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I am submitting herewith a thesis written by Brenda Faye Beaty entitled "Heat Inactivation of Trypsin Inhibitor in Vegetable-Type Soybeans and Physiological Responses of Rats to These Beans." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

J. L. Collins, Major Professor

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HEAT INACTIVATION OF TRYPSIN INHIBITOR IN VEGETABLE-TYPE SOYBEANS AND PHYSIOLOGICAL RESPONSES OF RATS TO THESE BEANS

A Thesis

Presented for the

Master of Science

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ABSTRACT

The objective of this investigation was to determine the effect of boiling water on the inactivation of trypsin inhibitor in green vegetable-type soybeans and to determine the effect of feeding the heat-treated soybeans to rats on weight gain, protein efficiency ratio, pancreas weight, liver weight, percentages of lipid and protein in the liver and histology of the pancreas.

Trypsin inhibitor activity decreased rapidly with a short heating time. In soybeans heated one minute, 62% of the TIA was destroyed, whereas in soybeans heated nine minutes, 96% of the TIA was destroyed.

Rats fed raw soybeans weighed less than rats fed heated soybeans or casein. An inverse relationship was found between increased weight gain and decreased TIA as heating time was extended.

Protein efficiency ratio (PER) increased as the heating time was extended. The PER of raw and heated soybeans was lower than the PER of casein.

The pancreas weight of rats fed raw soybeans was greater than that of rats fed heated soybeans or casein. The pancreas weight of rats fed heated soybeans was not different from that of rats fed casein. The occurrence of pancreatic hypertrophy in rats fed raw soybeans and a decrease in pancreatic hypertrophy in rats fed heated soybeans are related to inactivation of trypsin inhibitor. Histological examination of the enlarged pancreas of rats fed under heated soybeans indicated that enlargement is due to an increase in the number of cells or hyperplasia.

The liver weight of rats fed raw soybeans was less than that weight of rats fed heated soybeans or casein. There was a trend for increased liver weight as heating time was extended.

The lipid content of liver of rats fed raw soybeans was greater than that of rats fed heated soybeans or casein. In general, there was no trend relating heat treatment to lipid content of rat liver, other than the finding that raw soybeans produced the largest amount of lipid.

Rats fed raw soybeans had less protein in their livers than the rats fed heated soybeans or casein. There was a trend for an increase in protein content as the heating time was extended.

The measurement of various physiological responses in rats fed raw and heat-treated soybeans indicates that holding soybeans in boiling water for nine minutes is adequate to inactivate the trypsin inhibitor, although additional time is needed to achieve maximum protein efficiency ratio.

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CHAPTER I

INTRODUCTION

The soybean, native to eastern Asia, has become the leading source of high quality plant protein and edible oil in the United States and certain other countries (25,79). By virtue of the large world production, low cost, and desirable nutritional and functional properties, the soybean has the potential for making a substantial contribution to the world protein needs.

In industrialized countries, most of the soybean protein is used as a protein supplement in livestock feed (79). However, the soybean offers an excellent source of high quality protein for human consumption. Presently, limited use is made of soybean products as ingredients for such items as baked goods, meat products, meat analogs, infant foods, beverages, breakfast cereals, dietary foods and snack foods (49).

While the soybean is composed of high quality protein, it also contains various antinutritional factors which elicit diverse nutritional, biological, and physiological responses in animals (71). Raw soybeans and under heated soybeans and soybean products inhibit growth, depress metabolizable energy and fat absorption, reduce protein digestibility, cause pancreatic hypertrophy, stimulate hyper- and hyposecretion of pancreatic enzymes, and reduce amino acid, vitamin, and mineral availability (69,71,72). Almost all these effects are

interrelated and affects an animal's inability to use the essential nutrients fully rather than a response to toxic substances (71).

One of the antinutritional factors having received the most investigation is the trypsin inhibitors. Trypsin inhibitors account for 30-50% of the growth inhibitory effect of raw soybean meal and for nearly all the pancreatic hypertrophic response (71).

Proper heat treatment exerts a beneficial effect upon the nutritional value of raw soybeans. Contributing to improved nutritional value is the simultaneous destruction of trypsin inhibitor. Moist heat is most effective for destroying trypsin inhibitors in soybeans (2,71,72).

More recently, the mature green soybean is being recommended as an excellent source of protein. By its very nature, this crop could be grown and produced by gardeners. Since no studies could be found on heat inactivation of trypsin inhibitor in the vegetable-type soybean, this study was conducted. The objective was to determine the effect of boiling water treatments on inactivation of trypsin inhibitor in vegetable-type soybeans and to determine the effect of feeding the heated soybeans to rats on weight gain, protein efficiency ratio, pancreas weight, liver weight, percentages of lipid and protein in the liver, and histology of the pancreas.

CHAPTER II

REVIEW OF LITERATURE

I. IMPORTANCE OF SOYBEAN AS FOOD

The soybean (Glycine max L.) ranks highly among the leguminous crops in its content of high quality protein and edible oil. For this reason, soybeans can be considered a protein concentrate without defatting (25). Few other legumes have attracted as much attention as the soybean with regard to the tremendous possibilities of their use in animal feeds, human foods, and industrial application (25,33,79).

The soybean has been cultivated for thousands of years in the Orient (56,67,72,79). This crop makes an important contribution to the diet of Oriental people especially in the form of traditional foods. The soybean is a relatively new crop in the United States. Production of soybeans as an important commercial crop in the United States did not begin until the 1930's. However, in the late 1940's and early 1950's, the United States overtook China and eventually the entire Orient in soybean production (68). Today, soybeans are the most important cash crop in the United States (79). The United States soybean crop contributes more protein and oil to the food economy than any other single source (79).

II. NUTRITIVE VALUE OF SOYBEAN

The soybean varieties currently grown in the United States contain 39 to 45% (dry matter basis) high quality protein and 20 to 23% (dry matter basis) oil (33). Soybeans rank as one of the five great

proteinaceous foods, comparing favorably with meat, milk, fish, and eggs (58). The essential amino acid composition (gm/16 gm N) of defatted soy flour is: isoleucine, 4.6; leucine, 7.7; lysine, 6.2; methionine, 1.3; cystine, 1.2; phenylalanine, 5.3; threonine, 4.2; tryptophan, 1.4; and valine, 4.9 (49). Cystine and methionine are the first limiting amino acids of soybeans (49).

Soybean oil is quite desirable because it contains a considerable amount of unsaturated fatty acids. The total fatty acids consist of approximately 15% saturated, 25% oleic, 55% linoleic, and 5% linolenic acids (25).

Soybeans are a fair source of vitamins. Although the B vitamins are present in substantial amounts, soybean products are devoid of vitamins D and B_{12}. Concentrations of vitamin C are high in immature soybeans and soybean sprouts but are not present in mature soybeans. Vitamines E and K are found only in whole soybeans and full-fat soy flour (49).

The availability of most minerals from soybeans is quite low (24,31). Phytic acid is present in soybeans and interferes with the availability or utilization of certain minerals, particularly calcium and phosphorus (61,80).

III. ANTINUTRITIONAL FACTORS IN SOYBEANS

Raw soybeans contain many interrelated substances which cause differing biological and physiological responses in various species of

animals (71,72). Raw full-fat and defatted soy flours inhibit growth, depress metabolizable energy and fat absorption, reduce protein digestibility, cause pancreatic hypertrophy, stimulate hyper-and hyposecretion of pancreatic enzymes, and reduce amino acid, vitamin, and mineral availability (72). Almost all these effects are interrelated and affects an animal's inability to use the essential nutrients fully rather than a response to toxic substances. The nontoxicity of raw soybean was shown by Booth et al. (16) who reported that growth inhibition and pancreatic hypertrophy are readily reversible. After rats had been fed for 38 days to develop maximum pancreatic hypertrophy, the animals were switched to a basal diet for an additional 154 days; growth and pancreas weight became normal.

Hemagglutinins, proteins which agglutinate red blood cells, are distributed widely in the plant kingdom (35). Liener (46) isolated hemagglutinin from soybeans and reported that this substance may account for 25% growth inhibition which raw soybeans produce in rats. The growth-inhibiting properties of soybean hemagglutinins can be eliminated by proper heat treatment (46).

Nonprotein components such as saponins have been suggested as possible antinutritional factors. Whole soybeans contain about 0.5% saponin (71). Although saponins in some plants have antinutritional properties, saponins isolated from soybean meal do not harm chicks, rats, and mice, even when fed at a level three times greater than the level in diets containing soy flour (14). Sufficient evidence has been accumulated to remove saponins from the list of antinutritional factors in soybeans (14).

Kakade et al. (38) showed that the trypsin inhibitors make a significant contribution to the deleterious effects of unheated soybean meal since their selective removal by affinity chromatography not only produced a significant improvement in the protein efficiency ratio of the protein but also a significant reduction in pancreas size of rats. Studies by Rackis et al. (73) indicate that the greatest inhibition of rat growth and enlargement of the pancreas occurs with soybean meal fractions possessing very high trypsin inhibitor activity. Rackis (69) reported that trypsin inhibitors account for 30-50% of the growth inhibitory effect of raw meal and for nearly all of the pancreatic hypertrophic response.

IV. TRYPSIN INHIBITOR

Trypsin inhibitors are proteinaceous and thus have the ability to inhibit proteolytic activity of certain enzymes (72). Trypsin inhibitors actively inhibit the action of trypsin and chymotrypsin which are enzymes in the pancreatic juice responsible for hydrolysis of peptide linkages of certain amino acids during protein digestion (83).

Trypsin inhibitors are probably the most widely distributed among the inhibitors of proteolytic enzymes. In addition to soybeans, trypsin inhibitors are also present in lima beans, mung beans, kidney beans, navy beans, chick peas, field peas, potatoes, sweet corn, wheat, peanuts and other fruits and vegetables (51).

Seven or more trypsin inhibitors are reported for soybeans (71) but only two, the Kunitz (45) and the Bowman-Birk (13), have been

purified and studied in detail. The Bowman-Birk acetone-insoluble inhibitor differs from the Kunitz inhibitor not only in the fact that it is a more potent inhibitor of trypsin but it also displays powerful inhibitory activity toward chymotrypsin (51). Soybean trypsin inhibitor is slightly active against human trypsin, whereas, it is very active against human chymotrypsin and bovine trypsin and chymotrypsin (81). Both the Bowman-Birk inhibitor and the Kunitz inhibitor cause growth inhibition and pancreatic hypertrophy in rats and chicks (28).

V. EFFECT OF TRYPSIN INHIBITOR ON EXPERIMENTAL ANIMALS

Osborne and Mendel (63) are generally credited with having been the first to observe that soybeans, unless cooked for several hours, would not support the growth of rats, an observation which has since been extended to many other experimental animals (47). In addition to an inhibition of growth, the primary physiological response to unheated soybeans is the development of pancreatic hypertrophy.

Chernick et al. (22) were the first to report that chicks fed raw soybeans developed hypertrophy of the pancreas. This observation has since been confirmed for the rat (15,16,55,69). In rats, maximum pancreatic hypertrophy occurs in nine days of feeding raw meal (69). Increased pancreatic secretion is almost immediate in rats (55), whereas in chicks, hypertrophy and pancreatic juice secretion is delayed about 21 days following the feeding of soybean trypsin inhibitor (37,62,69). Adult rats and chicks fed raw soybean meal

maintain body weight in spite of prolonged pancreatic hypertrophy (16). Pancreatic hypertrophy occurs in laying hens (19) and germ-free chicks fed raw soybean meal (23).

Gorrill et al. (29) observed poor growth, but reduced pancreatic secretion, and no pancreatic hypertrophy in young calves fed raw soybean meal. Raw soy flour does not cause pancreatic hypertrophy in pigs, and pancreatic juice secretion is lower than with a heated soybean meal diet (66). Normal pancreas weight and pancreatic secretion occurs in dogs (65).

VI. INACTIVATION OF TRYPSIN INHIBITOR

Most plant protease inhibitors are destroyed by heat, an effect which is generally accompanied by a general enhancement of the nutritive valve of the protein (48,64,72). The degree of improvement in nutritive valve of soybeans parallels the destruction of the trypsin inhibitory activity by moist-heat treatment (48) and depends upon temperature, duration of heating, particle size, and moisture conditions (42,51,72). Excessive heat treatment markedly impairs nutritive valve of soybeans (42).

Almost all investigators agree that maximum protein efficiency is achieved by autoclaving mature, low moisture soybeans at 15 pounds per square inch pressure for no more than 30 minutes (17,42). At 100°C, only 15 minutes of atmospheric steaming, referred to as toasting, are required to achieve maximum protein efficiency and to inactivate 95% of the trypsin inhibitors of either full-fat or defatted soybean

flakes (69,70,78). The improvement in protein efficiency affected by atmospheric steaming at a level of 19% moisture is somewhat higher than at a level of 5% moisture (69,70).

The trypsin inhibitor of whole soybeans with an initial moisture content of 20% is completely destroyed by atmospheric steaming for 15 minutes, and, when the moisture content is raised to 60% or more by overnight soaking, boiling for only five minutes is sufficient to inactivate the inhibitor (2).

Trypsin inhibitors in full-fat soybeans are destroyed readily within two minutes by extrusion-cooking (3,60). The short cooking time minimizes damage to nutritional properties but adequately destroys the trypsin inhibitor. Roasting (9), infrared cooking (27), dielectric heating (18), and microwave processing (30) of soybeans improve nutritive value and destroys trypsin inhibitor activity; however, inactivation of trypsin inhibitor and maintenance of uniform nutritional value are both difficult to control.

Numerous studies have been undertaken to determine whether supplementation of the raw soybean protein with various amino acids would achieve the same effect as heating. Such experiments showed that the addition of methionine or cystine, limiting amino acids in the soybean, to unheated soybean meal improved protein utilization to essentially the same extent as proper heating (10,32). It is important to note, however, that additional methionine did not raise the nutritive value of raw soybean meal to the level of heated soybean meal similarly supplemented with methionine (50).

CHAPTER III

MATERIALS AND METHODS

I. SOURCE OF SOYBEANS

The soybeans, Prize variety, used in this study were grown in 1977 on the University of Tennessee Plant Science Farm at Knoxville by personnel of the Department of Plant and Soil Science. There was a May and June planting, and the plantings were designated as replications 1 and 2, respectively. The soybeans were allowed to mature to a stage just prior to the loss of chlorophyll. The plants were cut by hand and transported to the McLeod Food Technology Building.

II. PREPARATION OF SOYBEANS FOR TREATMENTS

The soybean pods were removed from the plants by hand, immersed in water at 80°C for one minute, and cooled in tap water to facilitate shelling. The beans were removed from the pods by passing them through a Taylor roller-type sheller (Model 675-7084). The beans were immersed in boiling water for 0, 1, 2, 3, 6 and 9 minutes. Ice water was used for immediate cooling of the beans. The beans were individually quick frozen in an air-blast freezer at -29°C and transferred to a still freezer at -17°C. After freezing, the beans were placed in a forced air dehydrator at 43°C and held for 48 hours. The dried beans were ground in a Viking hammer mill using a screen with openings of one mm.

After grinding, the beans were placed in a closed glass container with a desiccant and held at 1°C until preparation of diets.

III. MOISTURE DETERMINATION ON SOYBEANS

Moisture content of the soybeans was determined by the A.O.C.S. Official Method AC 3-44 (7). Five gram samples of the ground soybeans were dried at 130° ± 3°C for three hours in a direct heat oven. Moisture was determined in triplicate on each treatment.

IV. CRUDE LIPID DETERMINATION ON SOYBEANS

The crude lipid content of the oven-dried soybean material was determined by the A.O.C.S. Official Method AC 3-44 (7) on a Goldfish Extraction Apparatus. The soybean material was extracted with petroleum ether for five hours. Five samples of two grams each were analyzed per treatment. The crude lipid content was expressed as the percentage of ether extract in the sample based on dry weight.

V. CRUDE PROTEIN DETERMINATION ON SOYBEANS

Percentage of protein (N × 6.25) was determined by the Kjeldahl method according to the A.O.C.S. Official Method (AC 4-41 (7). Four samples of the oven-dried, defatted soybean material weighing one gram each were analyzed per treatment.

VI. TRYPSIN INHIBITOR ACTIVITY DETERMINATION

Trypsin inhibitor activity was determined by the method developed by Kakade and associates (39). Duplicate determinations were performed

on one gram samples of the oven-dried raw and heated soybean material. One gram of finely ground sample (100 mesh) was extracted with 50 ml. of 0.01 N NaOH. With raw soybean material the extraction time was one hour, whereas, three hours were needed to extract the maximum amount of trypsin inhibitor activity from heated samples. The pH of the soybean suspensions was within 8.4 to 10.0, and the suspensions were diluted to the point where one ml. of the soybean suspensions produced trypsin inhibition of 40 to 60%.

Portions (0.0,0.6,1.0,1.4 and 1.8 ml.) of soybean suspension were pipetted into duplicate sets of test tubes and adjusted to 2.0 ml. with water. After 2 ml. of trypsin solution were added to each test tube, the tubes were placed in a water bath at 37°C. To each tube 5 ml. of benzoyl-DL-arginine-p-nitroanilide (BAPA), a synthetic substrate, were added; ten minutes later the reaction was terminated by adding one ml. of 30% acetic acid. After thorough mixing, the contents of each tube were filtered on Whatman No. 3 paper, and absorbance of the filtrate was measured at 410 nm. against a reagent blank. A double beam Perkin-Elmer Spectrophotometer (Coleman Model 124) was used to make absorbance readings. The reagent blank was prepared by adding one ml. of 30% acetic acid to a test tube containing trypsin and water (2 ml. each) before the 5 ml. of BAPA solution were added. Because the soybean samples showed no appreciable absorption at 410 nm. no sample blank was prepared.

One trypsin unit (TU) is arbitrarily defined as an increase of 0.01 absorbance units at 410 nm. per 10 ml. of the reaction mixture

under the conditions previously stated. Trypsin inhibitor activity is expressed in terms of trypsin units inhibited (TIU) (39).

VII. EXPERIMENTAL DESIGN FOR FEEDING STUDY

The experimental design included two levels of dietary protein, seven treatments, and five rats per treatment. Two replications were conducted. The format in Table 1 was adequate to test the effectiveness of boiling water treatments on the inactivation of trypsin inhibitor.

VIII. THE USE OF LABORATORY ANIMALS

Albino weanling male rats, Sprague-Dawley strain, were purchased and used in the feeding study. The rats were housed in a small animal laboratory in McLeod Food Technology Building. The temperature in the laboratory was controlled at 21°C ± 2 degrees to permit favorable growing conditions for the rats. The rats were housed individually in cages measuring 18 cm. wide × 24 cm. long × 18 cm. high with 1 cm. hardware screening on the bottom and front. The cages were held in steel racks, six cages per row and five rows per side of each rack. The rats were randomly divided into treatment groups and were randomly placed in cages using a Random Numbers Table (53).

IX. DIET FORMULATION AND FEEDING STUDY

The control diets used in the feeding study were patterned after those used by Rackis and McGhee (74). The control diets, calculated to

Table I. Experimental Design for Feeding Study.[1]

Treatments-time minutes in boiling water	Replications			
	1		2	
	Protein Level		Protein Level	
	10%	19%	10%	19%
Control (casein)	-	-	-	-
0	-	-	-	-
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
6	-	-	-	-
9	-	-	-	-

[1]Five rats were fed per treatment.

contain 10 and 19% protein, are shown in Table II. A 10% protein diet was fed to the rats in order to measure Protein Efficiency Ratio. A 19% protein diet was fed to the rats in order to meet the rat's growth requirement for the essential amino acids, cystine and methionine which are the two most limiting amino acids in soybeans. A nomagram for rat growth was used to determine the level of protein required in the diet to meet the rat's growth requirement for these two essential amino acids (5).

In the experimental diets, the raw and heat-treated soybeans were substituted for 100% of the casein and substituted partially for corn oil and dextrose to maintain a 10 and 19% protein diet and to maintain a constant caloric level. Corn starch, vitamin mix, and salt mix remained constant for the control and the experimental diets at 20, 2, and 5%, respectively. The amount of cellulose was adjusted in the experimental diets to provide a weight of 100 grams. All diets were isocaloric providing 372 kcal. per 100 grams of ration. The composition of the individual experimental diets is listed in Table A-1 and A-2 of the Appendix.

The feed was placed in glass or aluminum feeding cups. Each cup contained a small mesh hardware cloth which rested on top of the feed. These feeders were designed to allow the rat easy access to the food, but to reduce wasting and scattering of feed in order to obtain more accurate feed consumption data. As the level of feed in the cup dropped, the screen level dropped also. Fresh chlorinated water was supplied to the rats in plastic water bottles attached to the front of the cage. The rats were given food and water ad libitum.

Table II. Composition and Caloric Content of Control Diets.

Ingredients	10% protein		19% protein	
	gram	kcal	gram	kcal
Casein	11.05	44.20	21.00	84.00
Corn oil	8.00	72.00	8.00	72.00
Dextrose	43.95	175.80	34.00	136.00
Corn starch	20.00	80.00	20.00	80.00
Vitamin mix[1]	2.00	-	2.00	-
Salt mix[2]	5.00	-	5.00	-
Cellulose	10.00	-	10.00	-
	100.00	372/100 grams	100.00	372/100 grams

[1]Vitamin Diet Fortification Mixture (Nutritional Biochemicals Corporation, Cleveland, Ohio.) Contains: 4.5 g vitamin A concentrate, 0.25 g vitamin D concentrate, 5.0 g alpha tocopherol, 45.0 g ascorbic acid, 5.0 g inositol, 75.0 g. choline chloride 2.25 g menadione, 5.0 g p amino benzoic acid, 4.5 g niacin, 1.0 g riboflavin, 1.0 g pyridoxine hydrochloride, 1.0 g thiamine hydrochloride, 3.0 g calcium pantothenate, 20.0 mg biotin, 90 mg folic acid, and 1.35 mg vitamin B-12.

[2]Jones Foster Salt Mixture (United States Biochemical Corporation, Cleveland, Ohio.) Contains: 800.8 g $CaCO_3$, 0.476 g $CoCI_2 \cdot H_2O$, 0.999 g $CuSO_4 \cdot 5H_2O$, 56.6 g $FeSO_4 \cdot 7H_2O$, 9.35 g $MnSO_4 \cdot 2H_2O$, 120.3 g $MgSO_4$, 1.66 g KI, 816.6 g KH_2PO_4, 292.5 g NaCI, and 0.545 g $ZnCI_2$ (36).

X. EVALUATION OF FEEDING STUDY

Feed consumption was recorded during the study. The rats were weighed every two days on a Pennsylvania Model 1-10-L Automatic fan dial laboratory scale. At the end of 28 days, the animals were sacrificed by anesthetizing with carbon dioxide and decapitating with a Wahmann rat guillotine. The liver was excised, weighed and frozen until determination of crude lipid and protein. The pancreas was excised, trimmed free of fat and extraneous tissue, and weighed. A small section of the pancreas was removed and used for histological examination. The pancreas was then discarded. The liver and the pancreas were weighed on a Mettler automatic read-out balance.

Body weight, protein efficiency ratio, and pancreas weight were determined.

XI. PREPARATION OF PANCREAS SECTION FOR HISTOLOGICAL EXAMINATION

The pancreas sections were fixed by immersion in 10% neutral buffer formalin. Tissues were fixed for three days. Sections of pancreas were dehydrated through graded alcohols, 50%, 75%, 95%, and 100%, and then cleared in xylene followed by cedar wood oil and chloroform. Tissues were then infiltrated and embedded in paraffin at 60°C. Sections measuring five microns thick were cut on a Lipshaw 45 standard microtome and affixed with egg albumen on glass slides. Sections were then stained with Harris hematoxylin, a basic staining dye, and counterstained with eosin Y, an acid staining dye. The sections were then mounted in permount (52).

XII. CRUDE LIPID DETERMINATION ON LIVERS

After excising, the livers were placed in Whirl-pak bags and stored in a still freezer at -17°C. The frozen livers were freeze-dried to a constant weight in a Virtis Model FFD-15 Sublimator. The dried livers were ground in a IKA-Universalmühle M20 Micro Mill for five minutes and further ground using a mortar and pestle. Crude lipid content was determined in triplicate by the A.O.A.C. Official Method 7.045 (6) on a Goldfish Extraction Apparatus. Two gram samples of the dried liver material were extracted with petroleum ether for ten hours.

XIII. TOTAL PROTEIN DETERMINATION ON LIVERS

Protein content was determined on the defatted liver material by the Kjeldahl method (N × 6.25) according to the A.O.A.C. Official Method 2.049 (6). Four replications were conducted by analyzing one gram samples.

XIV. STATISTICAL ANALYSIS

The statistical analysis system (SAS) computer program at the University of Tennessee Computer Center was used for the calculation of analysis of variance between treatments (12). Significance among means was determined by Duncan's Multiple Range Test (26). A two-tailed t-test was used to compare means of protein efficiency ratio, weight gain, pancreas weight, liver weight, protein content of liver, and lipid content of liver of rats fed soybeans against respective means of rats fed casein (12,76).

CHAPTER IV

RESULTS AND DISCUSSION

Analysis of variance showed no significant difference in trypsin inhibitor activity (TIA) between the two plantings of soybeans. Therefore, the soybeans from the two plantings were designated as replications 1 (May planting) and 2 (June planting).

I. PARTIAL COMPOSITION OF SOYBEANS

The partial composition of raw and heat-treated soybeans is presented in Table III. The proportional amounts of crude protein and ether extract increased as the heating time was extended. The protein level ranged from 32.33% to 33.71%; ether extract, 21.57% to 26.29% (dry weight basis). These values are in agreement with published values (82). The published values for ash and fiber content of soybean were used for calculations involving diet formulation (82).

II. TRYPSIN INHIBITOR ACTIVITY OF SOYBEANS

The mean TIA of raw and heat-treated soybeans is presented in Table IV. Raw (unheated) soybeans contained 51.90 trypsin inhibitor units (TIU) per milligram dry ground soybean material. Activity decreased rapidly at first but progressively as the period of heating was extended. In soybeans heated nine minutes, 96.13% of the TIA was destroyed.

Table III. Partial Composition of Vegetable-Type Soybeans Heated in Boiling Water.

Time (minutes) in boiling water	Components, %		
	Moisture[a]	Crude protein[b]	Ether extract[c]
0	13.77 ± 0.02	32.33 ± 0.62	21.57 ± 0.21
1	12.45 ± 0.10	32.65 ± 0.89	22.79 ± 0.22
2	11.77 ± 0.80	33.08 ± 0.50	23.03 ± 0.24
3	11.61 ± 0.60	33.37 ± 0.89	23.68 ± 0.31
6	11.24 ± 0.28	33.46 ± 0.70	25.71 ± 0.41
9	10.77 ± 0.30	33.71 ± 0.15	26.29 ± 0.48

[a] N = 3 with ± 1 standard deviation.

[b] N = 5 with ± 1 standard deviation; calculated on dry, fat-free weight basis.

[c] N = 3 with ± 1 standard deviation; calculated on dry weight basis.

Table IV. Trypsin Inhibitor Activity of Raw and Heat-treated Vegetable-type Soybeans.

Time (minutes) in boiling water	Trypsin Inhibitor Activity TIU/mg Dry Sample[a]	Destruction of Trypsin Inhibitor Activity, %
0 (raw)	51.90	
1	19.54	62.35
2	12.81	75.32
3	5.23	89.92
6	3.01	94.20
9	2.01	96.13

[a]TIU = trypsin inhibitor units; N = 2

The asymptotic curve in Appendix Figure 1 (page 61) represents the TIA of the raw and heat-treated soybeans. The coefficient of determination was 0.9576 and the regression equation was

$\hat{y} = 2.8388 + 48.6907e^{-0.9576t}$.

III. WEIGHT GAIN OF RATS

The analysis of variance for the effect of holding soybeans in boiling water on the weight gain of rats is presented in Table V. Heating had a significant effect (0.01 level) on the weight gain.

The mean weight gain of rats fed a diet containing soybeans or casein is presented in Table VI. The rats fed raw soybeans weighed less (0.01 level) than the rats fed soybeans heated one to nine minutes. This was observed in rats fed a 10% and a 19% protein diet. The rats fed a 19% protein diet showed a trend for increased weight gain as the heating time was extended. Consequently, an inverse relationship exists between increased weight gain (Table VI) and decreased TIA as heating time was extended (Table IV). A similar relationship has been reported by other investigators (38,69,74).

The weight gain of rats fed soybeans from each of the heat treatments was lower (0.01 level) than the weight gain of rats fed a diet containing casein. This was observed with the 10% and 19% protein diets.

There is lack of agreement among investigators concerning the relationship between soybean trypsin inhibitors and growth inhibitory

Table V. Analysis of Variance for the Effect of Holding Vegetable-type Soybeans in Boiling Water on the Weight Gain of Rats.

	10% Protein Diet				19% Protein Diet			
Source	D.F.	S.S.	M.S.	F-ratio	D.F.[1]	S.S.	M.S.	F-ratio
Total	59	40694.3333	689.7345		57	36847.9310	646.4549	
Holding time	5	38182.1333	7636.4266	164.15**	5	36129.8533	7225.9706	523.27**
Residual error	54	2512.2000	46.5222		52	718.0777	13.8092	

[1]Two rats died during the study.

**Significant at the 0.01 level.

Table VI. Mean Weight Gain of Rats Fed a Diet for 28 Days Containing Heated Vegetable-type Soybeans or Casein.

10% Protein Diet		19% Protein Diet	
Time (minutes) in boiling water	Weight gain (grams)	Time (minutes) in boiling water	Weight gain (grams)
0 (raw)	34.1^{b}**	0 (raw)	72.6^{f}**
1	96.0^{a}**	1	133.9^{e}**
2	100.4^{a}**	2	138.8^{d}**
3	102.2^{a}**	3	139.1^{d}**
6	103.9^{a}**	6	144.2^{c}**
9	104.4^{a}**	9	146.9^{c}**
Casein diet	127.0	Casein diet	174.3

^{a,b}Means followed by the same letter are not different at 0.01 level; N = 10.

$^{c-f}$Means followed by the same letter are not different at 0.01 level; N = 10 with two exceptions: for 0 and 6 minute times, N = 9.

**Weight gain is different from weight gain of rats fed casein at 0.01 level.

properties of raw soybean meal. Some investigators believe that the trypsin inhibitors stimulate biosynthesis of enzymes in the pancreas and thereby increase the essential amino acid requirements of the animal because of enhanced secretion of pancreatic enzymes into the intestinal tract (28,40,54,72). Methionine and cystine are the first-limiting amino acids in soybeans. There is increased metabolic conversion of methionine to cystine in the pancreas following ingestion of trypsin inhibitor (11). As a result, the greater need for sulfur amino acids cannot be compensated for by providing dietary proteins. An imbalance in the metabolic amino acid pool would enhance the growth-inhibiting effect of the raw soybean meal.

According to Almquist and Merritt (4), the action of trypsin inhibitor involves a general interference with digestion resulting in a substantial amount of the most-limiting amino acids being excreted (in chicks) or absorbed too late to be of value to the animal (in rats). In rats a considerable portion of protein from raw soybeans escapes hydrolysis in the small intestine and must be absorbed from the large intestine in which case it has little utility for growth. To further support their theory, Almquist and Merritt showed that the addition of trypsin inhibitor, in the form of raw soybean meal, to rations containing proteins with marginal levels of lysine, arginine, isoleucine, or tryptophan caused chicks to become markedly deficient in these amino acids.

Khayambashi and Lyamn (41) noted that the effect of soybean trypsin inhibitor on the secretion of pancreatic enzymes may be

a hormonal response. Many investigators believe that trypsin inhibitors in soybean elicit the formation or secretion of cholecystokinin-pancreozymin (CCK-PZ) a hormone in the duodenal mucosa that regulates protein digestion (41,44,57). Repeated administration of CCK-PZ caused pancreatic hypertrophy, stimulated pancreatic juice secretion, and inhibited growth of rats (72).

Lyman and Lepkovsky (54) suggested that the growth depression caused by the trypsin inhibitor may be the result of the endogenous loss of essential amino acids derived from a hyperactive pancreas. In the rat where the fecal loss of endogenous nitrogen and sulfur is relatively small, the loss could be incurred as result of bacterial degradation in the lower part of the intestinal tract (21).

IV. PROTEIN EFFICIENCY RATIO

Analysis of variance for the effect of holding the soybeans in boiling water on the PER of the protein is presented in Table VII. Heating the soybeans had a significant effect (0.01 level) on the PER of protein.

It is common practice to calculate PER (grams weight gained/gram protein consumed) on a basis with casein equal to 2.50. The PER values of the heat-treated soybeans were adjusted to casein and are presented in Table VIII. As the heating time was extended, the PER of the soybean protein increased. These data suggest that had the heating time been extended beyond nine minutes, the PER would have approached that of casein. An increase in heating time of soybeans accompanied by

Table VII. Analysis of Variance for the Effect of Holding Vegetable-type Soybeans in Boiling Water on the Protein Efficiency Ratio of the Soybean Protein.

Source	D.F.	S.S.	M.S.	F-ratio
Total	59	2.7691	0.0469	
Holding Time	5	2.6490	0.5298	240.82**
Residual Error	54	0.1201	0.0022	

**Significant at the 0.01 level

Table VIII. Mean Protein Efficiency Ratio of Heated Vegetable-type Soybeans or Casein Fed to Rats for 28 Days.

Time (minutes) in boiling water	Protein Efficiency Ratio (PER)
0 (raw)	1.20^{e}**
1	1.55^{d}**
2	1.60^{c}**
3	1.63^{c}**
6	1.70^{b}**
9	1.90^{a}**
Casein diet	2.50

$^{a-e}$Means followed by the same letter are not different at 0.01 level; N = 10.

**PER is different from PER rats fed casein at 0.01 level.

a decrease in TIA and an increase in PER has been previously reported (38,69,72,74).

The PER of soybeans heated in boiling water at all time periods was lower (0.01 level) than the PER of casein.

V. PANCREAS WEIGHT OF RATS

Analysis of variance for the effect of holding soybeans in boiling water on the pancreas weight of rats is presented in Table IX. The weight was significantly affected at the 0.01 level by the heat treatment.

The mean weight of pancreas of rats fed a diet containing the heated soybeans or casein is presented in Table X. The pancreas weight of rats fed raw soybeans was greater (0.01 level) than the pancreas weight of rats fed soybeans heated one to nine minutes. This was observed in rats fed a 10% protein diet or a 19% protein diet. There was no significant difference in pancreas weight of rats fed soybeans heated one to nine minutes in the 10% or the 19% protein diets.

The occurrence of pancreatic hypertrophy in rats fed raw soybeans and a decrease in pancreatic hypertrophy in rats fed heat-treated soybeans have been reported previously (16,38,69,71,74). This beneficial effect of heat treatment is related to inactivation of trypsin inhibitor. It has been observed that with only 50-60% destruction of trypsin inhibitor activity, pancreatic hypertrophy no longer occurred in rats (72). With only one minute of heating soybeans

Table IX. Analysis of Variance for the Effect of Holding Vegetable-type Soybeans in Boiling Water on the Pancreas Weight of Rats.

	10% Protein Diet				19% Protein Diet			
Source	D.F.	S.S.	M.S.	F-ratio	D.F.[1]	S.S.	M.S.	F-ratio
Total	59	0.7332	0.0124		57	0.7218	0.0127	
Holding time	5	0.6700	0.1340	111.66**	5	0.6441	0.1288	85.87**
Residual error	54	0.0632	0.0012		52	0.0777	0.0015	

[1]Two rats died during the study.

**Significant at the 0.01 level.

Table X. Mean Weight of Pancreas of Rats Fed a Diet for 28 Days Containing Heated Vegetable-type Soybeans or Casein.

10% Protein Diet		19% Protein Diet	
Time (minutes) in boiling water	Pancreas weight grams/100 grams body weight	Time (minutes) in boiling water	Pancreas weight grams/100 grams body weight
0 (raw)	0.739^{a}**	0 (raw)	0.758^{c}**
1	0.436^{b}N.S.	1	0.452^{d}N.S.
2	0.445^{b}N.S.	2	0.465^{d}N.S.
3	0.444^{b}N.S.	3	0.457^{d}N.S.
6	0.443^{b}N.S.	6	0.463^{d}N.S.
9	0.450^{b}N.S.	9	0.461^{d}N.S.
Casein diet	0.446	Casein diet	0.455

^{a,b}Means followed by the same letter are not different at 0.01 level; N = 10.

^{c,d}Means followed by the same letter are not different at 0.01 level; N = 10 with two exceptions: for 0 and 6 minutes, N = 9.

**Pancreas weight is different from pancreas weight of rats fed casein at 0.01 level.

N.S. Pancreas weight is not different from pancreas weight of rats fed casein.

in boiling water, there was a destruction of 62.35% TIA (Table IV) (page 21) and pancreatic hypertrophy no longer occurred (Table X).

The pancreas weight of rats fed raw soybeans was greater (0.01 level) than the pancreas weight of rats fed casein. This was observed in rats fed the 10% and the 19% protein diets. The pancreas weight of rats fed soybeans heated one to nine minutes did not differ from the pancreas weight of rats fed a casein diet in the 10% protein diet or the 19% protein diet.

The mechanism, whereby the trypsin inhibitor in raw soybeans causes pancreatic enlargement, is not clear. Khayambashi and Lyman (40) reported that rat pancreas, which had been perfused with blood plasma from a rat fed the trypsin inhibitor, secreted increased amounts of enzymes. They concluded that the trypsin inhibitor might cause the pancreas to liberate a hormone-like agent which stimulates the secretory function of the pancreas. It has been reported that trypsin inhibitors in soybeans elicit the formation or secretion of cholecystolcinin-pancreozymin (CCK-PZ), a hormone which stimulates pancreatic juice secretion and causes pancreatic hypertrophy (41,44,57,72).

The physiological response of the pancreas to the stimulation by soybean trypsin inhibitor is a situation analogous, perhaps, to the hypertrophy produced in the thyroid when it is hyperstimulated by thyroid-stimulating hormone.

VI. HISTOLOGICAL EXAMINATION OF PANCREAS SECTIONS

Photomicrographs of pancreas of rats fed a casein diet (control), raw soybeans, soybeans heated for one minute, and soybeans heated for nine minutes are presented as Figure 1A, 1B, 1C, and 1D, respectively.

Figure 1A: A Photomicrograph of the Pancreas of a Rat Fed a Casein Diet (control). The acinar cells (a) are pyramidal with a comparatively large nucleus and a normal amount of secretory material. The intercalated ducts are obvious (Arrow) (H and E; × 250).

Figure 1B: A Photomicrograph of the Pancreas of a Rat Fed a Raw Soybean Diet. The acinar cells are variable in size (a,a´) and some are binucleated. Most have small nuclei. Some small cells form "nests" (a´) and others (a) have a large amount of secretory material especially near the islets of Langerhans (il). The intercalated ducts are not obvious (Arrow) (H and E; × 250).

Figure 1C: A Photomicrograph of the Pancreas of a Rat Fed Soybeans Heated in Boiling Water for one Minute. The acinar cells are more uniform in size and content of secretory material (a). The intercalated ducts are obvious (Arrow) (H and E; × 250).

Figure 1D: A Photomicrograph of the Pancreas of a Rat Fed Soybeans Heated in Boiling Water for Nine Minutes. The acinar cells (a) resemble the controls. The intercalated ducts are obvious (Arrow) (H and E; × 250).

The pancreas of animals fed raw soybean meal showed a number of changes relative to the control. Except for those acini adjacent to

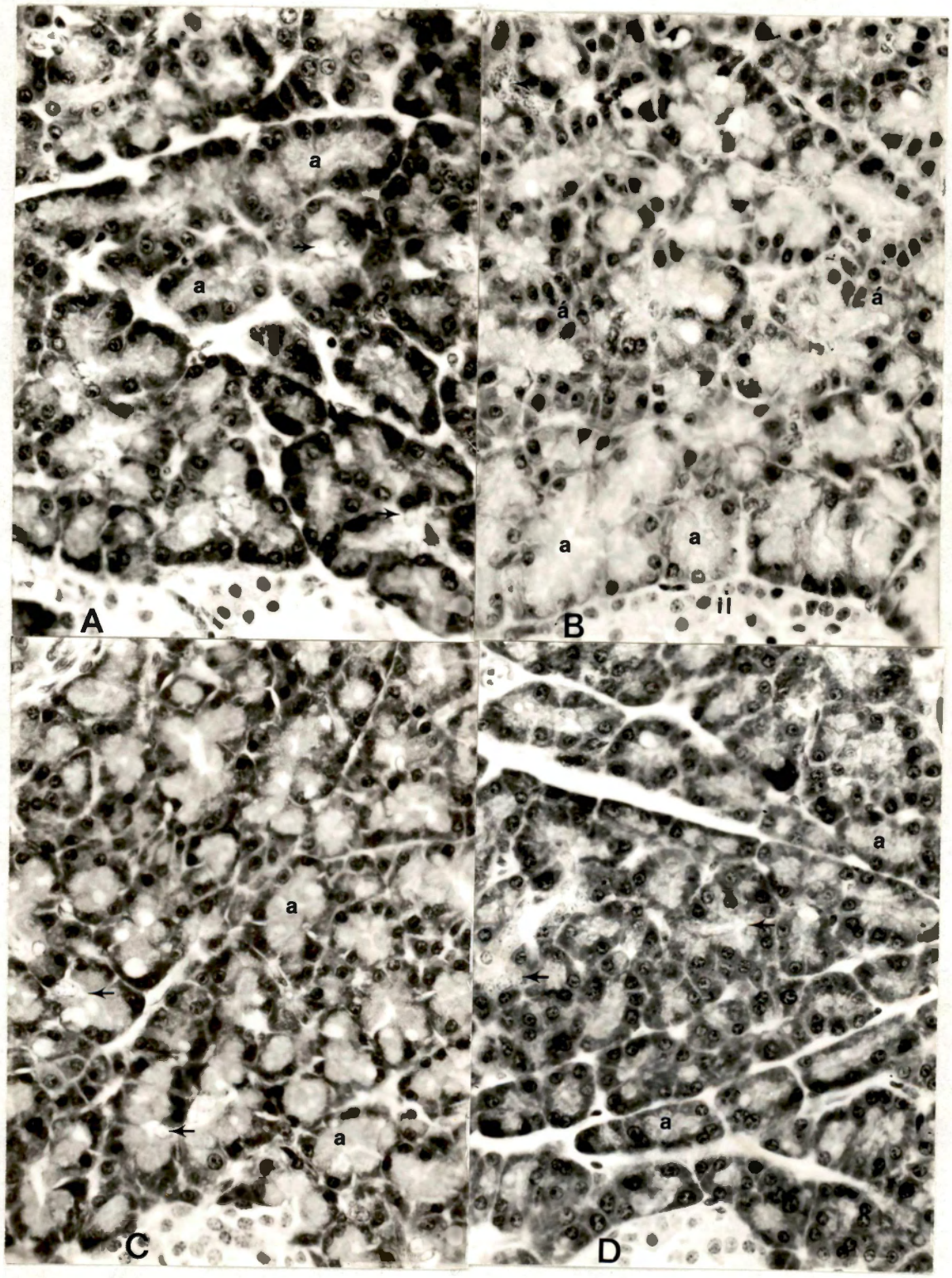


Figure 1. Photomicrograph of the pancreas of a rat fed (A) a casein diet (B) a raw soybean diet (C) soybeans heated one minute, and (D) soybeans heated nine minutes.

the pancreatic islets, the amount of secretory product per cell (and even per acinus) varied considerably. Those cells (and acini) with the least amount of secretory product had small nuclei (a few of these were binucleated), were very basophilic and had much smaller over-all dimensions than those seen in the controls. These cells could be seen as "nests" in the pancreatic parenchyma, indicating that whole acini were affected, rather than individual, unrelated cells. These regions showed a relative decrease in the number of intercalated duct cells (Figure 1A).

The pancreas of animals fed soybeans heated in boiling water for one minute showed a number of changes relative to Figure 1B. The pancreas of rats fed soybeans heated for one minute is representative of heating soybeans in boiling water one to three minutes. The acinar cells are more uniform in size than the acinar cells in the pancreas shown in Figure 1B. The amount of secretory material is more uniform and not localized as seen in the pancreas of rats fed raw soybeans. There is an increased number of intercalated duct cells as compared to Figure 1B. There is a graded effect in that the pancreas of rats fed soybeans heated one to three minutes is more similar to the control than to the pancreas of rats fed raw soybeans.

The pancreas of animals fed soybeans heated in boiling water for nine minutes is very similar to the pancreas of animals fed a casein diet. The acinar cells are pyramidal with visible nuclei and a normal amount of secretory material. The intercalated ducts are visible. The trend toward normalcy of the pancreatic tissue as heating time was extended is related to the inactivation of trypsin inhibitor.

Many investigators report that histological and biochemical examination of the enlarged pancreas of rats fed raw soybeans reveals true hyperplasia, which is characterized by an increase in the number of cells in the pancreatic tissue (8,20,37,75). Other investigators believe that pancreatic enlargement is the result of an increase in cell size rather than the number of cells (43,77). It is concluded from this histological study that pancreatic enlargement is most probably a consequence of an increase in the number of cells or hyperplasia.

VII. LIVER WEIGHT OF RATS

Analysis of variance for the effect of holding soybeans in boiling water on the liver weight of rats is presented in Table XI. Heating had a significant effect (0.01 level) on the liver weight.

The mean liver weight of rats fed a diet containing raw soybeans, soybeans heated in boiling water or casein is presented in Table XII. The liver of rats fed raw soybeans weighed less than the liver of rats fed soybeans heated from one to nine minutes. This was observed for rats fed a 10% protein diet and a 19% protein diet. The liver weight of rats fed soybeans heated from one to nine minutes was not different.

Liver weight of rats fed raw soybeans in the 10% protein diet and in the 19% protein diet was lower (0.01 level) than the liver weight of rats fed a casein diet. In the 10% protein diet, the liver weight of rats fed soybeans heated for one and two minutes was lower (0.05 level) than the liver weight of rats fed casein. The weight of

Table XI. Analysis of Variance for the Effect of Holding Vegetable-type Soybeans in Boiling Water on the Liver Weight of Rats.

	10% Protein Diet				19% Protein Diet			
Source	D.F.	S.S.	M.S.	F-ratio	D.F.[1]	S.S.	M.S.	F-ratio
Total	59	10.9514	0.1856		57	18.8970	0.3315	
Holding Time	5	3.6994	0.7399	5.51**	5	15.6924	3.1385	50.95**
Residual error	54	7.2520	0.1343		52	3.2046	0.0616	

[1]Two rats died during study.

**Significant at the 0.01 level.

Table XII. Mean Weight of Liver of Rats Fed a Diet for 28 Days Containing Heated Vegetable-type Soybeans or Casein.

10% Protein Diet		19% Protein Diet	
Time (minutes) in boiling water	Liver weight grams/100 grams body weight	Time (minutes) in boiling water	Liver weight grams/100 grams body weight
0 (raw)	3.145^{b}**	0 (raw)	3.629^{d}**
1	4.003^{a}*	1	4.449^{c}N.S.
2	4.082^{a}*	2	4.469^{c}N.S.
3	4.139^{a}N.S.	3	4.492^{c}N.S.
6	4.151^{a}N.S.	6	4.542^{c}N.S.
9	4.174^{a}N.S.	9	4.620^{c}*
Casein diet	4.132	Casein diet	4.347

^{a,b}Means followed by the same letter are not different at 0.01 level; N = 10.

^{c,d}Means followed by the same letter are not different at 0.01 level; N = 10 with two exceptions: for 0 and 6 minutes, N = 9.

**Liver weight is different from liver weight of rats fed casein.

*Liver weight is different from liver weight of rats fed casein.

liver from rats fed soybeans heated three to nine minutes was not different from the weight of liver of rats fed casein. In the 19% protein diet, the liver weight of rats fed soybeans heated for nine minutes in boiling water was higher (0.05 level) than the liver weight of rats fed casein. The liver weight of rats fed soybeans heated one to six minutes was not different from casein.

In general, there was a trend for increased liver weights as the heating time for the soybeans was extended. This applies to both the 10% protein diet and 19% protein diet. Addis et al. (1) reported that rats fed a diet inadequate in protein content, had marked changes in different organs, including a reduction in liver weight. The reduced liver weight of rats fed raw soybeans is related to the high trypsin inhibitor content of raw soybeans as shown in Table IV (page 21). Trypsin inhibitor interferes with complete digestion of protein and subsequent hydrolysis of protein. Therefore, a protein-adequate diet accompanied by a high level of trypsin inhibitor is actually one that is low in available protein.

VII. LIPID CONTENT OF LIVER OF RATS

Analysis of variance for the effect of holding soybeans in boiling water on the lipid content of rat liver is presented in Table XIII. The heat treatment had a significant effect (0.01 level) on the lipid content of rat liver.

The mean lipid content of the liver of rats fed a diet containing raw or heated soybeans or casein is presented in Table XIV. Lipid

Table XIII. Analysis of Variance for the Effect of Holding Vegetable-type Soybeans in Boiling Water on the Lipid Content of Rat Liver.

Source	10% Protein Diet				19% Protein Diet			
	D.F.	S.S.	M.S.	F-ratio	D.F.	S.S.	M.S.	F-ratio
Total	17	15.8883	0.9346		17	20.8361	1.2257	
Holding time	5	15.6609	3.1322	165.72**	5	20.6170	4.1234	225.32**
Residual error	12	0.2274	0.0189		12	0.2191	0.0183	

**Significant at the 0.01 level.

Table XIV. Mean Lipid Content of Liver of Rats Fed a Diet for 28 Days Containing Heated Vegetable-type Soybeans or Casein.

10% Protein Diet		19% Protein Diet	
Time (minutes) in boiling water	Lipid Content of Livers (%)	Time (minutes) in boiling water	Lipid Content of Livers (%)
0 (raw)	10.62^{a}**	0 (raw)	9.34^{d}**
1	7.85^{c}**	1	$6.82^{e,f}$N.S.
2	8.03^{c}**	2	6.11^{h}**
3	8.63^{b}**	3	6.95^{e}N.S.
6	8.96^{b}**	6	$6.43^{g,h}$**
9	8.16^{c}**	9	$6.45^{f,g}$
Casein diet	7.13	Casein diet	6.80

$^{a-c}$Means followed by the same letter are not different at 0.01 level; N = 3.

$^{d-h}$Means followed by the same letter are not different at 0.01 level; n = 3.

**Lipid content of liver is different from lipid content of rats fed casein at 0.01 level.

*Lipid content of liver is different from lipid content of rats fed casein at 0.05 level.

N.S. Lipid content of liver is not different from lipid content of rats fed casein.

content of the liver of rats fed raw soybeans was greater than that of rats fed soybeans heated one to nine minutes. This was observed in both levels of protein. Lipid content of liver of rats fed the soybeans in the 19% protein diet was lower than that of rats fed the soybeans in the 10% protein diet although this was not determined statistically. The mean percentage of lipid in the livers of rats fed 10% protein was 8.71; of rats fed 19% protein, 7.02.

In the 10% protein diet, lipid content of liver of rats fed soybeans subjected to various heat treatments was greater (0.01 level) than lipid content of liver of rats fed the casein diet. In the 19% protein diet, the lipid content of liver of rats fed raw soybeans was greater (0.01 level) than the lipid content of liver of rats fed a casein diet. Also, lipid content of liver of rats fed soybeans heated for two, six, and nine minutes was less (0.01 level) than the lipid content of liver of rats fed a casein diet. The mean weight values for the remaining heated soybeans were not different from casein. In general, there appears to be no trend relating heat treatment to lipid content in rat liver, other than the finding that raw soybeans produced the largest amount of lipid.

Diets high in TIA, like those having an inadequate amount of protein, caused increased lipid content in the liver of rats. There are many mechanisms involved in the production of fatty liver. Fatty acids from ingested dietary fat are reesterified to triglycerides in the liver and the triglycerides interact with protein (apoprotein, lipid-acceptor protein), cholesterol and phospholipid to form

lipoprotein which transports lipid out of the liver (34,60). Fatty liver as a result of ingestion of a low-protein diet may be caused by diminished lipoprotein formation or to impaired release of complete lipoproteins (34). A decrease in the synthesis of protein from amino acids or diminished protein and phospholipid synthesis will lead to diminished lipoprotein formation and fatty liver. Trypsin inhibitor present in the raw soybeans might have prevented complete protein synthesis which may have led to diminished lipoprotein formation and fatty liver in rats.

VIII. PROTEIN CONTENT OF LIVER OF RATS

The analysis of variance for the effect of holding soybeans in boiling water on the protein content of rat liver is presented in Table XV. Heating had a significant effect (0.01 level) on the protein content of rat livers.

The mean protein content of liver of rats fed a diet containing raw or heated soybeans or casein is presented in Table XVI. The rats fed raw soybeans had less protein in their liver than the rats fed soybeans heated from one to nine minutes. There was a trend for an increase in protein content as the heat treatment of soybeans was extended. Addis et al. (1) reported rapid and extensive changes in the total amount of protein in the liver of rats under conditions of protein deficiency. The liver of a rat loses protein rapidly when the rat is fed a diet inadequate in protein content. Trypsin inhibitor interferes with protein digestion and thus, a diet of raw soybeans is

Table XV. Analysis of Variance for the Effect of Holding Vegetable-type Soybeans in Boiling Water on the Protein Content of Rat Liver.

Source	10% Protein Diet				19% Protein Diet			
	D.F.	S.S.	M.S.	F-ratio	D.F.	S.S.	M.S.	F-ratio
Total	23	137.8207	5.9922		23	219.5420	9.5453	
Holding time	5	137.1481	27.4296	733.41**	6	218.9981	43.7996	1450.32**
Residual error	18	0.6726	0.0374		18	0.5439	0.0302	

**Significant at the 0.01 level.

Table XVI. Mean Protein Content of Liver of Rats Fed a Diet for 28 days Containing Heated Vegetable-type Soybeans or Casein

10% Protein Diet		19% Protein Diet	
Time (minutes) in boiling water	Protein Content of Livers (%)	Time (minutes) in boiling water	Protein Content of Livers (%)
0 (raw)	52.30^{d}**	0 (raw)	52.92^{h}**
1	58.01^{c}**	1	58.34^{g}**
2	58.22^{c}**	2	58.84^{g}**
3	59.20^{b}**	3	59.27^{f}**
6	61.00^{a}**	6	61.29^{e}N.S.
9	61.17^{a}**	9	61.34^{e}N.S.
Casein diet	60.24	Casein diet	61.00

$^{a-d}$Means followed by the same letter are not different at 0.01 level; N = 4.

$^{e-h}$Means followed by the same letter are not different at 0.01 level; N = 4.

**Protein content of liver is different from protein content of liver of rats fed casein at 0.01 level.

N.S. Protein content of liver is not different from protein content of liver of rats fed casein.

virtually a low-protein diet. The low protein content of liver of rats fed raw soybeans would be expected and would be in agreement with the findings of Addis et al. (1).

In the 10% protein diet, the protein content of liver of rats fed soybeans heated zero to three minutes was less (0.01 level) than the protein content of liver of rats fed casein. This was also observed in the 19% protein diet. The protein content of liver of rats fed soybeans heated six and nine minutes was greater than the protein content of liver of rats fed a casein diet in the 10% protein diet. In the 19% protein diet, the protein content of the liver of rats fed soybeans heated six and nine minutes was not different from the protein content of the liver of rats fed a casein diet.

CHAPTER V

SUMMARY

This investigation was undertaken to determine the effect of boiling water on the inactivation of trypsin inhibitor in green vegetable-type soybeans and to determine the effect of feeding these soybeans on certain physiological responses in the rat.

The soybeans were held in boiling water for 0 (unheated), 1, 2, 3, 6 and 9 minutes. They were prepared for analysis and diet preparation. The soybeans were analyzed for moisture, crude lipid, crude protein and trypsin inhibitor activity. The raw and heat-treated soybeans were fed to rats, and data were collected on weight gain, protein efficiency ratio, pancreas weight, liver weight, percentages of lipid and protein in the liver, and histology of the liver.

Trypsin inhibitor activity decreased rapidly with a short heating time. In soybeans heated one minute, 62% of the TIA was destroyed, whereas in soybeans heated nine minutes, 96% of the TIA was destroyed.

The rats fed raw soybeans weighed less than the rats fed heated soybeans. The rats fed 19% protein showed a trend for increased weight gain as the heating time was extended. Consequently, an inverse relationship was found between increased weight gain and decreased TIA as heating time was extended. The weight gain of rats fed raw and heated soybeans was lower than the weight gain of rats fed casein.

Protein efficiency ratio (PER) increased as the heating time was extended. This was related to the decrease in TIA as heating time was extended. The PER of raw and heated soybeans was lower than the PER of casein.

The pancreas weight of rats fed raw soybeans was greater than that of rats fed heated soybeans. There was no difference among the pancreas weight of rats fed heated soybeans. The pancreas weight of rats fed raw soybeans was greater than that of rats fed casein. The pancreas weight of rats fed heated soybeans was not different from that of rats fed casein. The occurrence of pancreatic hypertrophy in rats fed raw soybeans and a decrease in pancreatic hypertrophy in rats fed heat-treated soybeans is related to inactivation of trypsin inhibitor. Histological examination of the enlarged pancreas of rats fed under heated soybeans indicated that enlargement is due to an increase in the number of cells or hyperplasia.

The liver of rats fed raw soybeans weighed less than the liver of rats fed heated soybeans. Liver weight of rats fed heated soybeans was not different from each other. Liver weight of rats fed raw soybeans was lower than that of rats fed casein. In general, there was a trend for increased liver weight as the heating time for the soybeans was extended.

The lipid content of liver of rats fed raw soybeans was greater than that of rats fed heated soybeans or casein. In general, there was no trend relating heat treatment to lipid content of rat liver, other than the finding that raw soybeans produced the largest amount of lipid.

Rats fed raw soybeans had less protein in their livers than the rats fed heated soybeans or casein. There was a trend for an increase in protein content as the heating time of soybeans was extended.

The measurement of various physiological responses in rats fed raw and heat-treated soybeans indicates that holding soybeans in boiling water for nine minutes is adequate to inactivate the trypsin inhibitor. Additional time is needed to achieve maximum protein efficiency.

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APPENDIX

Table A-1. Composition[1] and Caloric Content of 10% Experimental Diets.

	0 min. heating		1 min. heating		2 min. heating		3 min. heating		6 min. heating		9 min. heating	
Ingredients	gram	kcal	gram	kcal	gram	kcal	gram	kcal	gram	kcal	gram	kcal
Soybeans	31.36	133.59	30.28	131.17	29.66	130.38	30.00	133.98	30.69	141.87	30.67	143.91
Corn oil	0.77	6.93	0.63	5.67	0.66	5.94	0.22	1.98	-	-	-	-
Dextrose	37.87	151.48	38.79	155.16	38.92	155.68	39.01	156.04	37.53	150.13	37.02	148.09
Corn starch	20.00	80.00	20.00	80.00	20.00	80.00	20.00	80.00	20.00	80.00	20.00	80.00
Vitamin mix	2.00	-	2.00	-	2.00	-	2.00	-	2.00	-	2.00	-
Salt mix	5.00	-	5.00	-	5.00	-	5.00	-	5.00	-	5.00	-
Cellulose	3.00	-	3.30	-	3.76	-	3.77	-	4.78	-	5.31	-
	100.00	372.00	100.00	372.00	100.00	372.00	100.00	372.00	100.00	372.00	100.00	372.00

[1]This is composition of replication one experimental diets. Replication two experimental diets vary slightly in composition due to small differences in moisture, protein, and lipid content of soybeans.

Table A-2. Composition[1] and Caloric Content of 19% Experimental Diets.

	0 min. heating		1 min. heating		2 min. heating		3 min. heating		6 min. heating		9 min. heating	
Ingredients	gram	kcal	gram	kcal	gram	kcal	gram	kcal	gram	kcal	gram	kcal
Soybeans	59.58	253.78	57.54	249.24	56.35	247.80	57.01	254.58	58.32	269.58	58.28	273.52
Corn oil	-	-	-	-	-	-	-	-	-	-	-	-
Dextrose	9.56	38.22	10.69	42.76	11.10	44.30	9.36	37.42	5.61	22.42	4.62	18.48
Corn starch	20.00	80.00	20.00	80.00	20.00	80.00	20.00	80.00	20.00	80.00	20.00	80.00
Vitamin mix	2.00	-	2.00	-	2.00	-	2.00	-	2.00	-	2.00	-
Salt mix	5.00	-	5.00	-	5.00	-	5.00	-	5.00	-	5.00	-
Cellulose	3.86	-	4.77	-	5.55	-	6.63	-	9.07	-	10.10	-
	100.00	372.00	100.00	372.00	100.00	372.00	100.00	372.00	100.00	372.00	100.00	372.00

[1]This is composition of replication one experimental diets. Replication two experimental diets vary slightly in composition due to small differences in moisture, protein, and lipid content of soybeans.

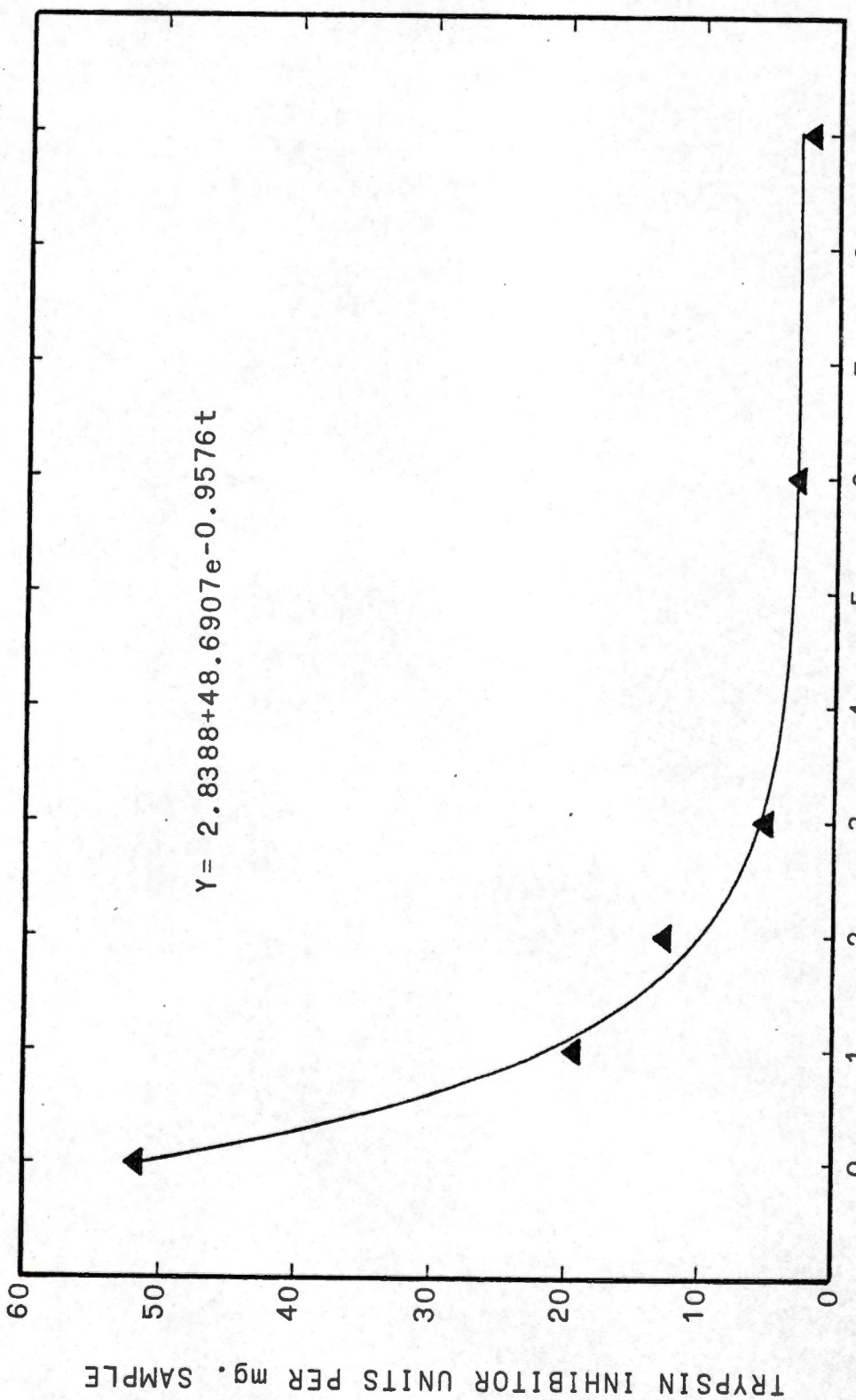


Figure A-1. Trypsin inhibitor activity regressed against time of heating.

VITA

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