THE BIOLOGICAL VALUE OF SOYBEAN IN RELATION TO PROTEIN AND CALCIUM AS REVEALED BY ANIMAL FEEDING

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CHAPTER I

INTRODUCTION

The original home of the soybean was Manchuria. There and all over the Orient this bean has played a prominent role as human food (93) (66). Soybeans, also called soja bean and Manchurian pea have been recognized as a valuable food and a chief source of protein for hundreds of millions of people in certain parts of the globe (6). Soybeans are a rich source of other nutrients as well.

Soybeans were grown in parts of Europe as early as 1600 B. C. but not until 1800 A. D. did the soybean gain entrance to America. The Commodore Perry expedition brought back two varieties of this seed in 1854 known as the white-seeded and the red-seeded variety (104).

After World War I the culture and production of the soybean spread to the western world where it began as a minor forage crop changing the landscape of the United States in the middle west. There was much early resistance to the soybean, but this was gradually overcome. Today, soybeans are rated as the number three crop in cash value in the United States, exceeded only by cotton and corn (8).

The plant is an erect, branching, summer legume. During its early growth, there is a resemblance to the field, or navy bean. The varieties range in maturity from the very early at 75 days to the very late at 200 days. The plant may be used at several stages of growth. While it is yet in the green state, the soybean is often plowed under for organic nourishment of the soil (135). Hay production represents another stage of growth. This harvest takes place when the pods are filled out but the leaves have not yet started to fall (33). The fully matured beans are used for flour, meal, and oil.

Occidentals, for the most part, depend upon meat and dairy foods for their source of animal protein while the Orientals rely largely upon fish and pork. The pig was used for food since this animal was not profitable for field work and produced in litters (78). Protein supply may be of vegetable or of animal origin. The protein in vegetables is of inferior quality and of low concentration, ranging from 1 to 2 per cent with the exception of legumes and peanuts (106). Most vegetable proteins contain a high content of indigestible cellulose which reduces their effectiveness as a food. The soybean is an exceptional bean because it can be made into a digestible food and is rich in protein (39).

Even with these findings about soybeans this plant protein still does not reach the American dining room tables mainly because of its strong, unpleasant flavor. The people of the Orient quite by accident discovered the sprout of the soybean as food. They found that the sprout was more delicious than the bean itself. One method of getting this bean removed from its state of being a bean is to make a curd out of the liquid obtained from it (117). To do this, the bean is softened in water, crushed and made into a soybean milk. The milk is boiled and solidified by a reagent such as magnesium or calcium salts (31). The resulting substance is as soft and silken as marshmallows (117). In our country bean curd or "tofu" may be said to be a milk substitute. It is found in approximately the same ratio in the Japanese home as milk is found in the American home.

This little bean is indeed a significant food which was relied upon heavily for fortifying the Asiatic diet since the dairying pattern never penetrated that part of the world. It was also an important food during World War II when the main supply of protein became insufficient on the island of Japan.

> Those Japanese who ate adequate quantities of properly cooked soybeans along with their rice rations also fared reasonably well in regard to the essential amino acids in spite of the war time shortage of fish (46, p. 46).

The soybean has great potential (138). It represents one of the great untapped sources of protein. It could feed a nation at war. In 1943 there was a production of 216 million bushels equivalent to 5 billion pounds of soybean protein which is roughly equivalent to the 5,867,000,000 pounds of protein produced by the United States in the form of beef, veal, lamb, mutton, pork, edible packing house products, eggs, milk, chicken, turkey, beans, and green peas (102). Soybeans could supply millions of tons of additional protein yearly. This bean played an important role in Germany during the first years of World War II. They constituted a material part of the ration of the huge German army.

> While Hitler and Stalin were still "friends" the supply was siphoned from the Orient to Germany over the single-track railroad across the wastes of Siberia (33, p. 12).

Statement of the Problem

The author has always had an interest in nutrition. Each place she has lived she has been interested in the food of that area, state or country and has wondered if these people received more nutrient value from the food they ate than American people and if they were healthier because of it. Following a navy husband to Cuba, it was noted that the pimento was served in restaurants and homes as often as three times a day. Since it is an excellent source of vitamin C, surely these Cubans were benefited by the repeated occurrence of this nutrient in their diet more than Americans who use it only for garnishing purposes.

While living in Japan, the author became intensely interested in the Japanese diet. Obesity was rare among the Japanese, and vitality seemed to abound. Through inquiries from the neighbors, the maid and a course in cooking, the author found that these people really do exist on a low caloric and a low fat diet. The Japanese cook solely with the poly-unsaturated oils, mainly sesame as it hides the off-flavors which may be present in the meat. Milk and milk products are almost absent from their diet but the food which they eat that is absent from the diet of people in the United States is the soybean. This is their main supplementary source of calcium and protein. It is most palatable in the form of soy-bean curd. This substance is eaten daily, and in a measure, takes the place of the milk which is not present.

It was the experience of living in Japan that prompted the author to determine the worth of the bean curd as a protein food and as a source of calcium. Protein and calcium are recognized as being necessary for good growth and health of young and old people. However, during growth, deficiency of these two nutrients often produces stunting and poor development.

In this study the author wishes to determine the effectiveness of soybean curd as a principal source of protein and calcium in the food supply of white rats. It is planned to feed one group of weanling white rats a ration adequate in all known nutrients except complete protein. When the animals are depleted, soybean curd will be added as the only source of the deficient nutrient. A third group of animals will be fed nothing but soybean curd ad libitum to determine whether this food will maintain life and growth in the young animal. A fourth group, a control group, will receive ad libitum all the nutrients known to be essential for optimum health of white rats.

Assumptions

The following factors are accepted as true:

1. It is possible to select components of a ration for rats which will be adequate in all nutrients except calcium. The diet will contain only traces of calcium.
2. It is possible to select components of a ration for rats which will be adequate in all nutrients except protein. This diet will contain only three per cent of inadequate vegetable protein.
3. The white rat is a suitable animal to be used to demonstrate the degree of adequacy of soybean curd as food.

Hypotheses

It is hypothesized that:

1. Soybean curd is a sufficiently complete food to promote life and growth in white rats.
2. Soybean curd furnishes enough calcium to promote growth in white rats that have been depleted of calcium.
3. Soybean curd furnishes enough protein to promote growth in white rats that have been depleted of animal protein.

After approximately one month of feeding the animals it is anticipated that the animals being fed deficient diets of protein and calcium will be depleted. Weight, length, breadth, and visual evidences of health will be evaluated at this time. The depleted animals will then be fed soybean curd to determine the effectiveness of it as a source of protein and calcium. In approximately one more month of feeding all of the animals will be compared to the control animals.

CHAPTER II

REVIEW OF LITERATURE

History of the Soybean

The early history of the soybean is lost in antiquity. Edward Dies calls the bean ancient, predating the Pyramids, the Tower of Babel, and the building of the Temple belonging to Solomon (83). It is known through legend that the soybean has supplied a substantial part of the protein in the diet of the Chinese for at least 4000 years (102). Story tellers of the Far East have related a tale about the saving of a bandit-besieged caravan from starvation by the food of the beans of a wild vine-like plant. The plant was later identified as the wild soybean. In this way the soybean became the very staff of life for the Chinese people (83).

> As firmly as history of the development of man in the western world is related to "bread and meat", the history of the eastern world is linked with "millet, rice, and soybeans" (102, p. 187).

Needless to say, the plant was extensively cultivated in China long before written records were kept (92). The first record of the plant is contained in a treatise written by Emperor Sheng Nung in 2838 B. C. (83). It is said to be one of the five sacred grains planted by one of the gods of agriculture, Hou Tsi. These grains--rice, soybeans, wheat, barley, and millet are essential to the continued existence of Chinese civilization (83).

The soybean is a native of Eastern Asia, originating from a wild plant. These plants are pubescent, that is the stems, leaves, and pods are covered with hairs. Perhaps the plant was called a wild plant because of the hair covering. These short, fine hairs were red in color and served as a protection to the plant against leaf hoppers and other devastating insects (101).

This leguminous plant grows from two to more than six feet in height. The flowers are small, sweet pea shaped and the bloom may, when mature, be yellow, green, brown, or black. Their shape may be round, oval, or flat. The yellow-seeded varieties are high in oil content, and are so hard that several hours' boiling fails to properly soften them (1). Salmon (112) says some of the newer varieties are palatable, high in nutritive value and easily grown. They should be considered as a standard vegetable to be included in all home gardens. The beans retain their shape when cooked. They become tender, but the texture and chewing qualities always remain much like that of nut meats (133).

The Chinese were aware of this staple protein food centuries ago and it has served them well. The pressure of increasing populations has always been a problem with the Oriental countries. Famine has devastated their economy many times and had it not been for this legume which resisted the attack of insects, the author speculates that the people would have surely starved in great numbers. These ingenious people have learned that a given measure of grain has several times the food value of animal protein when eaten directly by human beings. It will feed five times as many people as it would if fed to livestock and brought to the table in the form of meat and milk (97). Soybeans are the mainstay of millions of people living on small areas of land in other countries because the cost of soybean production is about one-third that of meat, and because the crop is a relatively foolproof one from the standpoint of insect and disease attack (50).

The Chinese eat large amounts of vegetables not only for economical reasons but also because of ingrained nutritional theories. From a health giving standpoint, vegetables in their estimation are far superior. The millions that profess the Buddhist faith are vegetarians. Considering the unhygenic circumstances in many areas of China, it was as prudent for the Chinese to abstain from meat and dairy products as it was for the ancient Hebrew people to refrain from partaking of pork. The Chinese have developed the art of cooking vegetables to a point which approaches perfection (78).

There was a period when the Chinese had a high state of culture and Europe and America were still barbarious. The Chinese are capable of sustained hard manual labor and great mental ability (61). The soybean has been a principal source of protein in the Chinese diet. This is something to consider.

The Japanese enjoy the highest standard of living in all of Asia (44). Still the simple, versatile soybean finds a recognized place in the diets of the rich and the poor, and forms an integral part of their Japanese traditional dietary pattern (94). Likewise these people have recognized that the soybean and its products are inexpensive for the amount of protein it will furnish in comparison to meat, eggs, and milk. The bean is especially eaten by those living in the hilly regions where fish are often unobtainable.

The soybean is the most important leguminous food in the world (113). As early as 1907 as much as fifty thousand acres of soybeans were grown in the United States. In 1956, there was on an order of twenty-four million tons of soybeans produced in the world (37). In 1965 soybean growers harvested the largest soybean crop ever produced in the United States (8). This amounted to 844 million bushels valued at over two billion dollars (8). The leguminous nature of the soybean makes it ideally adapted to the economy of the American farm. Because such plants have the ability, through their symbiotic relationship with nitrogen-fixing bacteria, to fix atmospheric nitrogen they fit excellently into crop rotation plans designed to conceive soil fertility and provide for the most economic use of expensive nitrogen fertilizers.

Composition of Protein

Protein contains carbon, hydrogen, and oxygen plus nitrogen. The atmosphere contains the great reservoir of nitrogen from which compounds are prepared. The free element is returned to the air after the decomposition of nitrogeneous compounds (68).

The average composition of protein is approximately as follows:

Carbon	51 per cent
Oxygen	26 per cent
Nitrogen	16 per cent
Hydrogen	7 per cent
Phosphorus	.4 per cent
Sulphur	.4 per cent

(11, p. 242).

The above percentages vary considerably with different proteins, but the percentage of nitrogen is fairly constant. Therefore, in order to estimate the total amount of protein in a given food or similar product, it has become a widespread practice to multiply the per cent of nitrogen content by the factor 6.25 on the assumption that all proteins contain, on the average, 16 per cent nitrogen. Phosphorus and sulphur are not always present. The following percentage has been found from the break-down of the globulin protein:

Glycinin

Carbon	51.12
Hydrogen	6.93
Nitrogen	17.53
Sulphur	0.79
Oxygen	22.63
	100.00

(98, p. 427).

There are a number of bacteria that convert nitrogen into compounds usuable by the plants. There are bacteria in the soil that convert free nitrogen into nitrates. These are called nitrogen-fixing bacteria because they form nitrogen compounds from free nitrogen. Some of the bacteria live free in the soil; others grow among the roots of leguminous plants upon which they live in a symbiotic relationship. Soybeans, peanuts, and clovers always have numerous small tubercles on their roots. These swellings contain nitrogen-fixing bacteria. The relationship between the plant and the bacteria that live together is one in which both organisms co-operate with each other.

Proteins are unquestionably the largest and most complex compounds that have ever been investigated (90). One index of their complexity is their enormous molecular weight. A simple protein, B-lactoglobulin in milk has been found to have a molecular weight of 42,000 (16). In comparison the soybean has a molecular weight of a magnitude of 300,000 (16). The reason for this difference lies in the protein composition and the fact that it is an oil-rich seed.

Proteins break down into amino acids during hydrolysis while in the organism they are built up from amino acids. Numerous amino acids are joined in a head-to-tail fashion to form peptide linkages (21). The bonded amino acids are then spoken of as a residue (124). The sequence of amino acids determines the primary structure, or the backbone, of the protein molecule (45). When several hundred amino acids join to form an extended chain molecule, it is easy to imagine that such a molecule may be arranged in space in various ways. It may retain a straight linear form or bunch up randomly in a ball, or arrange itself in a highly ordered helix. These arrangements are called secondary structures. The order of links in the chain is not the whole story as each chain is coiled and folded in a three dimensional pattern which is called a tertiary structure (124). An example of this tertiary structure is the globular soluble proteins. Chains with their own secondary structure line up and form aggregates. These macromolecules have a variety of forms which permits some to function as enzymes, some to act as structural material and some to aid in transmission of genetic information from cell to cell and from generation to generation (71) (41).

There are eight amino acids which are considered essential or indispensable for the diet of man. They are lysine, tryptophane, threonine, valine, methionine, leucine, isoleucine, and phenylalanine (23). These eight plus arginine and histidine are needed by growing rats (21). Proteins such as gelatin or zein, the chief protein in maize, are incomplete proteins with respect to their amino acid content and cannot sustain growth when fed as the only source of protein in a human or animal diet (21). Egg white protein, on the other hand, is a complete protein; that is, all of the amino acids that are essential in the diet are present in sufficient quantity to permit growth even if this substance is the sole source of edible protein (21).

Proteins occur in every particle of living matter (95). They are indispensable to life (91). These organic substances constitute the main structure of the animal and human body. This first ranking food nutrient has the special work in the human body of building tissue and of repairing worn out or broken-down cells. However, proteins may also furnish heat and energy if the diet is lacking in carbohydrate and fat. Hair, skin, muscles, nerve tissue, tendons, nails, feathers, horns, and hoofs are essentially all proteins (52). Familiar proteins are the hemoglobin from the blood, the casein from milk, and the albumin from the white of egg. The importance of protein as one of the three major nutrients in foodstuffs such as meat, eggs, or milk was appreciated long ago by intuition. The name "protein" was coined from the Greek verb "proteios" meaning to take first place (95). The word originated with a Dutch chemist and physician by the name of Mulder in 1838 (80).

The ultimate sources of all proteins are plants and bacteria (95). The plant receives water and nutrient salts through its roots. Carbohydrates are synthesized from water and carbon dioxide. This process goes on in the chlorophyll-bearing cells during daylight only. From the carbohydrate and salts the plant then manufactures other compounds such as amino acids, fatty acids, and water soluble vitamins (45). The making of protein requires neither chlorophyll nor sunlight. It has been shown experimentally that light is not an essential factor in protein synthesis, and the process occurs in the dark for the most part (11). However, Braverman (16) believes proteins are formed during the first minutes of illumination. Protein may be made in any part of the plant, but is principally carried on in the leaves. This is also the location for the synthesis of carbohydrate.

Composition of Soybean

The soybean is not really a bean. The name persists since no one has found a better one. It contains approximately 40 per cent protein which is twice as much as the ordinary bean (123). It also contains about 20 per cent oil while the ordinary bean has practically none (123). The available carbohydrate present in soybeans is between six and 12 per cent (133) (66). The starch content varies with the degree of ripeness when the bean is harvested. Ripe beans are almost starch free (48). The percentage is lower than that of many leguminous seeds and is largely in the form of sucrose rather than starch (29). When compared with the potato, the soybean has five times the calorie value, 20 times the protein value, and 200 times the fat value of the potato (61). When compared with meat, one pound of soybeans is equivalent in protein and fat to two pounds of beef (63). Other forms of carbohydrate are galactans, pentoses, and hemicelluloses (5). Since the soybean contains only traces of starch, it is a suitable food for diabetics (99) (66).

Soybean proteins are well-balanced in essential amino acids except for a slight deficiency in methionine (13). The amino acid content of the soybean is as follows:

> Methionine 1.8, cystine 1.1, lysine 5.4, tryptophane 1.7, leucine 9.2, isoleucine 2.4, phenylalanine 4.3, tyrosine 3.9, histidine 2.2, valine 1.6, arginine 8.3, glycine 0.75, alanine 1.7, aspartic acid 5.7, glutamic acid 19.0, proline 4.3, dapa 2.9, and ammonia 2.4 (10, p. 46).

The chief deficiency in legume proteins is a deficiency of total sulphur amino acids (109). Methionine in the diet is the only source of methionine in the animal tissue, but it may supply the sulphur for the synthesis of cystine and the methyl groups for the synthesis of choline and creatine. Methionine growth requirement may be affected by the presence or lack of dietary choline and cystine because these compounds in the diet may influence their rate of formation from methionine in vivo (3). The work of Henry and Kon suggest that the sulphur amino acid requirements of the rat decrease with age (58).

Many hundreds of analyses of the soybean have been made by the United States Department of Agriculture, Bureau of Chemistry and Soils (10). Their findings indicate a wide range of values of protein content which may be due to variety, soil, climate, and storage. A summary of these analyses follow in the table on the next page.

A serving of 100 grams of mature soybeans would supply 40 per cent of the protein, 25 per cent of the calcium, and over 60 per cent of the iron required daily by the adult. Salmon made a comparison of the

CHEMICAL COMPOSITION OF SOYBEANS

	Minimum Per Cent	Maximum Per Cent	Average Per Cent
Moisture	5.02	9.42	8.0
Ash	3.30	6.35	4.6
Fat	13.50	24.20	18.0
Fiber	2.84	6.27	3.5
Protein	29.60	50.30	40.0
Pentosan	3.77	5.45	4.4
Sugar	5.65	9.46	7.0
Starch-like substance by Diastase . .	4.65	8.97	5.6

(83, p. 11)

nutrient values of soybeans with other beans which is presented in the table below.

MAJOR NUTRIENTS OF SOYBEANS AND OTHER LEGUME SEEDS

FOOD	PROTEIN	CALCIUM	IRON	THIAMIN	RIBOFLAVIN	NICOTINIC ACID
	per cent	per cent	mg per 100 gm.	mg per 100 gm.	mg per 100 gm.	mg per 100 gm.
Soybeans, dry	40	0.212	10	1.1	0.32	4.8
Cowpeas, dry	21	0.100	6	0.9	0.30	---
Lima beans, dry	18	0.071	9	0.6	----	---
Navy beans, dry	22	0.140	9	0.6	----	---
Peanuts, dry	26	0.067	2	0.9	0.30	17.0

(112, p. 201)

It is evident from the table that the mineral content in soybeans is higher than in most legumes and grains. In comparing soybeans with grains the mineral content is higher in soybeans. For example, with wheat Baily, Capen and Le Clerc (6) state that the soybeans contain two to three times as much ash, four times as much potassium and sodium, two times as much phosphorus, about the same amount of sulphur, but only one-third as much chlorine as does wheat. These analysts give the following percentages for the mineral content of soybeans (air dry basis):

K	Na	Ca	Mg	P	Cl
1.913	0.343	0.210	0.223	0.406	0.024

Results from research on the soybean at the Wisconsin Station indicate that soybeans contain about 0.02 per cent of iron (114). The availability of iron in many biological materials was compared. The iron in most cereal grains has an availability of about 50 per cent whereas in soybeans, the percentage is much higher (114). From a table of the available iron content of many foods, four are as follows:

Material	Amount gm	Total Fe gm	Available Fe gm	Per cent available
Soybeans (non-roasted)	3.30	0.30	0.24	80
Pork liver (dry)	0.46	0.30	0.20	66
Spinach (dry)	0.57	0.30	0.06	20
Alfalfa	2.27	0.30	0.08	27

(115, p. 387)

Osborne and Mendel in their early research analyzed the ash constitutents of the soybean. Their findings are presented in table form below:

Per cent of the air dry material	soy cake meal	soy bean meal
Total ash	5.18	5.43
Calcium	0.34	0.18
Magnesium	0.27	0.30
Potassium	1.86	2.06
Sodium	0.17	0.14
Chlorine	0.01	0.005
Phosphorus	0.60	0.82

(99, p. 369)

The soybean has been sought after for its oil content in this country rather than its content of protein. In the twenties the demand for oil had resulted in large importations of soybeans (66). In 1966, soybeans and soybean products are the leading export crop in cash value in the United States (8). As production of this oil-bearing seed rose, increasingly larger amounts of the oil were used first for shortening, then for margarine, and more recently as salad oil. At the present time there are twenty edible oils and fats that are used for shortening. These fluctuate in price and are very competitive. Today the market conditions might indicate cotton and peanut oils as economically desirable. Next week the manufacturer might be using cotton and soy oils.

The oil content of twenty varieties of soybeans, representing the most promising strains grown at experiment stations, were analyzed for quantity and quality of oil. The samples varied from 17.41 to 22.9 per cent. The variety called Roanoke contained the highest per cent of oil (1).

Some of the leading oil seed varieties in 1929 were as follows: Illini, Dunfield, Mukden, and Scioto. These were direct introductions from the Orient or selections from such introductions (121). The fatty acid composition of several varieties of soybeans were studied in Urbana, Illinois, which is the heart of the soy belt and is known as the Regional Soybean Industrial Products Laboratory. Without cost to the government, the University of Illinois has provided laboratory, office, greenhouse, and field space for this work. The food utilization of soybeans in the United States is still so new that many problems must be solved before their main pattern of use is established (119).

Enzyme Inhibitors

The nutritive value of raw soybean is low (108). Soybeans contain substances which interfere with growth. These substances are known as enzyme inhibitors which curtail the activity of the trypsin enzyme secreted by the pancreas. This anti-trypsin factor is apparently ineffective in humans, but is effective in interferring with the growth of animals such as the rat. This inhibitor exists in the soybean protein (88).

This growth inhibitor was first pointed out by Osborne and Mendel during the first part of the nineteenth century. Their findings proved that soybeans would not support normal growth in rats unless cooked for several hours (99). Other researchers have also noted the presence of a tryptic inhibitor (134).

Kunitz isolated a crystalline protein from soybeans with strong trypsin inhibiting activity (75). He characterized the properties of the crystalline substance which he had isolated. This protein material has a strong affinity for trypsin and a much weaker affinity for chymotrypsin. It seems that more than one trypsin inhibitor exists in soybean (15).

Other inhibitors are hemagglutinins and there are yet other unidentified materials in the soybean which are noxious (4). Some but not all of these are heat-labile and are destroyed by toasting or cooking, while others are water-soluble. It is quite probable that some inhibitors may be removed by soaking and extraction (4).

The soybean is indigestible when prepared by simple means such as boiling (5). When digestion is incomplete, the undigested material may be observed in the feces (5). British prisoners of war in 1943 obtained some soybeans while they were in a Japanese prison camp. Their diet was limited since it consisted of almost nothing save milled rice and soybeans. The beans were boiled and in this form were found to be unpalatable and exceedingly indigestible. The beans were passed into the stools unaltered. Some Dutch prisoners suggested that a fermentation method be tried. It was found to be appetizing and readily digestible. This preparation was successfully used in treating protein and vitamin deficiencies (5). It appears that soybeans without suitable processing are imperfectly assimilated. Many attempts (47) were made to make the soybeans palatable and digestible. The only other satisfactory method proved to be one common in Indonesia which involved inoculation with a fungus. The beans gave rise to much digestive disturbance when used in quantity.

Several investigators (57) have found that heat in the preparation of certain foods does alter the nutritive value of the protein. When casein, meat, liver, kidney, cereals, and fish meal are heated to high temperatures, there is a definite decrease in their nutritional value.

This is not so with the soybean. There is much evidence through experimentation with animals that there is definite improvement in growth when they are changed from a raw soybean diet to a heat-treated soybean diet (57).

Sulfur and nitrogen balance studies made on rats indicated that soybean proteins are improved by heat. The claim that solvents such as hexane and methanol have a similar effect was not substantiated. It appears that soybeans have a sulfur and nitrogen complex which is absorbed but cannot be used for tissue building. Heating the bean makes the sulfur and nitrogen complex available (67).

Optimal heat processing such as steaming or mild-autoclaving has increased the availability of the amino acids, methionine and cystine (35). Experiments show that when processed soybean meal is supplemented with methionine, the protein efficiency ratio is increased which indicates that the protein contains adequate amounts of other essential amino acids (3).

Some of the nutrients of soybeans are altered during processing. Japanese nutritionists found the amino acid content to be reduced and the riboflavin increased (94) (127). Methionine is poorly utilized in unheated soybeans as demonstrated by tissue utilization. This was reflected by methionine requirements of rats fed unheated soybeans. It may be surmised tentatively that the growth-inhibitory factor in unheated soyflakes decreases tissue utilization of methionine (9). Soybean protein is a good source of lysine and rich in valine (13). The nutritive value of soybean proteins are related to two factors, the essential amino acids and the presence of a tryptic inhibitor that interferes with the tofu resembles beef in its content of protein and fat and has aptly been described by the Chinese as "the meat without bones" (62). Tofu is eaten by nearly every family in Japan with its breakfast miso-soup (55).

Studies have also been made on the palatability and acceptability of soybeans. Studies were made in Iowa City, Iowa, with infants to determine the number of babies who would accept soybean milk, the length of time they would take the milk, and the incidence of reaction to it. Infants did not tire of this food (7).

Varieties of soybeans are very numerous. Many hundreds of types and strains are known (22). The best all-around variety in the south is Rokusun. It ranks near the top in cooking quality and palatability. It is high in nutritive value as well (112). Six varieties which rated very good and might be accepted as a new vegetable by the American public are: Willomi, Hokkaido, 97155, Imperial, Jogun, and Funk Delivious (116). Best flavored flours came from Imperial and Hokkaido varieties (116).

Dr. Harper (51) calls the soybean the ancient food with modern potential. It is ancient because for nearly fifty centuries the people of the Orient have subsisted on the products of soybeans and hundreds of millions of these people have never known the taste of the milk of a cow (6). The first attempts with soy flour proved unsatisfactory as the soy flour had an adverse effect on the texture of the baked products. Newer techniques in processing have caused the bean to lose its objectionable flavor. Soy products are a potential source of several nutrients and offer variety for menus. Mass production could decrease costs (51). See the table on the following page.

Isolated soybean has many desirable physical properties such as

COMPARISONS OF SOY PRODUCTS AND SIMILAR FOODS*

(100 grams edible portion)

FOOD	PROTEIN	FAT	CALCIUM	IRON	VITAMIN A	THIAMINE	RIBOFLAVIN	NIACIN
	grams	grams	milli-grams	milli-grams	international units	milli-grams	milli-grams	milli-grams
Whole milk (3.7 per cent fat)	3.5	3.7	117	trace	150	0.03	0.17	0.1
Human milk	1.1	4.0	33	0.1	240	0.01	0.04	0.2
Soy milk	3.4	1.5	21	0.8	40	0.08	0.03	0.2
Cottage cheese, creamed	13.6	4.2	94	0.3	170	0.03	0.25	0.1
Soybean curd	7.8	4.2	128	1.9	0	0.06	0.03	0.1
Defatted soy flour	47.0	0.9	265	11.1	40	1.09	0.34	2.6
All-purpose wheat flour								
White, plain	10.5	1.0	16	0.8	0	0.06	0.05	0.9
White, enriched	10.5	1.0	16	2.9	0	0.44	0.26	3.5
Soy sauce	5.6	1.3	32	4.8	0	0.02	0.25	0.4
Soybeans	34.1	17.7	226	8.4	80	1.10	0.31	2.2
Peas	24.1	1.3	64	5.1	120	0.74	0.29	0.3

*Based on Agricultural Handbook No. 8, Agricultural Research Service, USDA, 1963. (51, p. 733) moisture-binding, emulsifying, stabilizing, thickening, film-forming, and dough-forming (65). Soy products which have these functional characteristics may be used in baking products, desserts, and gelatins (51).

The spun fibers have been fed to rats as the sole source of protein, and the results indicated that the spun protein provided was of good quality. The chemical composition of soybeans and isolated soy protein are compared. They are as follows: Soybeans contain (on a moisture-free basis) 45 per cent protein, 32 per cent carbohydrate, 20 per cent fat, and 5 per cent ash. Spun and isolated soy protein fiber contains 95 to 97 per cent protein, 2 to 4 per cent ash, and 0.1 to 0.2 per cent each of fat and carbohydrate (65). These latter products do not show any alterations in their amino acid content from the processing. These techniques, however, have removed the undesirable insoluble carbohydrate and destroyed the trypsin inhibitor which is present in raw soybeans (65). The fiber is a continuous strand or "tow" which can be produced in various physical forms, bits, cubes, or nuggets which are generally in the dehydrated state. The "tow", white and bland, may be processed by coloring and flavoring, and when reconstituted may be used in creamed dishes, casseroles, and combinations of vegetables (51).

Through new techniques, Proctor and Gamble company have prevented revision of the soybean oil, thereby making the flavor quite stable. These manufacturers have also produced a water-white oil for salads and for frying. The product has been named Sterling. Technology is producing materials having the aroma and flavor of meat and poultry which are combinations of hydrolyzed vegetable proteins containing polypeptides, peptones, and amino acids or their salts (65).

Biological Value of Soybean

The practice of measuring the biological value of any substance begun early in this century. McCollum experimented extensively with test animals. He started what was probably the first colony of rats late in 1907. He experimented with wild rats trapped in the barn and rejected them for albinos from a pet stock dealer (85). As a result, most of what we know about foods and diet has been learned through experiments upon animals. For such experiments the rat has been found to be the most satisfactory animal. The dietary habits of rats are much like ours, and taking their size into consideration, their life span is comparable to ours. Other animals are also used such as dogs, pigeons, piglets, chicks, mice, and monkeys. Small animals are chosen in preference to large ones for economical reasons. The growth rate of rats is 30 times faster than the human growth rate. One day in the life of a rat is the equivalent of 30 days in the human life span. It does not take long before altered food intakes are noted visibly and further substantiated by records kept during the length of the study.

William C. Rose worked with the problem of protein at the University of Illinois (110). In 1935 he and his associates completed the list of essential amino acids that must be present for the rat to reach normal growth and development. The presence of amino acids is critical (40).

The amino acid requirements are not rigid; they vary from species to species. Histidine and arginine are both essential for the rat, but are non-essential for man (54). There is a greater need for lysine by the young rat than the adult rat (91). All essential amino acids must be supplied simultaneously if the animal is to use them satisfactorily (43).

Animals have an inborn trait of being able to choose a nutritionally correct diet if they are in their natural surroundings (139). Cattle seeking and obtaining a salt lick is such an example. Their bodies need these minerals, and they involuntarily seek satisfaction through salt.

The differences in the biological value of proteins for rats are generally smaller when applied to man (4). Because of the rapid growth of the rat, their need for protein is highly sensitive (4).

Soybeans have their greatest value as a source of protein. At the Alabama Experiment Station the protein of cooked edible soybeans was nearly equal to casein in experiments at this station (112). The protein of the soybean is more efficient than most of the vegetable sources and approaches the quality of animal protein (84). Animals fed rations containing 15.0 to 18.7 per cent of soybean protein have grown normally. The rats on the 18.7 per cent soybean protein diet produced successive litters of young which have reproduced. This is sufficient evidence that the protein of the soybean fulfills all the physiological requirements (29).

The biological value of glycinin and raw soybean and autoclaved soybean protein were compared by determining the growth in young albino rats over a period of 8 weeks (30). The protein was fed at ten per cent level. Heat was not essential in promoting growth in rats when fed as an isolated protein, glycinin. Soybean glycinin protein was more effective in promoting growth than autoclaved soybean. The inhibitors of protein nature and other toxic factors are eliminated in preparing glycinin. From the table on the following page, it can be noted that isolated soybean protein has a biological value superior to the raw meal and approximately equivalent to autoclaved soybean meal.

Source of protein	Protein consumed (gm)	Gain in weight (gm)	Gain in weight (gm) per gm protein consumed
Raw soya-bean	29.1	22.6	0.78
Glycinin	31.5	48.8	1.55
Autoclaved soya-bean 15 (lb. for 1 hr.)	30.6	44.7	1.46

(30, p. 385)

The principle growth-limiting deficiency in raw soybean protein is that of methionine (3). Heated soybean protein is slightly deficient in methionine for the chick at 20 per cent protein level, but is complete in respect to all other amino acids required by the chick (3). Observation on the in vitro release of amino acids from soybean protein by the enzyme pancreatin suggested that the methionine of raw soybean was liberated more slowly by the proteolytic enzymes of the intestine than the other essential amino acids. Heat processing increases the rate of liberation of the methionine (87). It is now recognized that cooking or autoclaving increases the nutritive value of the soybean. It is believed that cooking increases the availability of cystine and methionine. These investigators (32) subjected soybean meal to an enzyme of the proteolytic type called papain. This unusual enzyme is found in the latex of the tropical plant papaya (69). The biological value of this digest was expected to be higher than the raw beans because all of the amino acids, including methionine, would be present simultaneously for utilization (32). From their findings it can be postulated that apart from the proteolytic inhibitors there is a separate factor which affects the nutritive value of soybean protein.

The acid hydrolysis of soybean protein liberates the amino acids in a certain order. The acidic are liberated first, then neutral, hydroxyl and basic amino acids are released. Amino acids with aromatic, heterocyclic and sulfur-containing groups are liberated at a slow rate. Proteins which contain tyrosine, histidine, proline, and valine are hydrolyzed with difficulty (27).

The average protein is a very sensitive material. Many reagents and conditions do not hydrolyze peptide bonds, but still destroy the biological nature and activity of a protein (60). When this has happened, the protein is said to have been denatured. To comprehend denaturation, one must visualize the protein molecule as one or more polypeptide chains cross-linked and coiled through a variety of chemical bonds of varying strengths. Modification of any of these links to a form which is not identical to the original native molecule indicates denaturation (45). Kauzmann defines denaturation as a process in which the spatial arrangement of the polypeptide chains within the molecule are changed from that typical of the native protein to a more disordered arrangement (70). It may occur as an unfolding or uncoiling of a pleated or coiled structure (60). Surface action, extreme pH and various other substances denature these fragile molecules (122). Heat denaturation proceeds on to a further stage, coagulation. This can be seen in the familiar example of the fried egg (19). See the table on the following page.

In a clinical study over a period of four years, Szujewski (31) fed eight to ten grams of soy protein daily to each of over 200 persons, patients with a variety of illnesses. Although their ages ranged from infants to quite elderly people, no untoward reactions were observed. All patients

THE COMPOSITION OF SOYBEANS AND SOYBEAN PRODUCTS[a, b] (*Glycine max*)
(All values for 100 g.)

	Water (g.)	Protein (g.)	Fat (g.)	Carbohydrate (g.)	Calories	Calcium (mg.)	Iron (mg.)	Yield[c,d] (kg.)
Dried mature beans	7.5	34.9	18.1	34.8	331	227	8.0	---
Flour, full fat	9.0	35.9	20.6	29.9	347	195	12.1	0.95
Flour, low fat	11.0	44.7	1.1	37.7	228	265	13.0	0.84
Milk	92.0	3.2	2.0	2.3	37	22	0.6	7.5
Curd[d]	85.1	7.0	4.1	3.0	71	100	1.5	3.5
Fermented beans (Chinese)[d]	45.0	17.0	10.0	6.0	---	100	3.7	2.0

[a] Unless otherwise specified, values are from B. K. Watt and A. L. Merrill, "Composition of Foods -- Raw, Processed, Prepared," U. S. Dept. Agr., Agr. Handbook No. 8, Washington, 1950.
[b] See also Tables IV, V, VI, and VII in Chapter 14.
[c] Yield from 1 kg. of beans.
[d] Taken from C. Chatfield, "Food Composition Tables," Food and Agr. Organization U. N. Nutritional Studies No. 3 (1953); "Food Composition Tables -- Minerals and Vitamins," ibid., No. 11 (1954).

(31, p. 221)

tolerated the soy protein well. Objective findings in all groups could be related as follows: clinical improvement in color of skin, in tissue turgor of the skin, and of mucous membranes of the mouth (31).

Determination of Protein Deficiency

Growth is a term that refers to ways in which a living system increases in size. Normal growth maintains a nice balance between all of the tissues (2). Body weight can be correlated with an increase of body protein (140). Two methods have been widely used for the evaluation of the nutritive value of proteins. The first determines, in growing rats, the maximum ratio of increase in body weight to protein consumned, known as the protein efficiency ratio (100). This value may be found by dividing the weight of the protein consumed into the weight increase of animals fed on that substance to determine the protein efficiency ratio. The second method is a balance-sheet method. This fundamental measure of the dietary protein was formulated by Karl Thomas of Germany in 1909 (129). This method measures the percentage of absorbed nitrogen retained for maintenance and in young rats, for growth, known as the biological value (90). Mathematically, biological value can be expressed as follows:

$$BV = \frac{\text{retained N}}{\text{absorbed N}} \times 100$$

This second method of measuring biological value of protein involves very definite experimental conditions. The procedure is rather difficult to ascertain.

Functions of Calcium

Early in the 20th century, Sherman (129) began studies on calcium and other minerals which were considered as essentials in the diet. Experiments were conducted to try to determine the amounts needed and how these minerals functioned in the body.

Calcium is found in the body in larger amounts than any other mineral. It serves as a body-builder and a body regulator (125). Ninety-nine per cent of the body calcium is found in hard tissues such as the teeth and the bones which normally contain large amounts of this mineral (86). It is the calcium that gives bone its strength and rigidity (126). Throughout life this mineral is needed as a regulator of body processes. It has been found that calcium is necessary for blood to clot, the action of certain enzymes and the control of the passage of fluids through the cell walls (53). Alternate contraction and relaxation of the heart muscle will occur only if calcium is in the blood in the right proportions (53). It is during the absorption process that this mineral is picked up by the blood. Calcium occurs in two forms: as the soluble inorganic ion and as a protein calcium complex (125). If the amount of calcium in the blood is below normal, the irritability of the nerves is increased (53).

Calcium is one of the nutrients most often in short supply. This has been found to exist by the National Research Council through diet surveys. Milk, ice cream, and cheese supply nearly two-thirds of the calcium in household foods. A one-inch cube of Cheddar-type cheese, in relation to its calcium content, is equivalent to two-thirds cup of milk; one-half cup of cottage cheese is equivalent to one-third cup of milk; 2 tablespoons of cream cheese is equivalent to 1 tablespoon of milk; and one-half cup of ice cream is equivalent to one-fourth cup of milk (103). It will be difficult to have enough calcium if the family uses little or no milk products. Certain cereals contain calcium. The amount depends on the extent to which grain has been milled. One cup of white wheat flour contains 18 milligrams of calcium, while one cup of graham flour provides 49 milligrams of the mineral (125). The calcium content in baked goods may be increased considerably if nonfat dry milk is used in their preparation. In enrichment programs calcium salts may be added to the flour as an optional nutrient (125). Baking powder and mold inhibitors also contain calcium. Self-rising flour contains calcium salts. This flour is very popular for making hot breads in the South (77).

Six authorities discussed the calcium factor in a symposium held by the Council on Foods and Nutrition of the American Medical Association. Hegsted of Boston (26) states that calcium requirements have not been substantiated with available data. This is demonstrated by the ability of both man and animals to maintain calcium balance over a wide range of calcium intakes. Hegsted argues that there is more evidence of harmful effects of high calcium intakes than of calcium deficiency. High intakes do not cause hypercalcemias, milk-alkali syndrome, kidney stones, and idiopathic hypercalciuria, but they may be detrimental in certain individuals.

Harrison (26) believes the calcium allowances which are set by the Food and Nutrition Board of the National Research Council are almost certainly above minimal needs. He emphasizes the requirement of Vitamin D for efficient intestinal absorption of calcium.

Nicolaysen (26) in Norway believes that human dietaries contain sufficient calcium to cover the needs of the majority of the population. Intent medical scrutiny of individuals in areas where calcium intake is low may still reveal that pathological conditions such as osteoporosis are more common situations than realized. Osteoporosis is a disorder of bone metabolism. There is a reduction in the amount of bone without any change in its chemical composition. The organic matrix appears abnormal as if it lacked calcium. The bone becomes thin and porous (125). Since calcium deficiency produces osteoporosis in animals, it might seem that this would occur in humans. The cause of osteoporosis in humans has not definitely been determined (96). Osteoporosis occurs in middle life and older age in both women and men (126).

Whedon and Lutwak (26) say that calcium levels must be kept within physiological limits for coagulation and neuromuscular functions. There are obligatory losses of calcium in urine, stool, and sweat. Calcium in the amount equivalent to output must be supplied from the diet. If dietary sources are insufficient, calcium is drawn from the body storehouse, bone.

The signs of deficiency of calcium are not known with certainty (26). The main obstacle to finding out such deficiency signs is that diets low in calcium usually are low in other essential nutrients. According to Walker (26), bone still contains 99 percent calcium whether the diet is low or not. Bone is a form of connective tissue which is highly specialized (28). This tissue is composed of minute, complex crystals of calcium and phosphorus set in the lattice of a material rich in protein, called the organic matrix. Bone is interspersed with connecting canals containing blood and lymph vessels into the intercellular fluid that bathes all the crystals (125). The formation of bone is a continuous process during the period of childhood and adolescence. It is dependent upon an adequate and steady supply of nutrients. After adolescence there is need of repair and rebuilding the bone. An adult does not outgrow his need for bone building nutrients in his diet (28).

The occurrence of transverse bands of dense tissue in bones has been recognized for many years (105). With the introduction of radiology transverse lines are seen in the growing ends of long bones of children. Such lines are noted in the bones of malnourished children and in children having had some illness such as measles or whooping cough (105). Platt and Stewart (105) have noticed that during severe illness a line is produced in the bone. They have suggested that it may be the result of protein deficiency. Illness increases the body's need for protein at a time when intake is unlikely to be adequate. These authors (105) also noted that when animals were fed a low-protein diet, the rate of development was slow. The increase in bone length in a given period may be only one-third that in animals receiving a normal diet.

Sound teeth are found in underdeveloped populations whose diets are low in calcium (26). This mineral has a metabolic role in blood coagulation, urolithiasis, cholelithiasis, and tetany. But the exact level has not been substantiated. Until knowledge of osteoporosis is more advanced with respect to significance of sex, age, race, socio-economic status, activity, and calcium intake it would not be advisable to re-establish the calcium requirements.

The urinary calcium and the endogeneous fecal calcium are the two major avenues of body calcium loss (12). A small amount of calcium does leave the body in prespiration. If sweating is heavy, there is more loss (24). Consolazio, et al, studied this factor. Calcium may be lost during heavy sweating conditions up to 20 mg. calcium per hour. This loss should be accounted for in establishing recommended allowances for calcium. It is doubtful if an individual consuming a low calcium diet ever really attains calcium balance under heavy sweating conditions (25).

Bronner, et al (17), studied calcium metabolism and the fate of intravenously injected radiocalcium in human beings. They found little correlation between chronological age and urinary calcium output. In all subjects of the demonstration the urine constituted the more important route of calcium excreted in the feces. Knapp (74) found that urinary calcium increased significantly with increase in weight. Age and sex are not factors.

Determination of Calcium Deficiency

From the review of the functions of calcium, it is evident that calcium has a definite role in the nourishment of the human body. Researchers are making determinations of calcium deficiency not only on animals but on human beings.

In Guatemala (42) it was found that Indian children suffering from extreme protein-calorie malnutrition are no further delayed in ossification stature than children on low-protein diets in the control villages, though the latter are obviously retarded in comparision with middle-class, Ohio-born, American, white children. Compact bone was measured and found to be low. The fact that the patients had no compact bones at 4 to 6 years suggested actual bone loss as well as simple failure to gain (42). There is marked deficiency in compact bone, especially in children with marasmus.

The dietary calcium requirements of the human being are a debated nutritional problem (125). Platt and Stewart (105) made a study of the growth in length and the mineral content of the bones of piglets on a diet deficient in protein and calories. The histological and radiological appearances of the bones were also noted. The growth of long bones was slow. There was a reduction in the formation of osteoblasts which are the bone-forming cells. The growth rate in length and the radiological appearance of the bone were improved by substituting an iso-caloric amount of protein for part of the carbohydrate of the low-protein diet. The addition of extra protein to the low-protein diets had no significant effect on bones. Small skull bones were noted. The unerupted molars of the lower teeth were especially crowded. The vertebrae were small and osteoporotic; the area of the cross section of the vertebral canal, though smaller in normal pigs of equal ages, was larger than that of vertebraes of about the same external dimensions from younger, normal pigs. Through this study Platt and Stewart demonstrated a direct relationship between adequate protein and the growth of long bones in piglets (105). In early life the diet should provide enough calcium to permit the growth which the person is genetically capable of achieving. Upon reaching adulthood he will have attained a fully mineralized skeleton (125) if sufficient bone-building nutrients have been supplied.

Growth in height stops when the epiphyses close, and a nutritional limitation may or may not delay this maturation process. Spies, et al (120), found that supplementation with dry skim milk did not hasten maturation in a malnourished adolescent group. The added milk induced a statistically significant acceleration in skeletal and general body growth in pre-adolescent groups. This was not maintained after the supplement was terminated.

A more tangible and objective test (20) can be made on young animals fed diets adequate in calcium and diets inadequate in calcium. The tibias of the rats are then stained in such a manner that their internal parts having calcified areas will show darker than the rest of the area. The rat fed an adequate diet in calcium will have a calcified area at the cartilage which has a deeper layer of cells than the cartilage of the rat which had a diet lacking in calcium. The metaphysis will be longer in the calcium fed rat. The bone trabeculae will be heavier and more numerous than the trabeculae of the rat fed no calcium. A rat fed a diet poor in calcium or devoid of calcium will have no storage of the mineral which will be shown by the staining method. A porous condition will also be revealed indicating the need of calcium.

The Rat as an Experimental Animal

The albino rat has long been used in experimental work, and especially for routine tests of protein quality (38). This standard test animal possesses many laboratory virtues and is, therefore, an almost universal choice (130). Recently and almost simultaneously, researchers in the United States, Israel, South Africa, and in Guatemala have demonstrated routine tests of protein quality on chicks. They have obtained values comparable with the rat (38). The rat is an omnivorous creature. He eats practically everything and reacts to food largely in the same way that humans do. Because of his short life span, growth will be noted on a short term demonstration.

The number of rats to choose for each diet is important. Inferences cannot be made from too small a sample, and a large sample has many problems. It is best to choose a number that can be kept under standardized conditions. Two different researchers have placed four rats on the same diet (89) (73). Two or more diets should be fed at the same time with at least four rats to a diet. The control diet will contain all the known nutrients and the other diets will be inadequate in some specific respect. In a short time the rat groups on the inadequate diets in respect to proteins will become depleted. Altschul has found that twelve days are required for a rat to loose 25 per cent of his initial weight and thus become depleted (4). A similar time is needed for repletion. Other researchers have found that depletion can occur with 7 days (18).

Experimental diets must be selected carefully to be sure that they will demonstrate clearly the adequacy of the materials fed. They can be simple and contain one or two items. Such a demonstration is as follows (136):

Rat (1) Enriched or whole grain bread and water;* all it wants.

Rat (2) Whole, pasteurized fresh milk and water;* all it wants.

Rat (3) Whole, pasteurized fresh milk and enriched or whole grain bread and water* (offered separately); all it wants.

*Distilled water is recommended but not required.

Or the diet can be patterned after a human diet such as is given by Eppright, Pattison and Barbour as follows:

PERCENTAGE COMPOSITION OF 3 EXPERIMENTAL DIETS

Ingredients	Diet 1 (all foods dried) Inadequate	Diet 2 (all foods dried) Inadequate plus milk	Diet 3 (all foods dried except last 3) Adequate
	(per cent)	(per cent)	(per cent)
Meat	15	15	15
Beans (Navy)	4	4	4
Potatoes (white)	10	10	10
Rolled oats	5	5	5
White flour	25	20	12
Butter	5	5	5
Lard	10	10	6
Sugar	20	10	5
Apples	5	5	5
Salt	1	1	1
Dried whole milk	0	15	15
Eggs	0	0	5
Lettuce	0	0	3
Carrots	0	0	4
Tomatoes	0	0	5
Total	100	100	100

(34, p. 321)

Diet I, inadequate diet, would deplete an animal of calcium.

The foods used in an experimental diet should be dried, ground, and mixed so that the diet is homogeneous. This method of procedure prevents the animal from choosing special foods in the diet (72). Block (13) found that baking and drying a protein food reduced the availability of the amino acid lysine to rats. Rats are unable to synthesize unsaturated fatty acids; the unsaturated fatty acids thus become essential components of their diet (69). The rat is among some of the mammals that are able to manufacture vitamin C within their own bodies (76).

A summary of the common nutrients required for the rat are as follows:

SUMMARY OF COMMON NUTRIENTS

Nutrient	Amount Per Day
Protein	25 - 30%
Calcium	40 - 50 mg.
Phosphorus	35 - 40 mg.
Sodium	0.5%
Chlorine	5 mg.
Vitamin A	4 mcg.
Thiamine	1 mcg.
Riboflavin	40 mcg.
Vitamin D	Not required if Ca:P ratio is between 1:1 and 2:1 (49, p. 98)

It has been found from recent experiments that protein fed as 10 per cent of the calories is utilized more efficiently than the same protein fed at the level of 20 per cent of the calories (4) (108) (38). The pattern of amino acids required for growth in rats was first established by Rose and co-workers. It is presented in the following table:

CLASSIFICATION OF AMINO ACIDS WITH RESPECT TO THEIR GROWTH EFFECTS IN THE RAT

Essential	Nonessential
Lysine	Glycine
Tryptophan	Alanine
Histidine	Serine
Phenylalanine	Cystine[a]
Leucine	Tyrosine[b]
Isoleucine	Aspartic acid
Threonine	Glutamic acid[c]
Methionine	Proline[e]
Valine	Hydroxyproline
Arginine[d]	Citrulline

(111, p. 753)

In more recent research at the University of Illinois a revised pattern of amino acids required by the growing rat was developed. Rama Rao, Norton and Johnson developed the table on the following page.

Cystine can replace about one-sixth of the methionine requirement but has no growth effect in the absence of methionine. Tyrosine can replace about one-half of the phenylalanine requirement but has no growth effect in the absence of phenylalanine. Glutamic acid and proline can serve individually as rather ineffective substitutes for arginine in the diet. This property is not shared by hydroxyproline. Arginine can be synthesized by the rat, but not at a sufficiently rapid rate to meet the demands of maximum growth. Its classification, therefore, as essential or nonessential is purely a matter of definition (111).

PATTERN OF AMINO ACIDS REQUIRED

BY THE GROWING RAT

	% of diet	
L-Histidine	0.25	
L-Lysine	0.90	
L-Tryptophan	0.11	
L-Isoleucine	0.55	
L-Valine	0.70	
L-Leucine	0.50	
L-Threonine	0.16	0.50 (bracketed: 0.50, 0.16, 0.34)
L-Methionine	0.34	
L-Cystine	0.42	0.72 (bracketed: 0.42, 0.30)
L-Phenylalanine	0.30	
L-Tyrosine		

Plus non-essential amino acid mixture[1] to make a total conventional protein content of 10% (1.6 gN).

[1]100 g of the non-essential amino acid mixture contains: 12.3 g DL-serine; 41.6 gL-glutamic acid; 12.7 g L-aspartic acid; 5.9 g DL-alanine; 23.7 g L-proline, and 3.8 g glycine. (107, p. 88)

CHAPTER III

METHOD OF PROCEDURE

The experiment must have every detail planned prior to the execution of it. The planning will include the securing of the test animals, the needed materials for housing them during the length of the experiment, the preparation of their rations, and the evaluation of the results.

It is planned to purchase 20 albino rats of weanling age from a supplier handling the Holtzman strain.[1] These descendants are selectively bred for rapidity of growth and relative freedom of disease. Upon receipt of the rats, each will be examined for well-being and placed in a separate cage which will be numbered in a consecutive order from one through 20. The cages will be placed on the shelves of a metal carrier in the animal feeding room of Home Economics Research. The animals will then be randomly selected for their diet groups, weighed and marked. Twenty numbers will be drawn out of the hat. To illustrate, these six numbers which are drawn may be 3, 6, 7, 10, 16, and 20. The rat in each of these cages will be placed on the Control Diet. Each cage will be left in the original position until the randomization is completed. Four numbers will be drawn from those remaining in the hat. In like manner this selected group will be placed on the Protein Deficient Diet.

[1]The animals were ordered from the Holtzman Company, 421 Holtzman Road, Madison, Wisconsin.

The next four numbers indicate those animals to be placed on the Soybean Curd Diet. The last six numbers indicate those animals to be placed on the Low Calcium Diet.

Prepared labels will then be placed on the cages indicating the diet ration and the date. Each rat will be weighed and either ear-marked or tail-marked as follows:

Control Diet - Group I

Rat 1 notch in right ear
Rat 2 notch in left ear
Rat 3 notch in right ear and red color
Rat 4 notch in left ear and red color
Rat 5 notch in right ear and yellow color
Rat 6 notch in left ear and yellow color

Protein Deficient Diet - Experimental Group II

Rat 7 notch in right ear and green color
Rat 8 notch in left ear and green color
Rat 9 notch in right ear and blue color
Rat 10 notch in left ear and blue color

Soybean Curd Diet - Experimental Group III

Rat 11 red marking on distal portion of tail
Rat 12 red marking on proximate portion of tail
Rat 13 green marking on distal portion of tail
Rat 14 green marking on proximate portion of tail

Low Calcium Diet - Experimental Group IV

Rat 15 blue marking on distal portion of tail
Rat 16 blue marking on proximate portion of tail
Rat 17 yellow marking on distal portion of tail
Rat 18 yellow marking on proximate portion of tail
Rat 19 purple marking on distal portion of tail
Rat 20 purple marking on proximate portion of tail

Six rats will be placed on Control Diet I, and six will be on Experiment Diet IV. The second group of four rats will be placed on an Experimental Diet II.

Each cage housing the animals will be round, eight to 12 inches in height and nine and one-half inches in diameter. It will be constructed of heavy galvanized wire mesh. The floor of the cage permits the passage of urine and feces into the pan beneath the cage. Ration cups will be marked I, II, III, IV according to the diet groups and placed in the cages; one to a cage. Heavy drinking cups will be filled with distilled water and offered at all times.

The diets will consist of control, protein deficient, soybean curd, and low calcium. The control animals, six in number, will receive a ration adequate in all known essentials. After one month of feeding, Experimental Diet IV, two animals from the Control Group I and two from the Experimental Group IV will be sacrificed, examined for internal changes, and a line test will be performed on one of the long bones to determine the extent of calcium depletion. The test will be as follows: kill two randomly selected rats which have been fed the Low Calcium Diet and perform the line test on one of the long bones in this manner:

a. Dissect and clean the bone.

b. Split it with a sharp knife longitudinally.

c. Rinse in distilled water.

d. Immerse for one minute in a 2 per cent silver nitrate solution. Rinse in distilled water.

e. Expose to light until the calcified areas have darkened. Observe under a hand lens (20, p. 514). See Appendix.

In like manner two animals on the Control Diet will be randomly selected, killed, and a line test performed on one of the long bones. Analyses from bones of animals fed a Low Calcium Diet will be compared with the bones of the animals fed a Control Diet.

The Control Diet will be a modified version of a control diet found in Laboratory Experiments in Nutrition (76). It is estimated that six pounds of Control Diet will feed six rats for one month, or 15 grams per day per animal. It is estimated that four pounds of diet mixture will be needed for the remaining period of feeding. For approximately two months of feeding animals, there will be a total of ten pounds or 4536.0 grams of Control Diet needed. The following ingredients will be included in mixing ten pounds of this diet:

	%	Gm
Milk	32	1451.50
Cornstarch	60	2721.60
Soybean Oil	3	136.08
Sodium Chloride	1	45.36
Calcium Carbonate	2	90.72
Brewer's Yeast	2	90.72
Cod Liver Oil	4 drops[a]	181.0 drops[a]
	100	4535.98

[a]The drops are not added in the total amount.

The Protein Deficient Diet will be taken from Laboratory Experiments in Nutrition (76). The diet will be modified since soybean oil will be substituted for lard. The diet will consist of the following:

	%	Gm
Cornstarch	92	834.6
Soybean Oil	3	27.2
Sodium Chloride	1	9.1
Calcium Carbonate	2	18.1
Brewer's Yeast	2	18.1
Cod Liver Oil	4 drops[a]	36.4 drops[a]
	100	907.2

[a]The drops are not added in the total amount.

The rats in Experimental Group II will eat this ration until depleted. The time has been estimated from one to two weeks or until 25 per cent of their initial weight has been lost plus or minus two grams.

Four animals will be eating this ration. It is estimated that two pounds of diet will be needed or 907.2 grams.

The Experimental Diet III will consist of soybean curd made from the yellowish-green soybeans. The procedure is as follows:

Wash one pound of soybeans and soak for ten hours (131) in two quarts of water (22). Pour off the water and measure the soaked beans. The soaked, drained beans were crushed in a mill made for this use.[2] The amount of fresh water to be used will be three times the volume of the beans. Part of the water should be added during the grinding process. The remainder of the water will be placed on the beans after they have been crushed. As the water is added to the ground bean mixture, milk will be formed.

Heat the raw soybean milk to boiling. Skim off the froth from the surface of the milk. Hold the mixture near the boiling point for thirty minutes. Strain through a sieve lined with a thin cloth. Save the supernatent material; discard the rest. The milk is measured and reheated. The precipitating agent is added and stirred vigorously. Approximately 1.5 ounces of one per cent calcium sulfate solution is needed for each quart of milk. Smith, Watanabe, and Nash found calcium sulfate to be superior to calcium or magnesium chloride. This salt causes the precipitate to be more of a gelatinous type. The texture is smooth (118). Allow the precipitated soy mixture to stand for a few minutes. After all the small particles flocculate the mixture is poured through a colander lined with a clean thin cloth. The supernatent fluid

[2]Quaker City No. F_4 grinding mill.

is discarded. The curd is transferred to lined storage pans. As the product stands, more liquid will tend to seep out from the curd and the product will hold together. Weights help press the curd, or cheese, to give desired consistency.

The Low Calcium Diet will be taken from a study on bone density which appeared in the Journal of Nutrition (137). The modified version is as follows:

	%	Gm
Cornstarch	60.5	2069.1
Casein	18.0	615.6
Soybean Oil	8.0	273.0
Brewer's Yeast	7.5	239.5
Salt Mixture	4.0	136.8
Cod Liver Oil	2.0	68.4
	100.0	3042.4

Six rats will subsist on this ration. It is estimated that the rats will become depleted within four weeks. Since this time of depletion is uncertain, two more weeks will be allowed. A diet for six weeks for six animals will require seven and one-half pounds. This amount is equivalent to 3042.4 grams.

Oklahoma grows soybeans which are superior in protein content (132), but not in the same quantities as the corn belt states. A straw-yellow or yellowish-green edible variety recommended by Dr. Chen for making soybean curd (22) will be identified. At this particular time of the year early spring varieties of soybeans will be in demand for planting. Top-notch varieties will be at a premium. Further, all seeds regardless of variety will have to be prepared for planting. The seeds will be innoculated or infected with nitrogen-fixing bacteria recommended by agriculturalists. Beans which have been treated in this manner are not acceptable for use in animal feeding demonstrations. Thirty pounds of soybeans will be purchased from Muskogee Farmers Association, 530 North Main Street in Muskogee, Oklahoma, at ten cents per pound. The beans will be of the Lee variety.

As the animals of the Experimental Diet II reach depletion, as indicated by loss of one-fourth their initial body weight, they will be placed on a repletion diet. In this diet 20 grams of cornstarch and three grams of soybean oil will be replaced by dry soybean curd as the source of protein. Other nutrients in the diet will be adequate. The repletion diet will be as follows:

	%	Gm
Dry Soybean Curd	23	828.0
Cornstarch	72	2592.0
Sodium Chloride	1	36.0
Calcium Carbonate	2	72.0
Brewer's Yeast	2	72.0
Cod Liver Oil	4 drops[a]	144.0 drops[a]
	100	3600.0

[a]The drops are not added in the totals.

When the low calcium animals have been fed the Experimental Diet IV for six weeks, they will be placed on a diet in which dry soybean curd will replace 16 grams of cornstarch and 14 grams of casein. Protein will be kept at the ten per cent level and the curd will be the only source of calcium. The diet composition is as follows:

	%	Gm
Cornstarch	44.5	1335.0
Casein	4.0	120.0
Dried Curd	30.0	900.0
Brewer's Yeast	7.5	225.0
Soybean Oil	8.0	240.0
Salt Mix	4.0	120.0
Cod Liver Oil	2.0	60.0
	100.0	3000.0

The rat will be supplied with fresh food and distilled water daily except Sunday. The papers under the cage will be replaced. Left over food will be weighed back and the food eaten calculated. The rats will be weighed to the nearest gram at bi-weekly intervals. Rats on Experimental Diet II and III will be weighed daily to detect change in weight since their diets will be quite restricted. Amounts of food sufficient for one week will be removed from the freezer and stored in a tightly covered container for accessibility.

Main housekeeping chores will be performed once a week. At that time the entire cage will be scrubbed in a disinfectant, rinsed, and dried. The carrier will also be washed in water containing disinfectant. Food and water will be placed in clean containers. Each cage with rat will be rotated weekly in such a manner that all cages will be moved through the various positions on the carrier. The rats will be cared for in this manner during the length of the experiment.

CHAPTER IV

RESULTS AND DISCUSSION

One practical test of any given food is its ability to produce growth. The growth rate of the rat is 30 times faster than the human growth rate. One day in the life of a rat is the equivalent of 30 days in the life of a human being. The length of this research plus weanling age is 98 days. This experiment could be compared in length to a human experiment from birth to over eight years of age.

In the Control Group, animal No. 3 was the largest animal at the termination of the experiment. It so happened that he was the smallest at the beginning of the experiment. See Table 1. He had consumed 1011 grams of diet and weighed 298 grams. The smallest control rat, no. 6, had eaten 987.3 grams of diet and weighed 248 grams. On the previous weighing he weighed 277 grams. During the course of the weekend he knocked over his heavy drinking cup and was without water and, therefore, lost 29 grams. Animal no. 3, the largest one, measured 15 3/4 inches in length, and had a rib cage which measured 5½ inches in circumference. The smallest rat in the Control Group, no. 6, measured 15 inches in length and had a rib cage of six inches. This animal may have been genetically capable of being a much larger animal but due to environmental conditions, he was unable to reach maximum growth. Early in the demonstration, he had diarrhea. Later he had a bloody discharge. There was a total of three instances in which he accidentally knocked over his water container.

TABLE 1 Weekly food eaten and weight gained by rats in Control Group I

	Rat No. 1		Rat No. 2		Rat No. 3		Rat No. 4		Rat No. 5		Rat No. 6	
Date	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
4/8-12	26.3	5.4	20.1	3.1	18.4	3.5	19.5	1.4	18.5	5.4	23.3	4.8
4/12-18	67.0	27.5	57.5	20.5	50.4	16.5	48.5	20.0	60.5	28.0	59.5	22.0
4/19-25	79.0	27.5	73.5	32.0	66.5	24.5	68.5	19.5	74.0	26.5	81.0	35.5
4/26-5/2	69.0	19.0	92.5	33.5	80.0	28.5	87.5	23.5	92.5	24.5	99.0	- 8.5
5/3-9	95.5	34.5	95.0	24.5	100.5	29.0	85.0	29.0	74.0	20.5	82.5	48.0
5/10-16	96.5	16.5	95.5	25.5	112.5	31.5	101.0	29.0	98.0	35.5	96.0	30.0
5/17-23	101.0	20.0	111.5	22.0	104.0	22.0	45.5[b]	3.0[b]	61.0[b]	14.0[b]	97.5	16.0
5/24-30	105.5	24.0	111.0	31.0	115.5	30.0					114.5	23.0
5/31-6/6	107.0	16.0	108.0	15.0	126.0	27.0					117.0	17.0
6/7-13	115.0	11.0	65.0	8.0	124.0	12.0					122.0	20.0
6/14-20	101.0	15.0	[a]	[a]	113.0	17.0					95.0	-18.0
Total	962.8	216.4	829.6	215.1	1010.8	241.5	455.5	125.4	478.5	154.4	987.3	189.8
Initial Wt.	59.6		56.9		56.5		56.6		58.6		58.2	
Terminal Wt.	276.0		272.0		298.0		182.0		213.0		248.0	

Mean Food Eaten 978.0 Mean Weight Gained 216.0 Mean Terminal Weight 274.0

[a]Died on June 12, 1966

[b]Sacrificed on May 19, 1966

Each time a larger, heavier jar was placed in the cage. A glass bubble was of no use with this animal as he had it unattached from the cage by the next feeding period.

Rats no. 4 and 5 were sacrificed on May 19. Random selection of two rats identified the largest and the smallest rat in the control group.

Rat no. 2 died on June 12. For three consecutive feedings his appetite waned. He had a bloody nose and diarrhea. His death may have been caused by respiratory trouble or a digestive disturbance. This animal is not calculated in the final results of the control group.

The animals on the Protein Deficient Diet were depleted at different rates. Animal no. 8 was depleted in 11 days; animal no. 7 was depleted in 14 days; animal no. 10 in 17 days, while animal no. 9 took 22 days. See Table 2. The animal that was depleted the earliest weighed the most at the end of the demonstration. As each animal reached depletion, he received Diet II modified to contain ten per cent dried soybean curd in place of an equal amount of cornstarch. The dry soy curd was not in powder form and the author felt that the rough particles might be hard or harsh to the intestinal track. After animal no. 7 had been on this diet for 16 days he died. This death may be attributed to the coarseness of the dried soybean curd which was very hard and rough. Upon two different occasions rat no. 7 had a bloody discharge. Upon one occasion rat no. 8 had a bloody discharge, also. This animal was able to survive the rough diet. At this time the researcher learned that she could pulverize the coarse, dry soybean curd by putting it through the Waring Blender. The resulting product was a finely ground substance. Acceptance of this product was much improved as indicated by food eaten.

TABLE 2 Weekly food eaten and weight gained by rats in Experimental Group II

	Rat No. 7		Rat No. 8		Rat No. 9		Rat No. 10	
Date	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
4/8-12	19.0	-3.0	15.0	-5.0	17.5	-3.5	19.5	-2.0
4/12-18	40.5	-2.0	29.5	-7.0	34.5	-5.0	33.0	-5.5
4/19-25	27.0[a]	-7.0	30.0[a]	-1.0	35.0	-2.0	31.5	-3.4
4/26-5/2	45.5	10.5	38.5	5.5	42.0	-1.5	43.0[a]	9.5
5/3-9	49.5	5.8	63.5	2.5	28.5	7.5[a]	34.5	-1.5
5/10-16	18.6[b]		55.5	29.5	43.5	22.5	30.5	10.5
5/17-23			59.0	21.5	54.5	25.5	46.0	14.0
5/24-30[c]			73.0	22.0	56.5	20.0	59.5	33.0
5/31-6/6			44.5	19.0	53.0	10.0	69.0	20.0
6/7-13			86.0	36.0	99.0	29.0	85.0	24.0
6/14-20			84.0	17.0	97.0	24.0	76.0	21.0
Total	200.1	12.0	578.5	140.0	561.0	125.5	527.5	118.0
Initial Weight		55.0		60.0		60.5		55.0
Terminal Weight		67.0		200.0		186.0		173.0

Mean Food Eaten 556.0 Mean Weight Gained 128.0 Mean Terminal Weight Gained 186.0

[a]Lost 25 per cent of initial body weight and started feeding coarse dried soybean curd.

[b]Died May 14, 1966.

[c]Started feeding finely ground dried soybean curd.

Animal no. 7 was also left out of the final analysis.

On the weekend of May 28 and 29 the temperature reached 86 degrees F. The animal laboratory may have been even hotter than this temperature during the afternoon as the laboratory was not air conditioned. The following Monday animal no. 9 was denuded of fur from the ventral portion of his body. There was no evidence of fur in the cage or in the area under the cage. Fur usually falls out in patches but this denuded area was as free from fur as if it had been shaved. The author suspects that the animal ate this portion of fur because he was too warm. The denuded area was available to him whereas the dorsal region would have been difficult to reach. This animal started growing new fur very soon and in three weeks he had enough to look normal again. Fur of all the animals in Group II was soft, lustrous, and normal looking.

Among the four rats on Experimental Diet II there was a direct relationship between the food eaten and the weight gained. During the experimental period the largest animal, no. 8, ate 578.5 grams of diet and had a terminal body weight of 200 grams, whereas animal no. 10 ate 527.5 grams and weighed 173 grams, the smallest animal of any rat in this group.

Further evidence of the development of rats on Experimental Diet II was exhibited in the length, and the rib-cage circumference. Animal no. 8 was 13½ inches long, and the circumference of his rib cage measured 5½ inches. Animal no. 10, the smallest of the group, was 13½ inches long, and had a rib-cage circumference which measured 4¾ inches.

Since all animals on Experimental Diet II increased their weight four times over their depletion weight, the soybean as a source of protein appeared to have a good biological value. If the finely ground, dry soybean curd had been fed from the time of depletion growth would probably have been more rapid.

In Experimental Diet III animal no. 14 was the largest among the group at the beginning and at the end of the experiment. See Table 3. Animal no. 13 was the smallest, weighing 74.0 grams.

The greatest gain of all the animals occurred on unstrained soy curd which contained the whole bean. After a period of three weeks the soy milk was strained before the making of the curd. The author recognized that the soybean curd was different than that she had used in Japan. She tried straining the soybean milk before the precipitant was added and found that this procedure produced the quality of curd she remembered. This new type of curd was offered to Group III animals. There was a smaller amount eaten than previously and a drop in the weight gain of the animals. Three weeks later finely ground, dry soybean curd was offered as a part of their food since the wet curd soured. The animals began to lose weight very soon. Acceptability of this dry diet was poor. During the last two weeks dry and wet soy curd was offered. Two animals, no. 12 and no. 14, gained only four grams in this period. The fact that all animals lived only on a soybean curd diet for 73 days is something to consider. This diet maintained life but not growth. The soybean curd appears to have considerable value as food but does not support growth as the whole bean did.

Animal no. 14 became paralyzed on May 30, 1966. Paralysis of the animal may have occurred because dry soybean curd was not well accepted and does not supply adequate vitamin and minerals and only an incomplete protein. He refused to eat and lost nine grams of body weight over one

TABLE 3 Weekly food eaten and weight gained by rats in Experimental Group III

Date	Rat No. 11 Food Eaten	Rat No. 11 Weight Gained	Rat No. 12 Food Eaten	Rat No. 12 Weight Gained	Rat No. 13 Food Eaten	Rat No. 13 Weight Gained	Rat No. 14 Food Eaten	Rat No. 14 Weight Gained
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
4/8-12	49.5	4.5	50.0	5.0	50.0	2.7	49.0	1.9
4/12-18	240.5	14.5	238.5	19.5	238.5	17.0	243.0	10.5
4/19-25	360.0[a]	17.5	351.0[a]	-2.0	340.0[a]	16.0	348.0[a]	12.5
4/26-5/2	275.0[a] 74.0[b]	8.5	273.5[a] 75.0[b]	9.0	271.0[a] 74.5[b]	11.5	260.0[a] 75.0[b]	7.0
5/3-9	281.5[b]	2.0	272.5[b]	4.5	236.0[b]	0.5	276.5[b]	1.5
5/10-16	178.0[b]	1.5	294.0[b]	-4.5	174.0[b]	- 0.5	233.5[b]	0.0
5/17-23	183.0[b]	-2.0	131.5[b]	2.5	129.5[b]	-13.5	165.0[b]	6.5
5/24-30	34.0[b] 22.5[c]	-7.5	33.5[b] 19.0[c]	-1.5	32.0[b] 17.5[c]	- 5.0	38.5[b] 16.5[c]	-11.0
5/31-6/6	24.0[c]	-3.5	15.0[c]	-8.5	17.0[c]	- 3.0	17.0[c]	- 7.0
6/7-13	69.0[b] 4.0[c]	-8.0	77.0[b] 5.5[c]	-7.0	98.0[b] 6.5[c]	- 6.0	80.0[b] 25.0[c]	4.0
6/14-20	116.0[b] 0.0[c]	-9.0	207.0[b] 1.5[c]	4.0	197.0[b] 3.0[c]	- 2.0	176.0[b] 3.0[c]	- 4.0
Total	925.0[a] 935.5[b] 50.5[c]	18.5	913.0[a] 1090.5[b] 41.0[c]	21.0	889.5[a] 941.0[b] 44.0[c]	17.7	900.5[a] 1044.5[b] 61.5[c]	21.9
Initial Weight		56.5		56.0		56.3		57.1
Terminal Weight		75.0		77.0		74.0		79.0
Mean Food Eaten 907.0[a]; 1002.87[b]; 51.75[c]			Mean Weight Gained 19.77			Mean Terminal Weight 76.7		

[a]Unstrained wet soy curd
[b]Strained wet soy curd
[c]Strained dry soy curd, fine
[d]Paralyzed May 30, 1966

weekend. The paralysis prevented him from reaching water and food a part of this time. Water and food were placed so the animal could reach them from his reclining position. He soon began to improve and was able to get around by using his two front feet. At the end of the experiment he was using all four feet. The two hind feet were still a little shaky. He climbed over the entire cage and remained active the last two weeks of the experiment. The fur of all the soybean curd eaters was the softest of any animals in the experiment. After dry soybean curd was offered, the fur did not seem as attractive as it did during the first part of the experiment.

In the Experimental Group IV, animal no. 20 was the largest. He had consumned 1116.0 grams of food and weighed 428.0 grams. The smallest, animal no. 17, ate 1017.0 grams of food and weighed 357 grams. See Table 4. This animal turned over his water upon two occasions with the result that his growth declined.

These animals grew very rapidly, and exceeded the Control Group by approximately 100 grams. The diet fed to these animals was adequate in all known nutrients needed by the rat with the exception of calcium. Supplements of Brewer's Yeast, Osborne and Mendel Salt Mixture and Cod Liver Oil were included in addition to casein, cornstarch, and soybean oil. These animals were not active and slept a great deal. Their front teeth were quite yellow in color while the control animals had white teeth. The fur of these animals was rougher than the fur of the control rats.

On May 19 four animals were randomly selected, two from Experimental Group IV, no. 15 and 18, and two from the Control Group I, no. 4 and 5.

TABLE 4 Weekly food eaten and weight gained by rats in Experimental Group IV

	Rat No. 15		Rat No. 16		Rat No. 17		Rat No. 18		Rat No. 19		Rat No. 20	
Date	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained	Food Eaten	Weight Gained
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
4/8-12	23.5	21.3	28.0	24.1	28.5	34.4	27.5	21.5	29.5	32.5	31.0	26.5
4/12-18	72.5	39.5	77.5	7.5	77.0	41.5	72.0	46.5	79.8	44.5	84.5	51.0
4/19-25	67.5	24.5	101.5	79.5	101.5	40.0	87.5	33.0	87.0	40.5	90.5	38.5
4/26-5/2	86.6	37.0	109.5	44.0	115.5	43.5	97.0	42.0	104.0	49.5	125.0	63.5
5/3-9	100.5	39.5	95.5	29.0	105.5	35.0	96.5	32.0	95.5	27.5	115.0	37.5
5/10-16	103.5	39.5	115.0	40.0	119.0	32.0	118.5	31.5	109.0	24.0	132.0	41.5
5/17-23	53.0[a]	9.0	103.0[b]	26.0	104.5[b]	7.0	52.0[a]	9.0	106.5	38.0	119.0	33.0
5/24-30			86.6	24.0	91.5	31.0			94.5	18.0	100.5	23.0
5/31-6/6			99.5	23.0	101.0	18.0			90.0	11.0	107.5	24.0
6/7-13			100.0	11.0	89.0	7.0			92.0	18.0	106.0	15.0
6/14-20			106.0	14.0	84.0	14.0			85.0	17.0	105.0	18.0
Total	507.0	210.3	1022.0	322.1	1017.0	303.5	551.0	215.5	972.8	320.5	1116.0	371.5
Initial Wt.		56.7		55.9		53.5		53.5		56.5		56.5
Terminal Wt.		267.0		378.0		357.0		269.0		377.0		428.0

Mean Food Eaten 1031.9 Mean Weight Gained 329.37 Mean Terminal Weight 385.0

[a]Sacrificed May 19, 1966

[b]Received diet adequate in all nutrients except calcium, dry finely ground soybean curd added in amount to supply calcium need.

These four rats were anesthetized, examined internally, and compared. The liver of animals no. 15 and 18 was almost twice as large as the liver of animals no. 4 and no. 5. The liver of animals no. 15 and 18 was lighter in color, granular, and had more fat around it than the organs of the control rats. The ribs of the animals in Experimental Group IV had twice as much space between them as the ribs in the Control Group I.

A line test was made on the long bones of the hind leg of each animal. This test revealed that there was more calcified areas exhibited in bones of rats in Control Group I than in the animals in Experimental Group IV.

Analysis was again performed on one animal from each group at termination of the experiment. Animals randomly selected were no. 3 and no. 19. Results were similar to analyses run May 19. The liver of animal no. 19 was 1½ times as large as the liver of the control animal. Organs of animal no. 19 had much fat. The line test revealed a definite line in the control animal and an indefinite line in experimental animal no. 19.

In the one month of feeding soybean curd as the only source of calcium, there was little evidence of further calcification as revealed in the line test.

CHAPTER V

SUMMARY AND CONCLUSIONS

Weanling albino rats were used as experimental animals in a study designed to determine the biological value of soybean curd as a source of protein and calcium. The effectiveness of soybean curd as the only source of food for the rats was also tested for its biological value.

A Control Diet I, containing all known nutrients needed by the rats, was fed to six weanling animals ad libitum. Experimental Diet II was adequate in all nutrients except protein. Four animals were fed this diet until they had lost one-fourth their initial weight. When protein depletion was identified, soybean curd was added to this experimental ration in sufficient amounts to supply the necessary quantity of protein. Experimental Diet III was composed of nothing but tofu and was fed ad libitum to four weanling rats. A Low Calcium Diet was fed to six animals for six weeks. At this time two control animals and two animals depleted of calcium were sacrificed and a line test performed on the long bones to demonstrate the degree of calcification which had taken place.

Three protein depleted animals increased their body weight fourfold when soybean curd was added to the Experimental Diet II. One animal in this group died after it had received tofu in the diet for 17 days. Soybean curd furnished enough protein to promote growth in white rats that had been depleted of this nutrient until they had lost one-fourth of their body weight.

Soybean curd, fed as the only source of food for four rats in Experimental Diet II for ten weeks, was found to be an incomplete food as these animals grew very little. However, all of the animals in this group lived for the duration of the experiment.

Experimental Diet IV, with soybean curd serving as a source of calcium, was found to be inadequate for four calcium depleted animals. From the line test performed on the long bones of the calcium deficient animals, it was possible to see that the calcified area was not as clear and definite as the same area in the control animals. Ribs of these animals were spaced rather far apart in comparison with the ribs of the control animals. Fur of the animals on the Low Calcium Diet was rougher than that of the Control Diet I animals. Although the author detected a slight difference after the feeding of dry soy curd as a source of calcium for one month, the change for the better was very slight.

The front teeth of the Low Calcium Diet rats were quite yellow as compared to the white teeth of the Control Diet animals. The final weight of the animals on Experimental Diet IV exceeded that of the Control Diet I animals, but the line test, and other evidences of poor calcium utilization, indicated that soybean curd was inadequate as a source of calcium during four weeks of feeding.

Findings from animal feeding experiments are often stimulating and can lead to new suppositions and inspirations. Since growth of animals on the Low Calcium Diet exceeded that of the control animals, the author believes a comparison of several low calcium diets fed to deplete weanling rats would make a revealing study. A longer experimental feeding period than ten weeks is advocated for calcium studies.

The whole, mature soybean and this bean in the early stages of growth are also suggested as subjects of animal feeding experiments to determine the biological value of these products. Observations made during the present study have caused the writer to believe that the whole soybean is more nutritious than the tofu or soybean curd. Thus, more information could be obtained about the potentiality of the soybean as food for animals and man.

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TABLE A Daily record of animal feeding Control Group I

Rat No. 1

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks	Temperature
	gm.	gm.	gm.	gm.	gm.		degrees
4/8	59.6	15.0				Notch - right ear	74
4/9		25.0	7.2	1.0	6.8	Healthy Looking	72
4/10-11		15.0	4.5	2.0	19.5		74
4/12	65.0	15.0	8.5	0.5	6.0	Clean	74
4/13		15.0	4.5	0.5	10.0		74
4/14		15.0	5.5	0.5	9.0		74
4/15		15.0	5.0	0.5	9.5		76
4/16-17	81.5	30.0	6.0	0.5	8.5		78
4/18		15.0	4.0	2.0	24.0	Sexual organs developing	78
4/19	92.5	15.0	4.5	0.5	10.0		80
4/20		15.0	7.0	0.5	7.5		74
4/21		15.0	6.0	0.5	8.5		74
4/22		15.0	4.0	0.0	11.0		76
4/23-24	107.0	40.0	2.0	0.0	13.0		76
4/25		20.0	9.0	2.0	29.0	Drank most of the water	76
4/26	120.0	20.0	6.0	1.0	13.0	Doubled weight in 19 days	76
4/27		20.0	7.5	0.5	12.0		78
4/28		20.0	9.0	0.0	11.0		76
4/29		20.0	8.0	0.5	11.5		74
4/30-5/1	138.5	40.0	8.0	0.5	11.5		74
5/2		20.0	25.0	5.0	10.0	Knocked over water	76
5/3	139.0	20.0	6.0	1.0	13.0		78
5/4		20.0	5.5	1.0	13.5		78
5/5		20.0	6.5	0.5	13.0	Lost some hair	79
5/6		20.0	6.0	1.0	13.0		82
5/7-8	151.5	40.0	8.0	0.5	11.5		84
5/9		20.0	7.5	1.0	31.5		74
5/10	173.5	20.0	4.5	0.5	15.0		84
5/11		20.0	4.0	0.0	16.0		78
5/12		20.0	6.0	0.5	13.5		74
5/13		20.0	8.0	0.5	11.5		74
5/14-15	188.0	50.0	1.5	0.5	18.0		76
5/16		25.0	25.5	2.0	22.5		80
5/17	190.0	25.0	11.0	0.0	14.0		80
5/18		25.0	9.0	0.0	16.0		70
5/19		25.0	11.0	0.0	14.0		74
5/20		25.0	8.0	1.0	16.0		78

TABLE A Daily record of animal feeding Control Group I (Cont.)

Rat No. 1

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks	Temperature
	gm.	gm.	gm.	gm.	gm.		degrees
5/21-22	205.0	50.0	10.0	1.0	14.0		80
5/23		25.0	20.5	1.5	27.0	Knocked over water	80
5/24	210.0	25.0	5.0	1.0	19.0		84
5/25		25.0	13.5	0.5	11.0		78
5/26		25.0	8.0	0.5	16.5		78
5/27		25.0	13.0	0.0	12.0		80
5/28-29	223.5	50.0	17.0	0.0	8.0		86
5/30		25.0	10.0	1.0	39.0		84
5/31	234.0	25.0	11.0	0.0	14.0		82
6/1		25.0	8.0	0.0	17.0		82
6/2		25.0	9.0	0.0	16.0		78
6/3		25.0	8.0	0.0	17.0		82
6/4-5	239.0	50.0	12.0	0.0	13.0		80
6/6		25.0	18.0	2.0	30.0	Bloody discharge	76
6/7	250.0	25.0	11.0	0.0	14.0		76
6/8		25.0	7.0	0.0	18.0		80
6/9		25.0	8.0	0.0	17.0		74
6/10		25.0	7.0	0.0	18.0		76
6/11-12	263.0	50.0	9.0	1.0	15.0		82
6/13		25.0	16.0	1.0	33.0		84
6/14	261.0	20.0	10.0	2.0	15.0		86
6/15		20.0	9.0	0.0	11.0		88
6/16		20.0	6.0	0.0	14.0		80
6/17		20.0	5.0	0.0	15.0		82
6/18-19	269.0	40.0	6.0	0.0	14.0		82
6/20	276.0	----	8.0	0.0	32.0		80
Total					962.8	15½" long 5¾" rib case	

TABLE A Daily record of animal feeding Control Group I (Cont.)

Rat No. 2

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	56.9	15.0				Notch - left ear
4/9		25.0	9.1	0.8	5.1	
4/10-11		15.0	10.0	0.0	15.0	Healthy looking
4/12	60.0	15.0	7.5	1.0	6.5	
4/13		15.0	4.5	1.5	9.0	
4/14		15.0	8.5	0.5	6.0	
4/15		15.0	7.5	0.5	7.0	Diarrhea
4/16-17	70.0	30.0	6.5	1.0	7.5	
4/18		15.0	5.5	3.0	21.5	
4/19	80.5	15.0	2.0	3.0	10.0	Scab on his nose
4/20		15.0	2.5	0.5	12.0	
4/21		15.0	4.0	2.0	9.0	
4/22		15.0	6.5	1.0	7.5	
4/23-24	97.5	40.0	2.0	0.5	12.5	
4/25		15.0	16.5	1.0	22.5	
4/26	112.5	20.0	1.5	1.0	12.5	Almost doubled weight in 19 days
4/27		20.0	5.0	4.0	11.0	
4/28		20.0	5.0	0.5	14.5	
4/29		20.0	10.0	0.5	9.5	
4/30-5/1	133.0	40.0	4.0	1.0	15.0	
5/2		20.0	8.0	2.0	30.0	
5/3	146.0	20.0	3.0	2.0	15.0	
5/4		20.0	5.5	1.5	13.0	
5/5		20.0	3.0	1.0	16.0	
5/6		20.0	5.0	1.5	13.5	
5/7-8	157.5	40.0	5.5	2.0	12.5	
5/9		20.0	8.0	7.0	25.0	
5/10	170.5	20.0	3.0	1.0	16.0	
5/11		25.0	3.0	0.5	16.5	
5/12		20.0	12.5	1.5	11.0	
5/13		20.0	8.0	0.0	12.0	
5/14-15	193.0	50.0	0.5	0.5	19.0	
5/16		25.0	27.0	2.0	21.0	
5/17	196.0	25.0	9.0	0.0	16.0	
5/18		30.0	2.0	0.0	23.0	
5/19		25.0	14.0	1.0	15.0	
5/20		25.0	6.0	1.0	18.0	
5/21-22	218.0	50.0	8.0	2.0	15.0	
5/23		25.0	25.0	0.5	24.5	Knocked over water
5/24	218.0	25.0	8.0	0.0	17.0	
5/25		25.0	6.5	2.5	16.0	
5/26		25.0	4.5	1.0	19.5	
5/27		25.0	9.0	1.5	14.5	

TABLE A Daily record of animal feeding Control Group I (Cont.)

Rat No. 2

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/28-29	234.0	50.0	20.0	0.5	4.5	
5/30		25.0	10.0	0.5	39.5	
5/31	249.0	25.0	10.0	1.0	14.0	
6/1		25.0	4.0	2.0	19.0	
6/2		25.0	7.0	2.0	16.0	
6/3		25.0	18.0	2.0	5.0	Spilled water
6/4-5	260.0	50.0	10.0	0.0	15.0	
6/6		25.0	8.0	3.0	39.0	
6/7	264.0	25.0	9.0	1.0	15.0	
6/8		25.0	6.0	1.0	18.0	
6/9		25.0	11.0	1.0	13.0	
6/10		25.0	12.0	2.0	11.0	
6/11-12	274.0	50.0	20.0	0.0	5.0	
6/13	272.0	----	43.0	4.0	3.0	
Total					829.6	15½" long. Died during the course of the weekend. Bloody nose. Evidence of diarrhea. Believe he died early in weekend. Possibly on Saturday.

TABLE A Daily record of animal feeding Control Group I (Cont.)

Rat No. 3

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	56.5	15.0				Notch - right ear
4/9		25.0	8.6	2.0	4.4	Red color
4/10-11		15.0	10.0	1.0	14.0	
4/12	60.0	15.0	8.5	1.0	5.5	
4/13		15.0	7.0	1.0	7.0	
4/14		15.0	8.0	0.1	6.9	
4/15		15.0	7.5	1.0	6.5	
4/16-17	69.5	30.0	6.0	1.5	7.5	
4/18		15.0	10.0	3.0	17.0	
4/19	76.5	15.0	6.5	1.0	7.5	
4/20		15.0	3.5	1.5	10.0	
4/21		15.0	7.5	1.5	6.0	
4/22		15.0	5.5	1.5	8.0	
4/23-24	90.5	40.0	1.0	1.5	12.5	
4/25		15.0	15.5	2.0	22.5	
4/26	101.0	20.0	4.0	2.0	9.0	
4/27		20.0	7.0	2.0	11.0	
4/28		20.0	5.0	3.0	12.0	
4/29		20.0	8.0	1.0	11.0	
4/30-5/1	118.0	40.0	3.0	5.0	12.0	
5/2		20.0	0.0	15.0	25.0	
5/3	129.5	20.0	2.5	1.0	16.5	
5/4		20.0	4.5	1.5	14.0	
5/5		20.0	4.0	1.5	14.5	
5/6		20.0	3.0	3.0	14.0	
5/7-8	141.5	40.0	4.0	1.5	14.5	
5/9		20.0	6.0	7.0	27.0	
5/10	158.5	25.0	1.5	1.0	17.5	
5/11		25.0	4.0	2.0	19.0	
5/12		20.0	11.5	1.0	12.5	Likes more water than most of others.
5/13		20.0	4.5	0.5	15.0	
5/14-15	175.0	50.0	0.5	1.5	18.0	
5/16		25.0	18.0	1.5	30.5	
5/17	190.0	25.0	8.5	2.0	14.5	
5/18		25.0	3.0	3.0	19.0	
5/19		20.0	8.5	5.0	11.5	
5/20		25.0	1.0	2.0	17.0	
5/21-22	203.0	50.0	3.0	9.0	13.0	
5/23		25.0	13.0	9.0	29.0	Knocked over water
5/24	212.0	25.0	5.0	7.0	12.0	
5/25		25.0	9.0	1.0	15.0	
5/26		25.0	1.0	2.0	22.0	
5/27		25.0	1.5	8.0	15.5	

TABLE A Daily record of animal feeding Control Group I (Cont.)

Rat No. 3

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/28-29	230.0	50.0	8.5	3.0	13.5	
5/30		25.0	9.0	3.5	37.5	
5/31	242.0	25.0	1.0	5.0	19.0	
6/1		25.0	1.0	5.0	19.0	
6/2		25.0	5.0	2.0	18.0	
6/3		25.0	1.0	4.0	20.0	
6/4-5	258.0	50.0	8.0	2.0	15.0	
6/6		25.0	13.0	2.0	35.0	
6/7	269.0	25.0	4.0	3.0	18.0	
6/8		25.0	4.0	4.0	17.0	
6/9		25.0	5.0	3.0	18.0	
6/10		25.0	4.0	2.0	19.0	
6/11-12	277.0	25.0	7.0	2.0	16.0	
6/13		25.0	11.0	3.0	36.0	
6/14	281.0	20.0	9.0	2.0	14.0	
6/15		20.0	3.0	2.0	15.0	
6/16		25.0	1.0	1.0	18.0	
6/17		25.0	10.0	3.0	12.0	Knocked over water
6/18-19	300.0	50.0	7.0	0.0	13.0	
6/20	298.0	----	5.0	4.0	41.0	Knocked over water
Total					1010.8	5½" rib case 15¾" long Randomly selected for line bone test.

TABLE A Daily record of animal feeding Control Group I (Cont.)

Rat No. 4

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	56.6	15.0				Notch - left ear Red color
4/9		25.0	10.0	0.5	4.5	
4/10-11		15.0	8.0	2.0	15.0	
4/12	58.0	15.0	6.5	2.0	7.5	
4/13		15.0	7.0	2.0	6.0	Wets in his feeding trough
4/14		15.0	7.0	1.0	7.0	
4/15		15.0	8.0	1.0	6.0	Selecting and discarding components of the diet.
4/16-17	64.5	30.0	8.5	0.5	6.0	
4/18		15.0	12.0	2.0	16.0	
4/19	78.0	15.0	5.0	1.0	9.0	
4/20		15.0	2.0	1.0	12.0	
4/21		15.0	3.5	0.5	11.0	
4/22		15.0	4.5	0.5	10.0	
4/23-24	88.5	40.0	10.0	0.5	4.5	
4/25		15.0	15.0	3.0	22.0	
4/26	97.5	20.0	4.5	0.5	15.0	
4/27		20.0	9.0	0.5	10.5	
4/28		20.0	4.0	2.0	14.0	
4/29		20.0	11.0	1.0	8.0	
4/30-5/1	114.0	40.0	5.5	0.5	14.0	
5/2		20.0	13.5	0.5	26.0	
5/3	121.0	20.0	8.5	0.5	11.0	
5/4		20.0	7.5	0.5	12.0	
5/5		20.0	5.5	1.0	13.5	
5/6		20.0	6.0	0.5	13.5	
5/7-8	138.0	40.0	8.0	0.5	11.5	
5/9		20.0	14.5	2.0	23.5	
5/10	150.0	25.0	4.0	1.0	15.0	
5/11		25.0	8.5	0.5	16.0	
5/12		20.0	15.0	0.5	9.5	
5/13		20.0	4.5	0.5	15.0	
5/14-15	170.0	50.0	2.5	0.5	17.0	
5/16		25.0	21.0	1.5	28.5	
5/17	179.0	25.0	9.5	0.0	15.5	16" long
5/18		25.0	8.0	0.0	12.0	5" rib case
5/19	182.0	-----	6.0	1.0	18.0	Sacrificed 2:45 p.m.
Total					455.5	

TABLE A Daily record of animal feeding Control Group I (Cont.)

Rat No. 5

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	58.6	15.0				Notch in right ear Yellow color
4/9		25.0	9.0	0.5	5.5	
4/10-11		15.0	11.5	0.5	13.0	
4/12	64.0	15.0	8.0	2.5	4.5	
4/13		15.0	4.0	3.0	8.0	
4/14		15.0	6.5	0.5	8.0	
4/15		15.0	2.5	1.0	11.5	
4/16-17	76.0	30.0	6.0	0.5	8.5	
4/18		15.0	8.0	2.0	20.0	
4/19	92.0	15.0	3.0	1.0	11.0	
4/20		15.0	4.0	0.5	10.5	
4/21		15.0	3.5	1.0	10.5	
4/22		15.0	4.5	1.5	9.0	
4/23-24	101.5	40.0	4.0	1.0	10.0	
4/25		15.0	16.0	1.0	23.0	Empty water container
4/26	118.5	20.0	0.0	0.0	15.0	Doubled weight in 19 days
4/27		20.0	7.0	0.5	12.5	
4/28		25.0	2.5	0.5	17.0	
4/29		20.0	12.5	0.5	12.0	
4/30-5/1	138.0	40.0	4.5	0.5	15.0	
5/2		20.0	18.0	1.0	21.0	
5/3	143.0	20.0	8.0	0.5	11.5	
5/4		20.0	7.5	0.5	12.0	
5/5		20.0	6.5	1.0	12.5	
5/6		20.0	10.0	0.5	9.5	
5/7-8	154.0	40.0	12.5	0.5	7.0	
5/9		20.0	17.5	1.0	21.5	
5/10	163.5	20.0	5.0	0.0	15.0	
5/11		25.0	3.0	0.0	17.0	
5/12		20.0	16.5	0.0	8.5	
5/13		20.0	5.5	0.5	14.0	
5/14-15	187.0	50.0	0.5	0.5	19.0	
5/16		25.0	25.0	0.5	24.5	
5/17	199.0	25.0	7.5	0.0	17.5	18" long
5/18		30.0	2.0	0.0	23.0	5¾" rib case
5/19	213.0	----	9.0	0.5	20.5	Sacrificed 2:45 p.m.
Total					478.5	

TABLE A Daily record of animal feeding Control Group I (Cont.)

Rat No. 6

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	58.2	15.0				
4/9		25.0	6.5	0.2	8.3	Notch - left ear Yellow color
4/10-11		15.0	10.0	0.0	15.0	Very clean
4/12	63.0	15.0	8.0	0.0	7.0	Very clean
4/13		15.0	8.5	0.5	6.0	Diarrhea
4/14		15.0	5.5	0.5	9.0	No diarrhea
4/15		15.0	5.0	0.5	8.5	As a group the more
4/16-17	77.0	30.0	7.5	0.5	7.0	granular portions
4/18		15.0	6.0	2.0	22.0	of diet are left
4/19	85.0	15.0	5.5	1.0	8.5	which indicates
4/20		15.0	7.0	0.5	7.5	selection of compon-
4/21		15.0	2.0	0.5	12.5	ents
4/22		15.0	2.5	1.0	11.5	
4/23-24	105.5	40.0	2.0	1.0	12.0	
4/25		20.0	3.0	8.0	29.0	
4/26	120.5	20.0	4.0	2.0	14.0	Doubled weight in 19 days
4/27		20.0	4.5	2.5	13.0	
4/28		25.0	1.0	2.0	17.0	
4/29		20.0	12.0	2.0	11.0	
4/30-5/1	142.0	40.0	2.0	1.0	17.0	
5/2		20.0	8.5	4.5	27.0	Turned over water
5/3	112.0	20.0	18.0	1.5	0.5	No feces. Lost weight
5/4		20.0	8.0	0.5	11.5	
5/5		20.0	0.5	3.5	16.0	
5/6		25.0	0.5	4.0	15.5	Shedding hair
5/7-8	147.5	50.0	6.0	3.0	14.0	
5/9		20.0	10.0	15.0	25.0	
5/10	160.0	25.0	2.5	3.0	14.5	
5/11		25.0	6.5	1.5	17.0	
5/12		20.0	7.5	3.5	14.0	
5/13		20.0	2.5	4.0	13.5	Bloody discharge
5/14-15	175.0	50.0	0.0	6.0	14.0	
5/16		25.0	25.0	2.0	23.0	
5/17	190.0	25.0	14.0	1.0	10.0	
5/18		30.0	1.0	4.0	20.0	
5/19		25.0	3.0	9.5	17.5	
5/20		25.0	6.0	8.0	11.0	
5/21-22	201.0	50.0	1.0	7.0	17.0	
5/23		25.0	12.0	6.0	22.0	
5/24	206.0	25.0	1.0	7.0	17.0	
5/25		25.0	5.0	4.0	16.0	
5/26		25.0	0.0	11.0	14.0	

TABLE A Daily record of animal feeding Control Group I (Cont.)

Rat No. 6

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/27		25.0	0.5	8.5	16.0	
5/28-29	219.5	50.0	8.0	8.5	8.5	
5/30		25.0	3.5	2.5	43.0	
5/31	229.0	25.0	1.0	4.0	20.0	
6/1		25.0	1.0	5.0	19.0	
6/2		25.0	8.0	4.0	13.0	
6/3		20.0	6.0	5.0	14.0	
6/4-5	237.0	50.0	5.0	2.0	13.0	
6/6		20.0	7.0	5.0	38.0	
6/7	246.0	25.0	1.0	5.0	19.0	
6/8		25.0	0.0	8.0	17.0	
6/9		25.0	0.0	8.0	17.0	
6/10		30.0	0.0	3.0	22.0	
6/11-12	260.0	50.0	8.0	6.0	16.0	
6/13		25.0	14.0	5.0	31.0	
6/14	266.0	20.0	10.0	2.0	13.0	Knocked over water twice. Filled at 5 p.m. Empty next night.
6/15		20.0	3.0	2.0	15.0	
6/16		25.0	1.0	2.0	17.0	
6/17		25.0	5.0	7.0	14.0	
6/18-19	277.0	50.0	7.0	8.0	10.0	
6/20	248.0	----	19.0	5.0	26.0	Must have knocked over water on Saturday.
Total					987.3	6" rib case 15" long

TABLE B Daily record of animal feeding--Protein Deficient Diet--Experimental Group II

Rat No. 7

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	55.0	15.0				Notch - right ear Green color
4/9		25.0	7.0	3.0	5.0	
4/10-11		15.0	11.0	3.0	14.0	
4/12	52.0	15.0	5.0	6.0	4.0	
4/13		15.0	6.0	3.5	5.5	No fecal dropping to speak of
4/14		15.0	7.0	2.0	6.0	
4/15	47.0	15.0	6.5	3.0	5.5	
4/16-17	47.5	25.0	6.0	3.5	5.5	
4/18	50.0	15.0	5.0	6.0	14.0	
4/19	48.0	15.0	10.0	1.5	3.5	
4/20	46.5	15.0	10.0	0.5	4.5	One dropping
4/21	45.5	15.0	10.0	0.5	4.5	
4/22	45.5	15.0	10.5	1.0	3.5	
4/23-24	45.0	30.0	10.0	1.0	4.0	
4/25	42.0	15.0*	18.0	5.0	7.0	Tail has red blotches on it.
4/26	47.5	15.0	7.0	1.0	7.0	Depleted
4/27	50.5	15.0	8.5	0.0	6.5	New diet and dry soy
4/28	53.5	15.0	6.5	1.0	7.5	
4/29	54.0	15.0	4.0	1.0	10.0	
4/30-5/1	55.0	30.0	1.0	6.5	7.5	
5/2	52.5	15.0	13.0	10.0	7.0	
5/3	54.0	15.0	7.5	3.0	4.5	
5/4	53.5	15.0	6.5	1.0	7.5	Bloody discharge
5/5	52.5	15.0	6.0	2.0	7.0	
5/6	54.5	15.0	8.0	3.0	4.0	
5/7-8	55.5	30.0	9.0	1.0	5.0	
5/9	58.0	15.0	2.5	6.0	21.5	
5/10	61.0	15.0	5.0	3.0	7.0	Bloody discharge
5/11	65.5	15.0	10.0	0.0	5.0	
5/12	65.0	15.0	13.0	0.0	2.0	
5/13	67.5	15.0	9.5	2.5	4.0	
5/14	67.0	----	14.5	0.0	0.5	Died
Total					200.1	

TABLE B Daily record of animal feeding--Protein Deficient Diet--
Experimental Group II (Cont.)

Rat No. 8

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	60.0	15.0				Notch - left ear Green color
4/9		25.0	3.5	7.0	4.5	
4/10-11		15.0	0.0	14.5	10.5	Wasteful
4/12	55.0	15.0	3.0	7.0	5.0	Wasteful
4/13		15.0	5.5	4.0	5.5	Wasteful
4/14		15.0	5.0	4.0	6.0	
4/15	48.5	15.0	6.0	6.0	3.0	
4/16-17	48.0	25.0	5.5	5.5	3.0	
4/18	48.0	15.0	16.0	2.0	7.0	Four droppings
4/19	48.0	15.0	9.5	1.0	4.5	
4/20	46.5	15.0	7.5	1.0	6.5	
4/21	46.5	15.0*	10.0	0.5	4.5	Depleted *Modified diet IV offered ad libitum.
4/22	48.5	15.0	9.5	0.5	5.0	
4/23-24	46.0	30.0	12.0	1.0	2.0	
4/25	47.0	15.0	15.5	7.0	7.5	
4/26	47.5	15.0	4.0	8.0	3.0	
4/27	46.5	15.0	10.0	1.0	4.0	
4/28	46.0	15.0	0.0	11.0	4.0	
4/29	46.0	15.0	6.0	2.0	7.0	
4/30-5/1	46.0	30.0	7.0	1.5	6.5	
5/2	52.5	20.0	7.5	8.5	14.0	
5/3	50.5	20.0	11.5	1.5	7.0	
5/4	57.5	20.0	4.5	0.5	15.0	
5/5	54.0	20.0	4.5	0.5	15.0	
5/6	55.0	20.0	6.0	3.5	10.5	Bloody discharge
5/7-8	55.0	30.0	8.5	6.0	5.5	
5/9	55.0	15.0	14.5	5.0	10.5	
5/10	57.5	15.0	6.0	2.5	6.5	
5/11	59.0	15.0	7.5	2.0	5.5	
5/12	64.5	15.0	6.0	2.5	6.5	
5/13	71.0	15.0	5.0	0.0	10.0	
5/14-15	79.0	30.0	3.0	1.0	11.0	
5/16	84.5	15.0	13.0	1.0	16.0	
5/17	87.5	15.0	8.0	1.0	6.0	
5/18	92.5	15.0	4.5	0.5	10.0	
5/19	95.0	15.0	7.0	1.0	7.0	
5/20	96.5	15.0	6.0	0.0	9.0	
5/21-22	100.0	30.0*	6.0	1.0	8.0	Begin blending soy bean curd.
5/23	106.0	15.0	9.5	1.5	19.0	Sex organs developing

TABLE B Daily record of animal feeding--Protein Deficient Diet--Experimental Group II (Cont.)

Rat No. 8

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/24	109.0	15.0	4.0	1.0	10.0	
5/25	107.0	15.0	8.5	0.5	6.0	
5/26	117.0	20.0	1.0	0.0	14.0	
5/27	119.5	20.0	8.5	0.5	11.0	
5/28-29	124.0	40.0	13.0	0.0	7.0	
5/30	128.0	20.0	12.0	3.0	25.0	
5/31	131.0	20.0	9.5	1.0	9.5	
6/1	139.0	20.0	13.0	2.0	5.0	
6/2	137.0	20.0	14.0	1.0	5.0	
6/3	139.0	15.0	15.0	1.0	4.0	
6/4-5	143.0	30.0	10.0	1.0	4.0	
6/6	147.0	15.0	11.0	2.0	17.0	
6/7	148.0	15.0	6.0	0.0	9.0	
6/8	148.0	15.0	4.0	1.0	10.0	
6/9	150.0	15.0	4.0	1.0	10.0	
6/10	156.0	20.0	1.0	0.0	14.0	
6/11-12	159.0	40.0	9.0	0.0	11.0	
6/13	183.0	20.0	8.0	0.0	32.0	
6/14	184.0	20.0	8.0	1.0	11.0	
6/15	194.0	20.0	6.0	1.0	13.0	
6/16	197.0	20.0	4.0	3.0	13.0	
6/17	195.0	20.0	10.0	1.0	8.0	
6/18	195.0	40.0	10.0	0.0	10.0	Knocked over water
6/20	200.0	----	9.0	2.0	29.0	
Total					578.5	5½" rib case 13½" long Soft fur Active

TABLE B Daily record of animal feeding--Protein Deficient Diet--
Experimental Group II (Cont.)

Rat No. 9

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	60.5	15.0				Notch in left ear Blue color
4/9		25.0	1.5	9.0	4.5	
4/10-11		15.0	9.0	3.0	13.0	
4/12	56.0	15.0	9.0	3.0	3.0	
4/13		15.0	7.0	4.0	4.0	One dropping
4/14		15.0	9.0	1.0	5.0	
4/15	53.0	15.0	7.0	2.5	5.5	Few droppings
4/16-17	52.5	25.0	6.5	3.0	5.5	
4/18	51.0	15.0	6.5	7.0	11.5	
4/19	51.5	15.0	5.0	2.5	7.4	Four droppings
4/20	50.0	15.0	7.0	1.5	8.5	Very clean
4/21	49.5	15.0	9.5	1.5	4.0	
4/22	49.0	15.0	8.5	2.0	4.5	
4/23-24	49.0	30.0	8.0	2.0	5.0	
4/25	49.0	15.0	17.0	7.0	6.0	Scaby tail
4/26	48.0	15.0	9.5	2.0	4.0	
4/27	48.5	15.0	10.0	0.5	4.5	
4/28	48.0	15.0	10.5	0.0	4.5	
4/29	48.5	15.0	11.5	0.0	3.5	
4/30-5/1	49.0	30.0	7.0	1.5	6.5	
5/2	47.5	15.0	7.5	3.5	19.0	
5/3	47.5	15.0	11.0	0.5	3.5	
5/4	47.5	15.0	11.0	0.5	3.5	
5/5	46.0	15.0*	11.5	0.5	3.0	Depleted after 23 days
5/6	49.0	15.0	10.5	0.5	4.0	
5/7-8	52.0	30.0	11.0	0.5	3.5	
5/9	55.0	15.0	15.0	4.0	11.0	
5/10	55.5	15.0	7.0	0.5	7.5	
5/11	59.5	15.0	10.0	0.5	4.5	
5/12	63.5	15.0	7.5	0.5	7.0	
5/13	68.5	15.0	9.0	0.0	6.0	
5/14-15	74.0	30.0	7.0	0.0	8.0	
5/16	77.5	15.0	17.5	2.0	10.5	
5/17	79.0	15.0	9.0	0.5	5.5	
5/18	81.5	15.0	8.0	0.0	7.0	
5/19	82.5	15.0	8.5	0.0	6.5	
5/20	85.5	15.0	5.5	0.0	9.5	
5/21-22	94.0	30.0	6.0	1.0	8.0	
5/23	103.0	15.0	10.5	1.5	18.0	Sex organs developing
5/24	109.5	15.0	6.0	0.5	8.5	
5/25	115.0	15.0	4.0	0.5	10.5	
5/26	114.5	15.0	7.0	0.0	8.0	

TABLE B Daily record of animal feeding--Protein Deficient Diet--
Experimental Group II (Cont.)

Rat No. 9

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/27	115.0	15.0	9.0	0.5	5.5	
5/28-29	115.0	30.0	13.0	0.0	2.0	
5/30	123.0	20.0	7.0	1.0	22.0	Lost most of the hair on the stomach
5/31	121.0	20.0	11.0	1.0	8.0	
6/1	123.0	20.0	13.0	2.0	5.0	
6/2	124.0	20.0	12.0	1.0	7.0	
6/3	121.0	15.0	11.0	3.0	6.0	
6/4-5	124.0	30.0	10.0	0.0	5.0	
6/6	133.0	15.0	8.0	0.0	22.0	
6/7	135.0	15.0	3.0	3.0	9.0	
6/8	136.0	20.0	1.0	3.0	11.0	
6/9	139.0	20.0	2.0	4.0	14.0	
6/10	147.0	25.0	2.0	2.0	16.0	
6/11-12	149.0	50.0	4.0	3.0	18.0	Loosing more hair around the front paws
6/13	162.0	25.0	16.0	3.0	31.0	
6/14	157.0	20.0	13.0	4.0	8.0	Growing hair back
6/15	175.0	20.0	2.0	3.0	15.0	
6/16	175.0	20.0	3.0	2.0	15.0	Organs of all rats are covered with hair.
6/17	176.0	20.0	6.0	2.0	12.0	
6/18-19	180.0	40.0	3.0	2.0	15.0	
6/20	186.0	----	5.0	3.0	32.0	
Total					561.0	13" long 5¼" rib case Soft fur Active Grew back all the hair which was lost.

TABLE B Daily record of animal feeding--Protein Deficient Diet--
Experimental Group II (Cont.)

Rat No. 10

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	55.0	15.0				Notch in right ear Blue color
4/9		25.0	2.5	7.0	5.5	
4/10-11		15.0	8.0	3.0	14.0	
4/12	53.0	15.0	9.0	2.0	4.0	
4/13		15.0	9.5	1.5	4.0	More droppings
4/14		15.0	10.0	1.0	4.0	
4/15	49.0	15.0	8.0	1.0	6.0	
4/16-17	48.0	25.0	7.5	2.0	5.5	
4/18	47.5	15.0	2.0	13.5	9.5	
4/19	46.5	15.0	10.0	1.0	4.0	Very clean
4/20	46.0	15.0	9.0	0.5	5.5	
4/21	45.0	15.0	8.0	1.0	6.0	
4/22	46.0	15.0	10.0	0.5	4.5	
4/23-24	44.0	30.0	10.5	0.5	4.0	
4/25	44.0	15.0	21.5	1.0	7.5	
4/26	44.0	15.0	11.0	1.0	3.0	
4/27	43.0	15.0	11.0	0.5	3.5	
4/28	42.0	15.0*	10.5	0.5	4.0	Depleted. New diet containing dry soy-bean curd added.
4/29	45.0	15.0	7.5	0.5	8.0	
4/30-5/1	49.0	30.0	5.5	0.0	9.5	
5/2	53.5	15.0	9.0	6.0	15.0	
5/3	52.0	15.0	8.0	0.5	6.5	
5/4	50.5	15.0	6.5	0.5	8.0	
5/5	50.5	15.0	8.0	0.5	6.5	
5/6	51.5	15.0	9.0	2.0	4.0	
5/7-8	51.5	30.0	10.0	3.0	2.0	
5/9	50.5	15.0	19.5	3.0	7.5	
5/10	50.5	15.0	10.0	1.0	4.0	
5/11	50.5	15.0	11.5	1.0	2.5	
5/12	50.0	15.0	9.5	2.5	3.0	
5/13	56.0	15.0	8.5	0.0	6.5	
5/14-15	59.0	30.0	8.0	0.0	7.0	
5/16	61.0	15.0	15.0	7.5	7.5	
5/17	61.5	15.0	12.0	0.0	3.0	
5/18	61.0	15.0	5.0	2.0	8.0	
5/19	57.0	15.0	10.0	0.0	5.0	
5/20	60.0	15.0	6.0	1.0	8.0	
5/21-22	64.0	30.0	10.0	0.0	5.0	
5/23	75.0	15.0	4.0	9.0	17.0	
5/24	78.5	15.0	7.5	1.0	6.5	

TABLE B Daily record of animal feeding--Protein Deficient Diet--Experimental Group II (Cont.)

Rat No. 10

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/25	86.0	15.0	5.0	0.5	8.5	
5/26	89.0	15.0	4.5	0.5	10.0	
5/27	95.5	15.0	4.0	1.0	10.0	
5/28-29	97.0	30.0	10.5	0.0	4.5	
5/30	108.0	15.0	9.0	1.0	20.0	
5/31	112.5	15.0	5.0	1.0	9.0	
6/1	117.0	15.0	1.0	2.0	12.0	
6/2	119.0	15.0	7.0	3.0	5.0	
6/3	120.0	15.0	3.0	2.0	10.0	
6/4-5	116.0	30.0	8.0	1.0	6.0	
6/6	128.0	15.0	1.0	2.0	27.0	
6/7	131.0	20.0	1.0	1.0	13.0	
6/8	132.0	20.0	6.0	1.0	13.0	
6/9	134.0	20.0	5.0	1.0	14.0	
6/10	139.0	20.0	4.0	2.0	14.0	
6/11-12	141.0	40.0	11.0	1.0	8.0	
6/13	152.0	20.0	15.0	2.0	23.0	
6/14	151.0	20.0	14.0	1.0	10.0	
6/15	158.0	20.0	10.0	1.0	9.0	
6/16	162.0	20.0	4.0	2.0	14.0	
6/17	167.0	20.0	8.0	2.0	10.0	
6/18-19	168.0	40.0	15.0	0.0	5.0	
6/20	173.0	----	3.0	9.0	28.0	
Total					527.5	13½" long 4¾" rib case

TABLE C Daily record of animal feeding--Soybean Curd Diet--
Experimental Group III

Rat No. 11

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	56.5	15.0				Red tail mark distal
4/9		35.0	0.0	0.0	15.0	
4/10-11		20.0	0.0	0.5	34.5	
4/12	61.0	30.0	0.0	0.0	20.0	
4/13	62.0	35.0	0.0	2.0	28.0	
4/14		45.0	2.5	2.0	30.5	
4/15		45.0	8.5	1.0	35.5	
4/16-17	68.0	90.0	4.5	1.0	39.5	
4/18		45.0	0.0	3.0	87.0	
4/19	75.5	50.0	2.0	1.5	41.5	
4/20		55.0	0.0	1.0	49.0	
4/21		55.0	2.0	1.0	52.0	
4/22		55.0	2.0	0.5	52.5	
4/23-24	83.0	120.0	0.0	2.0	53.0	
4/25		60.0	7.0	1.0	112.0	
4/26	93.0	60.0	1.0	3.0	56.0	
4/27		60.0	1.0	3.0	56.0	
4/28		70.0	2.0	5.0	53.0	
4/29	100.0	70.0	2.0	8.0	60.0	
4/30-5/1	107.0	90.0	15.5	0.5	50.0[a]	Strained milk before appt. made
5/2		45.0	15.0	1.0	74.0[b]	softer curd. Dry the curd on paper towel to absorb some of the water.
5/3	101.5	50.0	10.0	1.0	34.0[b]	
5/4		50.0	12.0	1.0	37.0[b]	
5/5		50.0	3.5	4.0	42.5[b]	
5/6		45.0	4.0	4.0	42.0[b]	
5/7-8	101.5	100.0	5.0	0.0	40.0[b]	
5/9		50.0	5.0	9.0	86.0[b]	
5/10	103.5	50.0	14.5	0.0	35.5[b]	
5/11		50.0	19.0	0.0	31.0[b]	
5/12		50.0	11.0	3.0	36.0[b]	
5/13		50.0	13.5	1.0	35.5[b]	
5/14-15	110.5	75.0	35.0	1.0	14.0[b]	
5/16		40.0	48.0	1.0	26.0[b]	
5/17	105.0	40.0	14.0	1.0	25.0[b]	
5/18		40.0	13.0	2.0	25.0[b]	
5/19		40.0	2.0	6.0	32.0[b]	
5/20	100.0	40.0	4.0	3.0	33.0[b]	
5/21-22	100.0	60.0	9.0	2.0	29.0[b]	
5/23	103.0	30.0	17.0	4.0	39.0[b]	

TABLE C Daily record of animal feeding--Soybean Curd Diet--
Experimental Group III (Cont.)

Rat No. 11

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/24	101.0	15.0[c]	5.0	1.0	24.0[b]	
		10.0[b]				
5/25	99.5	15.0[c]	0.0[b]	0.0	10.0[b]	
			10.0[c]		5.0[c]	
5/26	98.0	15.0[c]	11.0	0.0	4.0[c]	
5/27	96.5	15.0[c]	12.0	0.0	3.0[c]	
5/28-29	97.5	25.0[c]	13.0	0.0	2.0[c]	
5/30	95.5	15.0[c]	16.0	0.5	8.5[c]	
5/31	98.0	15.0[c]	9.5	0.5	5.0[c]	
6/1	98.0	15.0[c]	10.0	0.5	4.5[c]	
6/2	96.0	15.0[c]	12.5	0.0	2.5[c]	
6/3	94.0	10.0[c]	13.0	0.0	2.0[c]	
6/4-5	94.0	20.0[c]	8.0	0.0	2.0[c]	
6/6	92.0	10.0[c]	12.0	0.0	8.0[c]	
6/7	90.0	10.0[c]	8.0	1.0	1.0[c]	
6/8	89.0	20.0[b]	7.0	1.0	2.0[c]	
		10.0[c]				
6/9	93.0	10.0[b]	10.0	1.0	19.0[b]	
		10.0[c]				
6/10	93.0	10.0[c]	9.0	0.0	10.0[b]	
					1.0[c]	
6/11-12	85.0	40.0[b]	10.0	0.0	0.0	Began eating wet curd at once.
		10.0[c]				
6/13	84.0	40.0[b]	10.0[c]	0.0	40.0[b]	
		10.0[c]				
6/14	90.0	17.0[b]	22.0[b]	1.0	17.0[b]	Lively
		10.0[c]	10.0[c]	0.0	0.0[c]	
6/15	90.0	40.0[b]	15.0[b]	5.0	20.0[b]	
		10.0[c]	10.0[c]	0.0	0.0[c]	
6/16	89.0	40.0[b]	25.0[b]	0.0	15.0[b]	
		10.0[c]	10.0[c]	0.0	0.0[c]	
6/17	88.0	40.0[b]	5.0[b]	0.0	35.0[b]	
			10.0[c]	0.0	0.0[c]	
6/18-19	84.0	32.0[b]	32.0[b]	0.0	8.0[b]	
6/20	75.0	----	10.0[b]	1.0	21.0[b]	
Total[a]					925.0	10½" long
Total[b]					935.5	4¼" rib case
Total[c]					50.5	

TABLE C Daily record of animal feeding--Soybean Curd Diet--
Experimental Group III (Cont.)

Rat No. 12

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	56.0	15.0				4/7 New rat - 56.0
4/9		35.0	0.0	0.0	15.0	Proximate Red tail mark
4/10-11		20.0	0.0	0.0	35.0	
4/12		30.0	0.0	0.0	20.0	Drinking very little water
4/13		35.0	0.0	0.0	30.0	
4/14		45.0	6.5	0.0	28.5	
4/15		45.0	12.5	1.0	31.5	
4/16-17	66.5	90.0	3.0	0.5	41.5	
4/18		45.0	1.0	2.0	87.0	
4/19	80.5	50.0	1.0	3.0	40.0	
4/20		55.0	2.5	1.0	46.5	
4/21		55.0	1.5	1.5	52.0	
4/22		60.0	1.0	1.0	53.0	
4/23-24	75.5	120.0	2.0	1.5	56.5	
4/25		60.0	0.0	17.0	103.0	
4/26	78.5	60.0	1.0	1.5	57.5	
4/27		60.0	2.0	2.0	56.0	
4/28		70.0	0.0	4.0	54.0	
4/29		70.0	9.0	4.0	56.0	
4/30-5/1	83.0	90.0	20.0	0.0	50.0[a]	
5/2		45.0	5.0	10.0	75.0[b]	
5/3	87.5	50.0	6.5	1.0	37.5[b]	
5/4		50.0	9.0	4.0	37.0[b]	
5/5		50.0	7.0	2.0	41.0[b]	
5/6		45.0	6.0	7.0	37.0[b]	Coat is softer than any of the other groups
5/7-8	88.5	100.0	7.5	0.5	37.0[b]	
5/9		50.0	15.0	2.0	83.0[b]	
5/10	92.0	50.0	4.0	3.0	43.0[b]	
5/11		50.0	6.5	2.5	41.0[b]	Beautiful coat
5/12		50.0	3.0	3.0	44.0[b]	
5/13		60.0	0.0	2.0	48.0[b]	
5/14-15	92.0	80.0	20.0	5.0	35.0[b]	
5/16		50.0	32.0	2.0	46.0[b]	
5/17	87.5	50.0	10.0	3.0	37.0[b]	
5/18		60.0	5.0	2.0	43.0[b]	
5/19		40.0	39.5	1.0	19.5[b]	
5/20	88.5	41.0	13.0	1.0	26.0[b]	
5/21-22	86.0	60.0	30.0	0.0	11.0[b]	
5/23	90.0	30.0	27.0	1.0	32.0[b]	

TABLE C Daily record of animal feeding--Soybean Curd Diet--
Experimental Group III (Cont.)

Rat No. 12

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/24	88.5	15.0[c]	1.0	1.0	28.0[b]	
		10.0[b]				
5/25	89.5	15.0[c]	4.5[b]	0.0	5.5[b]	
			12.0[c]		3.0[c]	
5/26	88.5	15.0[c]	10.5	0.0	4.5[c]	
5/27	82.5	15.0[c]	14.5	0.0	0.5[c]	Dry soy alone. Acceptability poor
5/28-29	87.5	25.0[c]	13.0	0.0	2.0[c]	
5/30	88.5	15.0[c]	16.0	0.0	9.0[c]	Active
5/31	87.0	15.0[c]	12.5	0.0	2.5[c]	Dry
6/1	86.5	15.0[c]	12.5	0.0	2.5[c]	
6/2	84.0	15.0[c]	12.0	1.0	2.0[c]	
6/3	80.5	10.0[c]	13.0	0.0	2.0[c]	
6/4-5	80.0	20.0[c]	9.0	0.0	1.0[c]	
6/6	80.0	10.0[c]	13.0	2.0	5.0[c]	
6/7	80.0	10.0[c]	9.0	0.0	1.0[c]	
6/8	76.0	20.0[b]	6.0	0.0	4.0[c]	
		10.0[c]				
6/9	76.0	20.0[b]	0.0			
		10.0[c]	10.0[c]	1.0	19.0[b]	
6/10	77.0	10.0[c]	0.0[b]	2.0[b]	18.0[b]	
			10.0[c]	0.0[c]	0.0[c]	
6/11-12	73.0	10.0[c]	10.0[c]	0.0[c]	0.0[c]	
6/13	73.0	40.0[b]			40.0[b]	
		9.5[c]	9.5	0.0	0.5[c]	
6/14	72.0	40.0[b]	4.5[b]	2.5[b]	33.0[b]	Diarrhea
		8.0[c]	8.0[c]	0.0	1.5[c]	
6/15	74.0		2.8[b]	3.0[b]	35.0[b]	
		8.0[c]	8.0	0.0	0.0[c]	
6/16	75.0	40.0[b]	0.0	0.0	40.0[b]	
		8.0[c]	8.0[c]	0.0	0.0[c]	
6/17	75.0	40.0[b]	12.0	0.0	28.0[b]	
6/18-19	75.0	80.0[b]	26.0	0.0	14.0[b]	
6/20	77.0	----	20.0	3.0[b]	57.0[b]	
Total[a]					913.0	4" rib case
Total[b]					1090.5	11¼" long
Total[c]					41.0	Somewhat irritable

TABLE C Daily record of animal feeding--Soybean Curd Diet--
Experimental Group III (Cont.)

Rat No. 13

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	56.3	15.0				Distal tail mark Green color
4/9		35.0	0.0	0.0	15.0	
4/10-11		20.0	0.0	0.0	35.0[a]	
4/12	59.0	30.0	0.0	0.0	20.0[a]	
4/13		35.0	0.0	3.0	27.0[a]	
4/14		45.0	0.0	3.0	32.0[a]	
4/15		45.0	8.0	1.0	36.0[a]	
4/16-17	67.5	90.0	7.0	1.0	37.0[a]	
4/18		45.0	0.5	3.0	86.5[a]	
4/19	76.0	50.0	5.5	0.5	39.0[a]	
4/20		55.0	4.0	1.0	40.0[a]	
4/21		55.0	5.0	0.5	49.5[a]	
4/22		55.0	4.0	0.5	50.5[a]	
4/23-24	83.0	120.0	4.5	0.5	50.0[a]	
4/25		60.0	2.0	7.0	111.0[a]	
4/26	92.0	60.0	3.0	1.0	56.0[a]	
4/27		60.0	18.5	1.0	40.5[a]	
4/28		70.0	6.5	1.0	52.5[a]	
4/29		70.0	7.0	1.0	62.0[a]	
4/30-5/1	100.0	90.0	15.0	5.0	50.0[a]	
5/2		45.0	15.0	1.5	74.5[b]	
5/3	103.5	50.0	5.5	3.0	36.5[b]	
5/4		50.0	12.5	1.0	36.5[b]	
5/5		50.0	9.0	2.0	39.0[b]	
5/6		45.0	17.0	2.0	31.0[b]	Shedding
5/7-8	100.5	100.0	14.0	0.0	31.0[b]	
5/9		50.0	37.0	1.0	62.0[b]	
5/10	104.0	50.0	15.0	0.0	35.0[b]	
5/11		50.0	14.5	1.0	34.5[b]	
5/12		50.0	22.0	2.0	26.0[b]	
5/13		50.0	32.5	1.0	16.5[b]	
5/14-15	103.0	65.0	34.0	1.0	15.0[b]	
5/16		40.0	16.0	2.0	47.0[b]	
5/17	103.5	40.0	21.0	1.0	18.0[b]	
5/18		40.0	9.0	1.0	30.0[b]	
5/19		40.0	31.5	1.0	7.5[b]	
5/20	93.0	41.0	16.0	2.0	22.0[b]	
5/21-22	89.0	60.0	37.0	0.0	4.0[b]	
5/23	90.0	30.0	7.0	5.0	48.0[b]	
5/24	86.0	10.0[b]	1.0	1.0	28.0[b]	
		15.0[c]				
5/25	91.0		6.0[b]	0.0	4.0[b]	
		15.0[c]	11.0[c]		4.0[c]	

TABLE C Daily record of animal feeding--Soybean Curd Diet--
Experimental Group III (Cont.)

Rat No. 13

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/26	94.5	15.0[c]	10.5	0.0	4.5[c]	
5/27	89.0	15.0[c]	13.0	0.0	2.0[c]	
5/28-29	86.0	25.0[c]	15.0	0.0	0.0[c]	
5/30	85.0	15.0[c]	18.0	0.0	7.0[c]	
5/31	89.0	15.0[c]	10.0	0.0	5.0[c]	
6/1	90.0	15.0[c]	12.0	0.0	3.0[c]	
6/2	90.0	15.0[c]	12.0	0.0	3.0[c]	
6/3	86.0	10.0[c]	13.0	0.0	2.0[c]	
6/4-5	84.0	20.0[c]	9.0	0.0	1.0[c]	
6/6	82.0	10.0[c]	14.0	3.0	3.0[c]	
6/7	80.0	10.0[c]	9.0	0.0	1.0[c]	
6/8	82.0	20.0[b]			20.0[b]	
		10.0[c]	7.0	0.0	3.0[c]	
6/9	84.0	20.0[b]			18.0[b]	
		10.0[c]	9.5	0.0	0.5[c]	
6/10	80.0	10.0[b]	2.0		2.0[b]	
			10.0[b]	0.0	0.0[c]	
6/11-12	79.0	40.0[b]				Started eating wet
		10.0[c]	8.0	0.0	2.0[c]	curd immediately.
6/13	76.0	40.0[b]			40.0[b]	
		10.0[c]	10.0	1.0	0.0[c]	
6/14	78.0	40.0[b]	10.0[b]	5.0	35.0[b]	
		9.0[c]	9.0	0.0	1.0[c]	
6/15	83.0	40.0[b]	5.0[b]	0.0[b]	35.0[b]	
		9.0[c]	8.0	0.0[c]	1.0[c]	
6/16	80.0	40.0[b]	3.0[b]	0.0	37.0[b]	
		9.0[c]	8.0[c]	0.0	1.0[c]	
6/17	82.0	40.0[b]	24.0	0.0	16.0[b]	
6/18-19	80.0	80.0[b]	31.0	0.0	9.0[b]	
6/20	74.0	----	10.0	5.0	65.0[b]	
Total[a]					889.5	11¼" long
Total[b]					941.0	4½" rib case
Total[c]					44.0	Healthiest of the group.

TABLE C Daily record of animal feeding--Soybean Curd Diet--Experimental Group III (Cont.)

Rat No. 14

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	57.1	15.0				Green color Tail proximate
4/9		35.0	0.0	0.0	15.0[a]	
4/10-11		20.0	0.0	1.0	34.0[a]	
4/12	59.0	30.0	0.0	0.0	20.0[a]	
4/13		35.0	0.0	2.0	28.0[a]	
4/14		45.0	0.0	3.0	32.0[a]	
4/15		45.0	2.0	3.0	40.0[a]	
4/16-17	61.5	90.0	6.0	2.0	37.0[a]	
4/18		45.0	1.0	3.0	86.0[a]	
4/19	69.5	50.0	3.0	1.5	40.5[a]	
4/20		55.0	0.0	2.0	48.0[a]	
4/21		55.0	1.0	2.5	51.5[a]	
4/22		55.0	3.0	1.0	51.0[a]	
4/23-24	78.5	120.0	2.0	1.5	51.5[a]	
4/25		60.0	0.0	14.0	106.0[a]	
4/26	82.0	60.0	12.0	3.0	45.0[a]	
4/27		60.0	2.5	10.0	47.5[a]	
4/28		70.0	1.5	4.0	54.5[a]	
4/29		70.0	3.0	2.0	65.0[a]	
4/30-5/1	85.0	90.0	22.0	0.0	48.0[a]	
5/2		45.0	0.0	15.0	75.0[b]	
5/3	89.0	50.0	4.5	3.0	37.5[b]	
5/4		50.0	9.5	2.0	38.5[b]	
5/5		50.0	0.0	10.5	39.5[b]	
5/6		45.0	9.0	3.5	37.5[b]	
5/7-8	81.0	100.0	6.5	0.0	38.5[b]	
5/9		50.0	3.0	12.0	85.0[b]	
5/10	90.5	60.0	0.0	5.0	45.0[b]	
5/11		50.0	17.5	3.0	39.5[b]	Losing hair
5/12		50.0	13.0	2.0	35.0[b]	
5/13		50.0	8.0	4.0	38.0[b]	
5/14-15	99.5	75.0	11.0	4.0	35.0[b]	
5/16		50.0	24.0	10.0	41.0[b]	
5/17	90.5	40.0	17.0	3.0	30.0[b]	
5/18		60.0	5.0	1.0	34.0[b]	
5/19		40.0	24.0	1.0	35.0[b]	
5/20	96.0	41.0	15.0	1.0	24.0[b]	
5/21-22	93.5	60.0	35.0	0.0	6.0[b]	
5/23	97.0	30.0	23.0	1.0	36.0[b]	
5/24	98.0	10.0[b]			28.5[b]	
		13.0[c]	1.5	0.0	1.0[c]	

TABLE C Daily record of animal feeding--Soybean Curd Diet--
Experimental Group III (Cont.)

Rat No. 14

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/25	98.0	15.0[c]	5.0[b]		10.0[b]	
			10.0[c]	0.0	3.0[c]	
5/26	100.0	15.0[c]	10.5	0.0	4.5[c]	
5/27	97.0	15.0[c]	12.0	0.0	3.0[c]	
5/28-29	95.5	25.0[c]	14.0	0.0	1.0[c]	
5/30	86.0	1.0[c]	21.0	0.0	4.0[c]	Paralyzed and elimination trouble. Unable to get up to drink H_2O or eat.
5/31	88.0	5.0[c]	0.5	0.0	0.5[c]	5:30 p.m., still
6/1	85.0	10.0[c]	0.0	3.0	2.0[c]	alive. Ate and
6/2	85.0	10.0[c]	7.0	1.0	2.0[c]	drank some.
6/3	84.0	10.0[c]	6.0	0.0	4.0[c]	Had some elimination but acts shaky like palsy.
6/4-5	85.0	20.0[c]	6.0	0.5	3.5[c]	
6/6	79.0	10.0[c]	15.0	0.0	5.0[c]	Think he could not reach the remainder of the food.
6/7	84.0	10.0[c]	3.0	2.0	5.0[c]	
6/8	86.0	20.0[b]				
		11.0[c]	4.0	1.0	5.0[c]	
6/9	86.0	20.0[b]			20.0[b]	Noted infected front
		8.0[c]	8.0	0.0	3.0[c]	fourth toe on right front foot.
6/10	88.0				20.0[b]	
		10.0[c]	4.0	0.0	4.0[c]	
6/11-12	85.0	40.0[b]	5.0	1.0		
		10.0[c]			4.0[c]	
6/13	83.0	40.0[b]			40.0[b]	
		10.0[c]	6.0[c]	0.0	4.0[c]	
6/14	82.0	30.0[b]	10.0[b]	0.0	30.0[b]	Diarrhea
		6.0[c]	8.0	0.0	2.0[c]	
6/15	85.0	40.0[b]	5.0[b]	0.0	25.0[b]	
		5.0[c]	5.0[c]	0.0	1.0[c]	
6/16	84.0	40.0[b]	10.0[b]	0.0	30.0[b]	
		5.0[c]	5.0[c]	0.0	0.0[c]	
6/17	84.0	40.0[b]	20.0[b]	0.0	30.0[b]	11" long
					0.0[c]	4¼" rib case
6/18-19	82.0	80.0[b]	28.0	0.0	12.0[b]	Not cranky during
6/20	79.0	----	16.0	5.0	49.0[b]	measurements.
Total[a]					900.5	Can climb all over
Total[b]					1044.5	the cage.
Total[c]					61.5	

TABLE D Daily record of animal feeding--Low Calcium Diet--
Experimental Group IV

Rat No. 15

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	56.7	15.0				Blue color Distal mark
4/9		25.0	4.5	1.0	4.5	
4/10-11		15.0	6.0	0.0	19.0	
4/12	78.0	15.0	6.5	0.0	8.5	
4/13		15.0	2.5	0.0	12.5	
4/14		15.0	4.0	0.0	11.0	
4/15		15.0	3.0	0.5	11.5	Sex organs developed
4/16-17	102.5	35.0	4.0	0.0	11.0	
4/18		20.0	16.5	0.5	18.0	
4/19	117.5	20.0	10.0	0.0	10.0	
4/20		20.0	8.5	0.0	11.5	
4/21		20.0	10.0	0.0	10.0	
4/22		20.0	15.0	0.0	5.0	
4/23-24	130.0	40.0	10.0	0.0	10.0	
4/25		20.0	18.5	0.5	21.0	
4/26	142.0	20.0	8.0	0.5	11.5	
4/27		20.0	9.0	0.0	11.0	
4/28		20.0	7.0	0.0	13.0	
4/29		20.0	8.0	0.0	12.0	
4/30-5/1	158.0	40.0	8.5	0.0	11.5	Inactive
5/2		20.0	12.0	0.5	27.5	
5/3	179.0	20.0	6.0	0.0	14.0	
5/4		20.0	4.0	0.0	16.0	
5/5		20.0	3.0	0.0	17.0	
5/6		20.0	9.0	0.0	11.0	
5/7-8	210.0	40.0	3.5	0.0	16.5	
5/9		20.0	14.0	0.0	26.0	
5/10	218.5	20.0	4.0	0.0	16.0	
5/11		20.0	6.0	0.0	14.0	
5/12		20.0	4.0	0.0	16.0	
5/13		20.0	7.5	0.0	12.5	
5/14-15	240.0	45.0	2.0	0.0	18.0	
5/16		25.0	18.0	0.0	27.0	
5/17	258.0	25.0	9.0	0.0	16.0	18" long
5/18		30.0	5.0	0.0	20.0	7" rib case
5/19	267.0	----	13.0	0.0	17.0	Sacrificed
Total					507.0	

TABLE D Daily record of animal feeding--Low Calcium Diet--Experimental Group IV (Cont.)

Rat No. 16

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	55.9	15.0				Blue color Proximate mark
4/9		25.0	7.0	0.0	8.0	
4/10-11		15.0	5.0	0.0	20.0	
4/12	80.0	15.0	5.0	0.0	10.0	
4/13		15.0	3.5	0.0	11.5	
4/14		15.0	3.5	0.0	11.5	
4/15		15.0	4.5	0.0	10.5	Sex organs developed
4/16-17	109.0	35.0	4.0	0.0	11.0	
4/18		20.0	12.0	0.0	23.0	
4/19	87.5	20.0	13.5	0.5	6.0	
4/20		25.0	0.0	0.0	20.0	
4/21		20.0	10.5	0.0	14.5	
4/22		20.0	6.5	0.0	13.5	
4/23-24	151.5	40.0	1.0	0.0	19.0	
4/25		20.0	11.5	0.0	28.5	
4/26	167.0	20.0	7.0	0.0	13.0	Tripled weight
4/27		20.0	5.0	0.0	15.0	
4/28		25.0	3.0	0.0	17.0	
4/29		20.0	14.0	0.0	11.0	
4/30-5/1	196.5	50.0	2.0	0.0	18.0	
5/2		25.0	14.5	0.0	35.5	
5/3	211.0	20.0	11.5	0.0	13.5	
5/4		20.0	7.0	0.0	13.0	
5/5		20.0	4.0	0.0	16.0	
5/6		20.0	7.5	0.0	12.5	
5/7-8	230.0	40.0	7.0	0.5	12.5	
5/9		20.0	12.0	0.0	28.0	
5/10	240.0	25.0	3.5	0.0	16.5	
5/11		25.0	11.0	0.0	14.0	
5/12		25.0	6.0	0.0	18.0	
5/13		25.0	8.0	0.0	17.0	Knocked over water
5/14-15	275.0	50.0	5.0	0.0	20.0	
5/16		25.0	20.5	0.0	29.5	
5/17	280.0	25.0	10.0	0.0	15.0	
5/18		30.0	5.0	0.0	20.0	
5/19		20.0	17.0	0.0	13.0	
5/20		10.0*	4.0	0.0	16.0	
5/21-22	294.0	40.0	0.0	0.0	10.0	
5/23		15.0	10.0	1.0	29.0	Knocked over water
5/24	306.0	20.0	1.0	0.0	14.0	
5/25		20.0	6.5	0.0	13.5	
5/26		20.0	5.0	0.0	15.0	Very active

TABLE D Daily record of animal feeding--Low Calcium Diet--
Experimental Group IV (Cont.)

Rat No. 16

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/27		20.0	7.0	0.0	13.0	Acceptability of new diet good
5/28-29	318.0	40.0	15.0	0.0	5.0	
5/30		20.0	12.0	2.0	26.0	
5/31	330.0	20.0	6.0	0.5	13.5	
6/1		20.0	6.0	2.0	12.0	
6/2		20.0	4.0	1.0	15.0	
6/3		20.0	5.0	2.0	13.0	
5/4-5	349.0	40.0	5.0	1.0	14.0	
6/6		20.0	6.0	2.0	32.0	
6/7	353.0	20.0	6.0	1.0	13.0	
6/8		20.0	7.0	2.0	11.0	
6/9		20.0	5.0	1.0	14.0	
6/10		20.0	2.0	0.0	18.0	
6/11-12	358.0	40.0	9.0	1.0	10.0	
6/13		20.0	5.0	1.0	34.0	
6/14	364.0	20.0	4.0	1.0	15.0	
6/15		20.0	8.0	2.0	10.0	
6/16		20.0	3.0	0.0	17.0	
6/17		20.0	3.0	0.0	17.0	
6/18-19	380.0	40.0	4.0	0.0	16.0	
6/20	378.0	----	7.0	2.0	31.0	Knocked over water
Total					1022.0	6¾" rib case 17½" long

TABLE D Daily record of animal feeding--Low Calcium Diet-Experimental Group IV (Cont.)

Rat No. 17

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	53.5	15.0				Yellow color Distal mark
4/9		25.0	6.5	0.0	8.5	
4/10-11		15.0	5.0	0.0	20.0	
4/12	88.0	15.0	6.0	0.0	9.0	
4/13		15.0	4.0	0.0	11.0	
4/14		15.0	3.0	1.0	11.0	
4/15		15.0	4.5	0.5	10.0	Sex organs developed
4/16-17	103.5	35.0	2.5	1.0	11.5	
4/18		20.0	9.5	1.0	24.5	
4/19	129.5	20.0	6.5	0.5	13.0	
4/20		20.0	4.5	0.0	15.5	
4/21		20.0	9.0	0.0	11.0	
4/22		20.0	6.0	0.0	14.0	
4/23-24	150.5	40.0	5.5	0.0	14.5	
4/25		20.0	9.5	1.0	33.5	
4/26	169.5	20.0	6.0	0.0	14.0	Tripled weight
4/27		20.0	6.0	0.0	14.0	
4/28		25.0	2.0	1.0	17.0	
4/29		20.0	10.5	0.5	14.0	
4/30-5/1	195.5	50.0	1.0	0.0	19.0	Inactive
5/2		25.0	12.0	0.5	37.5	
5/3	213.0	20.0	11.5	0.0	13.5	
5/4		20.0	6.0	0.0	14.0	
5/5		25.0	0.5	0.5	19.0	
5/6		20.0	12.5	0.5	12.0	
5/7-8	240.0	40.0	4.5	0.0	15.5	
5/9		20.0	8.0	0.5	31.5	
5/10	248.0	25.0	3.0	0.5	16.5	
5/11		22.0	9.5	0.0	15.5	Turned over water
5/12		25.0	3.0	3.0	19.0	
5/13		25.0	6.0	0.0	19.0	
5/14	275.0	50.0	6.0	0.0	19.0	
5/16		20.0	19.0	1.0	30.0	
5/17	280.0	20.0	7.0	0.0	13.0	
5/18		30.0	1.0	0.0	19.0	
5/19		20.0	18.0	0.0	12.0	
5/20		10.0*	4.0	0.0	16.0	
5/21-22	284.0	40.0	0.0	0.0	10.0	
5/23		20.0	5.0	0.5	34.5	
5/24	287.0	20.0	1.0	0.0	19.0	Knocked over water
5/25		20.0	14.0	1.0	5.0	Very active - acceptability good.
5/26		25.0	2.0	0.0	18.0	

TABLE D Daily record of animal feeding--Low Calcium Diet--
Experimental Group IV (Cont.)

Rat No. 17

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/27		20.0	12.5	0.0	12.5	
5/28-29	311.0	40.0	15.0	0.0	5.0	
5/30		20.0	8.0	0.0	32.0	
5/31	318.0	20.0	8.0	0.0	12.0	
6/1		20.0	3.0	0.0	17.0	
6/2		20.0	5.0	0.0	15.0	
6/3		20.0	6.0	0.0	14.0	
6/4-5	340.0	40.0	6.0	0.0	14.0	
6/6		20.0	11.0	0.0	29.0	
6/7	336.0	20.0	9.0	0.0	11.0	
6/8		20.0	8.0	0.0	12.0	
6/9		20.0	5.0	0.0	15.0	
6/10		20.0	3.0	0.0	17.0	
6/11-12	344.0	40.0	17.0	0.0	3.0	
6/13		20.0	8.0	1.0	31.0	
6/14	343.0	20.0	7.0	1.0	12.0	
6/15		20.0	10.0	0.0	10.0	
6/16		20.0	7.0	0.0	13.0	
6/17		20.0	12.0	0.0	8.0	
6/18-19	350.0	40.0	7.0	0.0	13.0	
6/20	357.0	----	10.0	2.0	28.0	
Total					1017.0	8½" rib case 16½" long

TABLE D Daily record of animal feeding--Low Calcium Diet--
Experimental Group IV (Cont.)

Rat No. 18

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	53.5	15.0				Yellow color Proximate mark
4/9		25.0	6.0	0.5	8.5	
4/10-11		15.0	5.0	1.0	19.0	
4/12	75.0	15.0	5.0	1.0	9.0	
4/13		15.0	4.0	1.0	10.0	
4/14		15.0	4.5	2.0	8.5	
4/15		15.0	5.0	1.0	9.0	Sex organs developed
4/16-17	101.0	35.0	2.5	1.5	11.0	
4/18		20.0	8.0	2.5	24.5	
4/19	121.5	20.0	6.5	1.5	12.0	Very clean
4/20		20.0	3.0	2.0	15.0	
4/21		20.0	8.5	1.0	10.5	
4/22		20.0	8.0	0.5	11.5	
4/23-24	137.0	40.0	6.5	1.0	12.5	
4/25		20.0	6.0	8.0	26.0	
4/26	154.5	20.0	8.0	1.0	11.0	Tripled weight
4/27		20.0	5.0	2.0	13.0	
4/28		25.0	4.0	1.0	15.0	
4/29		20.0	10.0	2.0	13.0	
4/30-5/1	180.0	50.0	2.5	3.0	14.5	
5/2		25.0	17.0	2.5	30.5	
5/3	196.5	20.0	11.0	0.0	14.0	
5/4		20.0	8.0	0.0	12.0	
5/5		20.0	4.0	0.5	15.5	
5/6		20.0	7.5	0.5	12.0	
5/7-8	220.0	40.0	5.0	1.0	14.0	
5/9		20.0	6.0	5.0	29.0	
5/10	228.5	25.0	1.5	4.0	14.5	
5/11		25.0	1.0	8.0	16.0	
5/12		25.0	4.0	2.0	18.0	
5/13		25.0	1.0	7.0	17.0	
5/14	255.0	50.0	1.0	5.0	19.0	
5/16		25.0	12.0	4.0	34.0	
5/17	260.0	25.0	6.0	3.0	16.0	
5/18		30.0	1.0	5.0	19.0	
5/19	269.0	----	9.0	4.0	17.0	Sacrificed
Total					551.0	

TABLE D Daily record of animal feeding--Low Calcium Diet--
Experimental Group IV (Cont.)

Rat No. 19

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	56.5	15.0				Purple color Distal mark
4/9		25.0	6.0	0.0	9.0	
4/10-11		15.0	4.5	0.0	20.5	
4/12	89.0	15.0	6.0	0.0	9.0	
4/13		15.0	2.0	0.0	13.0	
4/14		15.0	2.0	0.0	13.0	
4/15		15.0	3.0	0.0	12.0	Sex organs developed
4/16-17	114.5	35.0	4.5	0.0	10.5	
4/18		20.0	12.0	0.5	22.3	
4/19	133.5	20.0	8.0	0.0	12.0	Very clean
4/20		20.0	6.0	0.0	14.0	Very clean
4/21		20.0	13.5	0.0	6.5	
4/22		20.0	8.0	0.0	12.0	
4/23-24	154.0	40.0	4.0	0.0	14.0	
4/25		20.0	11.0	0.5	28.5	
4/26	174.0	20.0	6.0	0.0	14.0	More than tripled weight
4/27		20.0	6.0	0.0	14.0	
4/28		25.0	1.5	0.5	18.0	
4/29		20.0	14.0	0.0	11.0	
4/30-5/1	198.0	50.0	3.5	0.0	16.5	Lazy
5/2		20.0	19.5	0.0	30.5	
5/3	223.5	20.0	7.0	0.0	13.0	
5/4		20.0	5.0	0.0	15.0	Lost some hair
5/5		20.0	5.5	0.0	14.5	
5/6		20.0	6.0	0.0	14.0	
5/7-8	237.0	40.0	8.0	0.0	12.0	
5/9		20.0	13.0	0.0	27.0	
5/10	251.0	25.0	3.0	0.5	16.5	
5/11		25.0	11.0	0.0	14.0	
5/12		25.0	6.0	0.0	18.0	
5/13		25.0	8.5	0.0	16.5	
5/14	270.0	50.0	9.0	0.0	16.0	
5/16		25.0	22.0	0.0	28.0	
5/17	275.0	25.0	11.0	0.0	14.0	
5/18		30.0	6.0	0.0	19.0	
5/19		25.0	13.0	0.0	17.0	
5/20		10.0*	8.5	0.0	16.5	
5/21-22	307.5	40.0	0.0	0.0	10.0	
5/23		15.0	10.0	0.0	30.0	
5/24	313.0	20.0	1.0	0.0	14.0	
5/25		20.0	8.0	0.0	12.0	

TABLE D Daily record of animal feeding--Low Calcium Diet--
Experimental Group IV (Cont.)

Rat No. 19

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/26		25.0	3.0	0.0	17.0	
5/27		20.0	12.5	0.0	12.5	
5/28-29	322.0	40.0	15.0	0.0	5.0	
5/30		20.0	6.0	0.0	34.0	
5/31	331.0	20.0	7.0	0.0	13.0	
6/1		20.0	5.0	0.0	15.0	
6/2		20.0	7.0	0.0	13.0	
6/3		20.0	6.0	0.0	14.0	
6/4-5	341.0	40.0	9.0	0.0	11.0	
6/6		20.0	16.0	0.0	24.0	
6/7	342.0	20.0	9.0	0.0	11.0	
6/8		20.0	4.0	0.0	16.0	
6/9		20.0	8.0	0.0	12.0	
6/10		20.0	2.0	0.0	18.0	
6/11-12	359.0	40.0	16.0	0.0	4.0	
6/13		20.0	9.0	0.0	31.0	
6/14	360.0	20.0	6.0	0.0	14.0	
6/15		20.0	7.0	0.0	13.0	
6/16		20.0	10.0	0.0	10.0	
6/17		20.0	11.0	0.0	9.0	
6/18-19	275.0	40.0	6.0	0.0	14.0	
6/20	377.0	----	15.0	0.0	25.0	
Total					972.8	Randomly selected for long bone line test. 6" rib case 18¼" long measured dead.

TABLE D Daily record of animal feeding--Low Calcium Diet--
Experimental Group IV (Cont.)

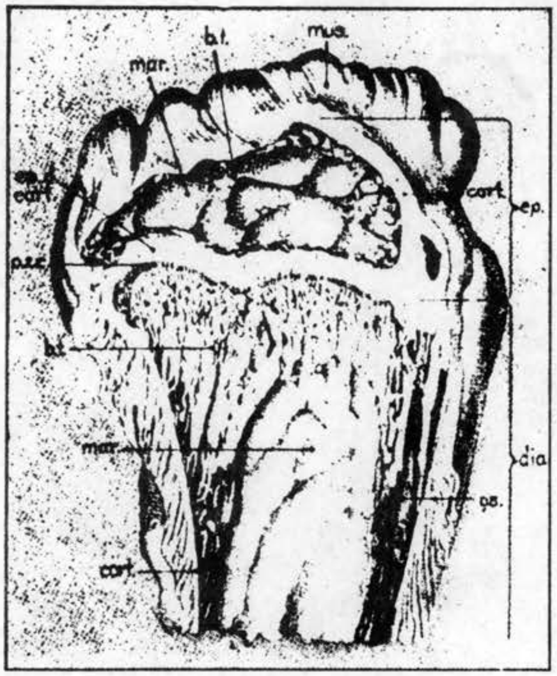
Rat No. 20

Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
4/8	56.5	15.0				Purple color Proximate mark
4/9		25.0	4.5	0.0	10.5	
4/10-11		15.0	4.5	0.0	20.5	
4/12	83.0	15.0	6.5	0.0	8.5	
4/13		15.0	3.0	0.0	12.0	
4/14		15.0	2.5	0.0	12.5	
4/15		15.0	2.0	0.0	13.0	Sex organs developed
4/16-17	111.0	35.0	2.0	0.0	13.0	
4/18		20.0	9.0	0.5	25.5	
4/19	134.0	20.0	9.0	0.5	10.5	
4/20		20.0	6.0	0.5	13.5	Clean
4/21		20.0	5.5	0.0	14.5	
4/22		20.0	6.5	0.0	13.5	
4/23-24	158.0	40.0	4.0	0.0	16.0	
4/25		20.0	17.5	0.0	22.5	Very clean
4/26	172.5	20.0	2.5	0.0	17.5	More than tripled weight
4/27		25.0	3.0	0.0	17.0	
4/28		25.0	6.5	0.0	18.5	
4/29		20.0	10.0	0.5	14.5	
4/30-5/1	207.0	50.0	0.0	0.0	20.0	Lazy
5/2		25.0	12.5	0.0	37.5	
5/3	236.0	25.0	5.5	0.5	19.0	
5/4		20.0	9.5	0.0	14.0	Lost some hair
5/5		25.0	0.0	0.0	20.0	
5/6		25.0	9.5	0.0	15.5	
5/7-8	260.0	50.0	11.0	0.0	14.0	
5/9		25.0	17.5	0.0	32.5	
5/10	273.5	25.0	8.0	0.0	17.0	
5/11		25.0	9.5	0.0	15.5	
5/12		25.0	3.0	0.0	22.0	
5/13		25.0	4.0	0.0	21.0	
4/13	305.0	50.0	2.5	0.0	22.5	
5/16		250.0	16.0	0.0	34.0	
5/17	315.0	25.0	9.0	0.0	16.0	
5/18		35.0	2.0	0.0	23.0	
5/19		25.0	16.0	0.0	19.0	
5/20		10.0*	4.5	0.0	19.5	
5/21-22	340.0	40.0	0.0	0.0	10.0	
5/23		20.0	9.5	0.0	31.5	
5/24	348.0	20.0	1.0	0.0	19.0	
5/25		20.0	7.5	0.0	12.5	

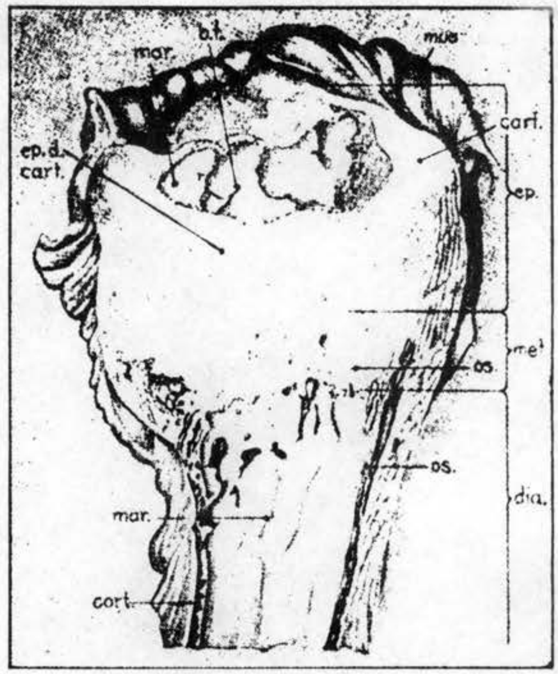
TABLE D Daily record of animal feeding--Low Calcium Diet--
Experimental Group IV (Cont.)

Rat No. 20

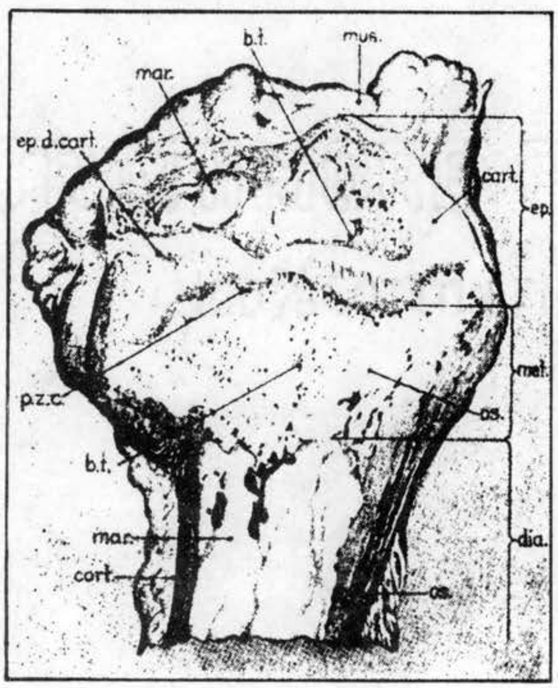
Date	Weight	Food Given	Food Left	Food Wasted	Food Eaten	Remarks
	gm.	gm.	gm.	gm.	gm.	
5/26		25.0	3.5	0.0	16.5	
5/27		20.0	13.5	0.0	11.5	
5/28-29	362.0	40.0	14.0	0.0	6.0	
5/30		20.0	5.0	0.0	35.0	
5/31	371.0	20.0	6.5	0.0	13.5	
6/1		20.0	3.0	0.0	17.0	
6/2		20.0	5.0	0.0	15.0	
6/3		20.0	3.0	0.0	17.0	
6/4-5	381.0	40.0	4.0	0.0	16.0	
6/6		20.0	11.0	0.0	29.0	
6/7	395.0	20.0	5.0	0.0	15.0	
6/8		20.0	4.0	0.0	16.0	
6/9		20.0	3.0	0.0	17.0	
6/10		20.0	2.0	0.0	18.0	
6/11-12	406.0	40.0	15.0	0.0	5.0	
6/13		20.0	5.0	0.0	35.0	
6/14	410.0	20.0	9.0	0.0	11.0	
6/15		20.0	1.0	0.0	19.0	
6/16		15.0	6.0	0.0	14.0	
6/17		20.0	2.0	0.0	13.0	
6/18-19	425.0	40.0	2.0	0.0	18.0	
6/20	428.0	----	10.0	0.0	30.0	
Total					1116.0	6½" rib case 17¼" long



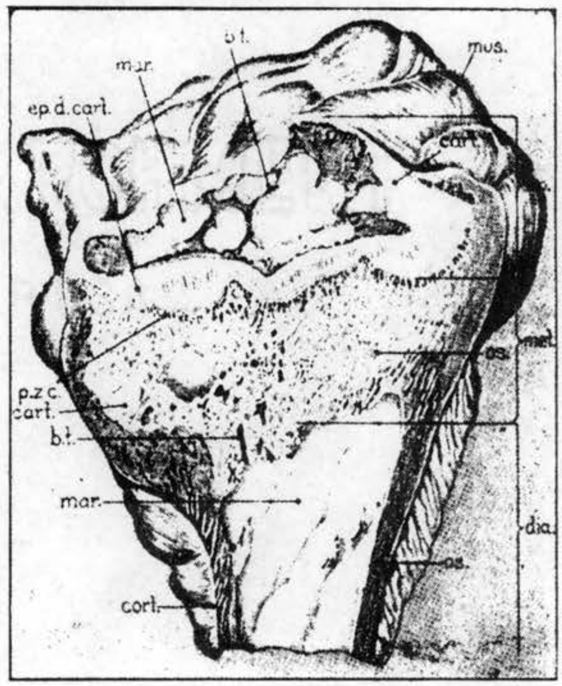
A. Normal Tibia
Tibia from a young rat which never had rickets.



B. Severe Rickets
Produced by rachitic diet.



C. + Healing Rickets
Indicating just perceptible healing.



D. + + Healing Rickets.
Indicating distinct healing.

Figure 1. Line test on normal and rachitic tibias.
(20, p. 230)

VITA

Evelyn Lane Williams

Candidate for the Degree of

Master of Science

Thesis: THE BIOLOGICAL VALUE OF SOYBEAN IN RELATION TO PROTEIN AND CALCIUM AS REVEALED BY ANIMAL FEEDING

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Biographical:

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Education: Attended grade school in Hagerman, New Mexico; graduated from Hagerman High School in 1934; attended Park College in Parkville, Missouri and University of Iowa, Iowa City, Iowa; received the Bachelor of Science degree from Mary Washington College, June, 1941, with a major in Food, Nutrition and Institution Administration; completed requirements for the Administrative Dietetic Internship at Oklahoma State University, August, 1963; completed the requirements for the Master of Science degree in Food, Nutrition and Institution Administration at Oklahoma State University, July, 1966.

Professional Experience: Taught the first four grades in West Line, Missouri 1938-39; taught Biology and Home Economics and was Manager, School Cafeteria in Dinwiddie, Virginia 1941-42; Army Dietitian at Station Hospital, Camp Lee, Virginia 1942-43; taught kindergarten in Lutheran Church School, Key West, Florida 1953-54; taught third grade in Norfolk, Virginia 1954-55; taught adult English in Yokosuka, Japan 1956-57; graduate assistant in Dairy Research Laboratory, Oklahoma State University 1963-65; Administrative Dietitian, Methodist Hospital and Medical Center, St. Joseph, Missouri 1965 (summer months).

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