulted.

Vitamin Analysis:

Biotin	0.3	Pantothenic Acid	4.2.
Choline	2.09	Para-Aminobenzoic Acid	0.7
Citrovorum Factor	0.92	Pyridoxine	5.4
Folic Acid	2.5	Riboflavin	1.2
Inositol	300.0	Thiamine	0.25
Niacin	6.0	Vitamin B_{12}	0.0005
Panthenol	None		

Amino Acid Analysis:

Arginine	8.3	Valine	5.5
Histidine	2.6	Glycine	4.1
Isoleucine	6.5	Alanine	3.6
Leucine	7.5	Aspartic Acid	6.2
Lysine	6.8	Cystine	0.6
Methionine	1.0	Glutamic Acid	19.5
Phenylalanine	5.0	Proline	2.5
Threonine	3.9	Serine	6.9
Tryptophane	1.0	Tyrosine	3.4

PURINA ASSAY PROTEIN RP-100*

Ralston Purina Assay Protein RP-100 is a purified high quality, chemically isolated soybean protein especially prepared for biological assay and nutritional laboratory research. Purina Assay Protein RP-100 can serve as the sole source of protein in your formulated laboratory diets and does not usually require supplementation with other proteins.

Special precautions are observed in the selection and treatment of soybeans and throughout the protein isolation process to preserve the excellent nutritive properties of the soybean protein. Judicious in-process treatment eliminates or minimizes negative nutritive factors. The non-protein components of the seed are effectively separated and removed. It is ready to use "as received" and does not require autoclaving, prior to use, to destroy enzymes or enhance its nutritive properties.

Purina Assay Protein RP-100 is utilized by nutritionists as the source of protein in purified and semi-purified diets for many experimental animals such as mice, rats, rabbits, guinea pigs, chicks, turkeys, pigs, calves, and sheep.

Typical Analysis

Protein (N X 6.25)(moisture free basis)	%94 or more
Moisture	%8 ± 1
Fat	%0.3 or less
Fiber	%0.2 or less
Ash	%1.5 or less
SO_2	%0.1 or less

*Ralston Purina Company, Checkerboard Square, St. Louis, Missouri 63199.

Typical Amino Acid Assay*

Essential		Non Essential	
Arginine	6.3	Alanine	3.6
Histidine	2.2	Aspartic Acid	10.5
Isoleucine	4.2	Cyst(e)ine	1.3
Leucine	7.4	Glutamic Acid	17.7
Lysine	5.3	Glycine	3.6
Methionine	1.2	Proline	5.6
Phenylalanine	4.9	Serine	4.5
Threonine	3.1	Tyrosine	3.5
Tryptophane	0.7		
Valine	4.3		

Typical Analyses (as is)**

Arsenic	0-0.4 ppm	Niacin	4.9 ppm
Calcium	0.39 %	Pantothenic Acid	0.63 ppm
Cobalt	0.02 ppm	Pyridoxine	1.3 ppm
Copper	6.8 ppm	Riboflavin	1.25 ppm
Fluorine	3.0 ppm	Thiamin	0.2 ppm
Iron	64.0 ppm	B-12	0.0025ppm
Lead	1.7 ppm		
Magnesium	57.5 ppm		
Manganese	1.2 ppm		
Molybdenum	1.9 ppm	Salt	0.03 %
Potassium	0.02 %	Chloride	0.018 %
Selenium	0.90 ppm	SO_2	0.1 %
Sodium	0.08 %		
Sulfur	1.02 %		
Zinc	11.6 ppm		
Phosphorus	0.56 %		

Product Description and Analyses

Standard Package	50 pound Fiber Drum
Light Tan Granular Powder	(thru 40 mesh, 420 microns)
Bulk Density	44 pounds (± 1 lb.) per cu ft
Storage Stability	Excellent (keep dry)

Laboratory diets employing Purina Assay Protein RP-100 may require supplementation with methionine or glycine depending on the animal's requirements and levels of Purina Assay Protein RP-100 used.

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* Values expressed as grams per 100 grams protein "as is", typical 87 percent or more protein, 8 percent H_2O.

** Elements, vitamins, and compounds (as shipped), typical 87 percent or more protein, 8 percent H_2O.

TEXTURED EDI-PRO*

Textured Edi-Pro is purified, isolated soy protein which has been converted into continuous filament or fiber form by a patented process. It is bland in flavor, light in color, tender, and has good protein nutritive values.

Typical Analysis

	As Shipped Acid-Salt State	Ready to Use
Protein	35.0 %	30.0 %
Moisture	55.2	69.1
Fat	.70	.61
Ash	7.74	.75
Potassium	.02	.006
NaCl	8.8	.0
Ca	.31	.34
P	.04	.03
S	.2	.2
Fe	43 ppm	24 ppm
Cu	9 ppm	6 ppm
pH	3.5	6.1

Typical Essential Amino Acid Content**

Arginine	7.90	Histidine	2.56
Isoleucine	7.64	Leucine	8.48
Lysine	6.21	Methionine	1.00
Phenylalanine	5.66	Threonine	3.85
Tryptophane	.81	Valine	8.45

Textured Edi-Pro represents a significant break-through for the food industry. Now the food technologist is able to engineer into new foods the properties of controlled structure, tenderness, composition,

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* Ralston Purina Company, Checkerboard Square, St. Louis, Missouri 63199.

** In grams per 100 grams of sample (dry basis).

nutrition and flavor. The versatility of this unique material allows the properties of absorbency, chewability, color, drying characteristics, and heat resistance to be easily controlled, thereby making it possible to fabricate products ranging from chewy meat-like foods to crunchy snacks and confections.

Textured Edi-Pro is now in commercial production in "tows" of 60,000 filaments or multiples thereof. Each filament is approximately .001 (one thousandth of an inch) in diameter. It is shipped in polyethylene bags and is moistened with an acid-salt brine to preserve its factory fresh quality until ready for processing.

TEXTURED SOY PROTEIN FIBERS*

Amino acid analysis of the protein of textured soy protein (Fibrotein) before supplementation and incorporation into finished products. (Expressed as grams per 100 grams of protein).

Lysine	5.7	Histidine	2.5
Arginine	8.6	Aspartic Acid	11.2
Threonine	3.1	Serine	4.3
Glutamic Acid	19.0	Proline	5.0
Glycine	3.6	Alanine	3.5
Valine	4.2	Methionine	1.0
Isoleucine	4.4	Leucine	7.5
Tyrosine	5.5	Phenylalanine	5.0

Typical analysis of spun fiber at 68 percent moisture:

Protein	28.4 (gms./%)
Moisture	68.0
Ash	1.8
Fat	0.14
Fiber	0.048
Sodium	1.3 to 1.5
Calcium	0.0416
Phosphorous	0.256

This is somewhat representative of soy protein as the first limiting amino acid is methionine (compared with amino acid analysis in Soyamel booklet).

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*Worthington Foods, Inc., Worthington, Ohio 43085.