THE UTILIZATION OF TOASTED SOY PROTEIN
BY GROWING RATS

by

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CHAPTER I

INTRODUCTION

In the Orient, the nutritional needs of the people are met largely through the use of foods of plant origin. Due to the high consumption of cereals, the caloric intake is largely to be satisfactory of which rice supplies the major portion in most of the areas in China, India and Japan. However, the protein intake is below what is adequate for the promotion of optimal growth and good health. This problem calls for the development of readily available, inexpensive and acceptable protein-rich foods in order that the proper and adequate nutrients can be obtained.

Soybean is native to eastern Asia and contains approximately 40% protein. Unlike cereals which are chiefly used in their original forms or prepared as breads and other baked goods, soybean can be made into numerous products and substituted for such foods as cow's milk, cheese, egg and meat. Because of the ample production and low cost, soybean and soybean products constitute the chief sources of protein for hundreds of millions of orientals.

The biological value of raw soybean protein is generally considered to be poor. The ground raw soybean, when fed to rats as the sole source of protein in an otherwise adequate ration, does not support appreciable growth. The biological value of raw soybean protein can be greatly improved, however, by either heat treatment of the raw soybean or supplementation of amino acids.

Toasted Soy Protein is a commercial preparation of 50% defatted soy protein which has been heat-treated to attain maximum nutritional potential of the soybeans. The purpose of this study was to test the nutritive value of Toasted Soy Protein when it is incorporated in a basal rice diet by using the growth rate, protein efficiency ratio, nitrogen retention value, liver nitrogen content and plasma methionine as criteria of judgement.

CHAPTER II

REVIEW OF LITERATURE

The Nutritive Value of Soybean Protein

Heating effect: Many investigators have reported that the use of heat in the preparation of certain foods definitely alters the nutritive value of proteins. When casein, meat, liver, kidney, heart muscle, cereals and fish meal were exposed to high temperatures for a considerable length of time, there resulted a decided decrease in their nutritive values. To the contrary, there is evidence of definite improvement in the growth of animals when their diets were changed from raw soybean to heated soybean. Osborne and Mendel (57), Vestal and Shrewbury (64) found that ground raw soybeans when fed to rats as the sole or principal source of protein in an otherwise complete ration did not support appreciable growth. However, normal growth resumed when cooked soybean was given.

The effect of the amount of heat used in the process of oil extraction upon the nutritive value of the protein in the residue known as soybean oil meal has been studied by Hayward et al. (33). The commercial soybean oil meal which has been extracted at different temperatures contains proteins which have about twice the nutritive value of that of the raw soybean. Clandinin et al. (22) reported that heating the solvent extracted soybean flakes in an autoclave at 15-pounds pressure for more than 40 minutes had an adverse effect upon the nutritive value of the resulting meal. Both lysine and methionine were either destroyed or made unavailable by prolonged autoclaving. The findings of Fritze et al. (30) indicated that the highest biological value was obtained when ground raw soybeans were autoclaved at 15-pounds pressure for 20 to 30 minutes.

Unavailability of amino acids in raw soybean protein: For many years the unavailability of amino acids has been considered a major factor in the poor growth, when raw soybean oil meal is the only source of amino acids in an otherwise complete ration. Hill and Borchers (37) reported that adding a mixture of essential amino acids to the diet failed to prevent or reduce the chick growth inhibitory factor caused by feeding 20% raw soybean with 27% casein. Almquist and Merritt (2) were able to get excellent growth response when the diet used by Hill and Borchers was supplemented with arginine and methionine. Recent reports of Borchers and Hayward (15, 35) have established that increasing the level of soybean oil meal from 25% to 40% in the ration resulted in similar growth rates between the animals fed raw or heated soybean oil meal. This observation prompted the hypothesis that, at a level of 25% raw soybean oil meal, certain of the amino acids contained therein were not available in adequate amounts for proper growth, or the need of the animal for certain amino acids was increased for undetermined reasons.

Fisher and Johnson (29) further demonstrated the utilization of amino acids in the raw soybean oil meal. These authors reported that a supplement of 14 amino acids in the same proportion as that in egg albumin was effective in overcoming the growth-depressing effect caused by raw soybean oil meal in chick rations. Borchers (16) reported that the growth inhibition in rats can be prevented by adding four specific amino acids to the diet: tyrosine, methionine, threonine and valine. Booth et al. (12) confirmed this observation by comparing the growth of rats fed raw soybean plus these four specific amino acids with a group fed autoclaved soybean meal without methionine supplement. Supplementation of amino acid mixtures varying from 4 to 14 amino acids to raw soybean meal did not overcome completely the growth depression effect, as compared with heated soybean meal supplemented with methionine (62). Almquist et al. (1) suggested that a greater degree of growth response is observed when unheated soybeans are supplemented with methionine than in the case with heated soybeans supplemented with methionine. Borchers (17) showed that the requirements of methionine supplementation for weanling rats fed unheated and heated soybean oil meal were 0.19% and 0.15% of the ration, respectively.

It has been well documented by Hayward et al. (34) that methionine is the first limiting amino acid in soybean protein. In Evans' work (28) it appeared that autoclaving the raw soybean increased the availability of the cystine and methionine. Proper supplementation with cystine and methionine to raw soybean (34) effected a substantial improvement in the growth rate of weanling rats. The observations that the digestibility of sulfur (42) and methionine (53, 48) was similar in animals fed raw or heated soybeans, suggest that the methionine derived from unheated soybean protein was absorbed in a form (42, 33) or at a rate (53) that did not permit adequate utilization for growth. The ineffective utilization could imply that absorption of methionine from the gut is blocked or that tissue utilization of methionine is impaired. Kwong et al. (46) reported that a specific impairment in methionine absorption is not involved in utilization of unheated soybean. In Barnes' (7) report, a tentative conclusion has been offered that the growth inhibitory factor in unheated soybean decreases tissue utilization of methionine and that this is not a general inhibitory effect upon the utilization of all amino acids.

The presence of the inhibiting substance: Bowman (18) and Ham (31) reported the presence of trypsin inhibiting substances in raw soybeans to explain the increase in protein utilization when soybean products are subjected to proper heating. Kunitz (45) crystallized and characterized the trypsin inhibitor from soybeans. Ham (32) found that the crude extracts from raw soybean had a retarding effect upon the growth of chicks when the extracts were included in a ration with either autoclaved soybean oil meal or a supplement composed of nutritionally adequate protein from animal sources. He believed that the retarding effect was due to an inhibition of intestinal proteolysis. But Klose et al. (44) and Westfall et al. (65) found that the crude extracts of raw soybeans reduced the growth rate even when they were fed with hydrolyzed protein. The feeding of a purified trypsin inhibitor which is identical with that isolated by Kunitz was shown to have an innocuous effect on growth, both in chicks and rats (13). Supplements of antibiotics have been observed to permit similar growth rates when animals were fed either raw or heated soybean oil meal as reported by Borchers et al. (14) and Hill et al. (38). Linkswiler et al. (49) indicated that antibiotics had a sparing effect on various nutrients, but that the digestibility of nutrients was not improved. Thus, it was felt that the trypsin inhibitor acted to depress growth in some other ways than to inhibit intestinal proteolysis. Liener (47) has identified soyin which is a toxic protein, associated with the antitrypsin, from the raw soybean. It apparently acts in a non-specific manner to limit food intake of the rat and, as a result, causes growth depression. Evidence has accumulated that the antitrypsin or some other factor in raw soybeans exerts a physiological effect involving the pancreas. Chernick (21) reported that chicks fed raw soybean meal developed hypertrophic pancreas which contained abnormally high concentrations of trypsinogen. It was suggested that these changes in concentration were the result of reaction to the soybean antitrypsin. Pancreatic hypertrophy and increased pancreatic enzymes in the intestines of chicks and rats following the ingestion of unheated soybean has also been noted by Lyman and Lepkovsky (51). Booth et al. (12) reported that pancreatic hypertrophy, poor growth and low food efficiency resulted from ingesting raw soybean meal as the sole source of dietary protein. Addition of the four amino acids: methionine, valine, tyrosine, and threonine, to a raw soybean diet corrected poor growth, increased food efficiency but did not prevent pancreatic hypertrophy. These results support that depressed growth caused by feeding raw soybean meal is due to direct stimulation of the pancreas which causes an excessive loss of amino acids contained in the pancreatic enzymes that are in turn excreted in the feces. However, Kwong et al. (46) in 1962 observed that fecal loss of endogenous nitrogen and the limiting amino acid, methionine, did not account for the poor nutritive value of unheated soybean products.

Evaluation of Protein Quality

There are many methods of assessing the nutritive value of proteins. Four of the main procedures are based upon the following:

1. Growth
2. Nitrogen balance
3. Tissue regeneration
4. Amino acid composition of the protein

Growth is perhaps the method most extensively used. Growth is often expressed in terms of body weight change. It follows definite mathematical patterns and can be correlated well with an increase of body protein, a major component of growth. Such a correlation was presented by Osborne, Mendel and Ferry (56) who fed each protein to be tested ad libitum, at several levels. Gain in body weight in grams per gram of protein eaten was calculated. This value is known as the protein efficiency ratio (PER). For each protein there is an optimum level at which maximum efficiency of protein utilization for growth takes place. Barnes (5) observed that this ratio varies with the level of dietary protein ingested. The maximum PER value of animal proteins is found to be at a lower dietary level than in the case of cereal proteins. Block and Mitchell (10) found a good correlation between PER and biological value as well as PER and chemical score based on amino acid composition. Bender (9) reported a good correlation between PER and net protein utilization. The net protein utilization is the proportion of the intake of nitrogen that is retained in the body. It is the product of biological value and digestibility. Hegsted (36) is of the opinion that there is a high correlation between the PER and weight gain, therefore, the latter alone can be used as an index of protein quality. Mitchell (54) pointed out the drawbacks of this method due to the differential effect of the quality of the diet and the amount of food intake on the composition of the tissues. It is possible that tissue components may vary, so the gain in weight is poorly correlated with nitrogen retention. Sure (63) observed that the PER value was found to be lower after an experimental period of 10 weeks than at 4 weeks. Moreover, the decrease of PER value was greater in dried whole egg and dried skim milk than in cereal proteins. Morrison (55) confirmed this and found that female rats tended to give maximum PER values at a lower dietary protein level than did males. Differences between casein and a plant protein mixture were greater during the early stages of development in both sexes. Further studies show that PER values may be different in different strains of rats. The results can be compared by use of casein as an internal standard (41). It has been demonstrated that the protein efficiency ratios of casein, rolled oats, and gluten supplemented with lysine and threonine are relatively insensitive to the change of protein level within a range of 9 to 16% of dietary protein. The difference that is often found between animal and vegetable proteins appears to result only from the lower level of the limiting essential amino acids in the vegetable protein. Jansen (41) concluded that comparing maximum PER as originally suggested by Osborne et al. (56) and again by Barnes (5) tended to obscure the difference in protein quality that can be shown readily at lower protein levels. Dietary protein in the range of 10 to 12% dry weight appears to be the most satisfactory level at which to assay protein quality by the PER value. Since there are many experimental variations involved in the determination, such as sex and age of the animal, type of carbohydrate used, length of experimental period, protein level employed, and feeding technique, it is necessary to develop a standard procedure for protein evaluation in order to obtain comparable results from different laboratories. The standard method adopted by the Association of Official Agricultural Chemists is the one proposed by Chapman (19) who uses weanling male rats of a single strain, 20-23 days of age, given 10% protein level, for a 4-week period.

Plasma Amino Acids

It has been known for years that the quality of any protein is based on the amount and proportion of its amino acids. Not all the amino acids present in a protein, however, are available to the animal. The origin and treatment may determine the actual nutritive value of the protein food in question.

As long ago as 1906, Howell (40) reported that the concentrations of amino acids in the portal vein were greater than in the jugular vein after a meal of protein. Since then, many studies have been made relating diet to the concentrations of free amino acids in both portal and systemic blood. One major objective is to establish the quantitative relationships between plasma amino acids in blood and the composition of ingested protein.

Hier (39) investigated the effect of feeding an excess of several amino acids singly to dogs and found that the level of the corresponding amino acid in the plasma increased. Richardson et al. (61) measured arginine, lysine, methionine, tryptophan and valine in the plasma of chicks fed diets containing soybean meal or peanut meal for 6 weeks. The plasma concentration of each of these amino acids correlated reasonably well with its concentration in the dietary protein with two exceptions: arginine in soybean meal and tryptophan in soybean and peanut meal. An addition of 0.4% DL-methionine to the diet of chicks increased the concentration of plasma methionine regardless of whether the diet was adequate or inadequate in methionine. Charkey et al (20) and Denton et al. (24) presented evidence that the concentration of any one amino acid in blood was usually in agreement with the relative concentration of that amino acid in the diet; and that the addition of supplemental amino acids to the diet resulted in an increase in blood level of the corresponding amino acid. Furthermore, a lower concentration of any one amino acid in the blood has been observed by these authors to be the reflection of a deficiency of the particular amino acid in the diet. Almquist (3) has drawn attention to the fact that a dietary deficiency of an amino acid would cause an excessively low level of that amino acid in the plasma. Particularly low levels of plasma methionine were found in chicks fed either soybean meal or peanut meal. All of these findings are consistent with the theory that an abnormally low level of a particular amino acid in the plasma indicates a deficiency of the amino acid in the dietary protein. Puchal and associates (58) investigated the plasma amino acids of young swine fed different sources of protein: dried skim milk, soybean meal, fish meal, meat meal, and cottonseed meal. The study indicated that the plasma concentrations of individual amino acids depended to a certain degree upon the amount of amino acids present in the diet, with the exception of some of the dietary non-essential amino acids. The concentration of free amino acids in the blood plasma seems to be related not only to the amount present in the protein ingested, but also to the nature of the protein in question.

CHAPTER III

PROCEDURE

Experimental Design

Toasted Soy Protein[1] (TSP) is a commercial product which has been heat-treated to bring out the highest possible biological value. The composition of TSP is shown in Table 1. This investigation was designed to test the nutritive value of the Toasted Soy Protein when incorporated with a basal rice diet, using casein as a comparison. Weanling male rats of a single strain were used. The diets provided 10% protein for a 4-week experimental period according to the standard procedure for protein evaluation (26). For many previous studies, it appears that the unavailability of methionine and the presence of a trypsin inhibitor decreased the nutritive value of raw soybean protein. For this experiment, recommended amount of methionine (17) was supplemented to one of the soybean diets to observe any improvement of its nutritive value.

Experimental Animals

Forty-two weanling male albino rats weighing between 40 and 55 grams, were purchased from a stock laboratory[2]. They were assigned by body weight to the five diets, with seven in each group and seven in an initial group. The initial group served as a basis to compare liver nitrogen and plasma methionine with the experimental groups.

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1 TSP was given through the courtesy of General Mills, Minneapolis, Minn.

2 Dublin Laboratory Animals, Dublin, Va.

Table 1

Analysis of Toasted Soy Protein-200, Micro-Milled[1]

Protein (Nx6.25) %	50.0 Minimum
Fat, %	2.0 Maximum
Available Carbohydrate, %	13.5 Minimum
Ash, %	6.0 Maximum
Fiber, %	3.0 Maximum
Moisture, %	10.0 Maximum
Trypsin Inhibitor	inactive
Protein efficiency ratio (Casein - 2.5)	2.4

[1] Source - General Mills, Inc., Specialty Products Division, Minneapolis, Minnesota.

Rice flour[1] was used as the basal diet with Toasted Soy Protein added as follows:

Diet 1: Basal diet, 6% protein from rice flour.

Diet 2: Basal diet supplemented with 4% Toasted Soy Protein.

Diet 3: Basal diet supplemented with 4% Toasted Soy Protein and 0.15% methionine.

Diet 4: Basal diet supplemented with 4% casein.

Diet 5: Basal diet supplemented with 6.5% Toasted Soy Protein.

The composition of the five diets is given in Table 2. The composition of the vitamin mixture is given in Table 3. The diets were well-mixed and stored in a freezer (-20° C) until used.

Administration of Food and Daily Care of Animals

Each animal was housed in separate metabolic cage. Food and tap water were given ad libitum. Spilled or leftover food was retrieved and weighed. The actual food intake was recorded. The animals were weighed every other day and were kept under a temperature of 72° F. and 50% humidity.

Collection Period

The experiment continued for a period of 28 days. At the end of the third week, a 7-day balance study was conducted. Metal meshes were put under the metabolic cages to separate fecal material from urine.

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[1] Chicago Dietetic Supply House, Inc., Chicago, Ill.

Table 2

Composition of Diets[a]

	Diet I	Diet II	Diet III	Diet IV	Diet V
	%	%	%	%	%
Protein	6	10	10	10	12.5
Rice flour	(6)	(6)	(6)	(6)	(6)
TSP		(4)	(4)		(6.5)
Casein[c]				(4)	
Methionine[c]			0.15		
Carbohydrate[d]	77	73	72.85	73	70.5
Fat[e]	10	10	10	10	10
Minerals[c,f]	4	4	4	4	4
Vitamins[c]	1	1	1	1	1
Non-nutritive fiber[c]	2	2	2	2	2
	100	100	100	100	100

[a] Detail of diet composition is given in Appendix Table I.

[b] Figures in parentheses indicate the percentage of the source of protein.

[c] Nutritional Biochemicals Corporation, Cleveland, Ohio.

[d] Rice Starch, Ruger Chemical Company, Inc., Long Island, N. Y.

[e] Mazola Corn Oil

[f] Jones, T. H., and C. Foster. 1942. A mixture for use with basal diet either low or high in phosphorus. J. Nutrition 24: 245.

Table 3

The Composition of the Vitamin Mixture[2]

Vitamins	mg/100 gm diet
Ascorbic acid	10.00
B_{12}	0.02
Biotin	0.02
Calcium-pantothenate	5.00
Choline chloride	100.00
Folic acid	0.20
Inositol	100.00
Menadione	0.50
Niacin	3.00
P-aminobenzoic acid	10.00
Pyridoxine	0.50
Riboflavin	1.20
Thiamine.hydrochloride	0.60
Alpha-tocopherol acetate	5.00
Oleum-percomorphum[1]	15.00

[1] Provide 900 and 127 U.S.P. units of vitamins A and D, respectively in 15 mg.

[2] Source - Yang, S. P., H. E. Clark and G. E. Vail, 1959, Nutritive value of turkey proteins. A preliminary study. J. Am. Dietet. Assn. 35: 1251.

The urine from each animal was collected in a bottle in which 3 ml of 20% hydrochloric acid was used as a preservative. Daily collections were transferred to flasks and stored in a freezer. At the end of the 7-day period, the meshes and funnels were rinsed with hot distilled water and the washings were added to the collection flasks. The urine collections were filtered through a suction funnel and made to a volume of 250 ml with distilled water. Aliquots were taken for nitrogen analysis. The fecal materials were collected daily into a 125-ml Erlenmeyer flask containing 20 ml of 20% hydrochloric acid. Twenty ml of 2.75 N hydrochloric acid were added to the total fecal collections and autoclaved at 15 pounds pressure for 16 hours. The fecal hydrolysates were then filtered and diluted to 250 ml. Aliquots were taken for nitrogen analysis.

Plasma and Liver Samples

An initial group of seven animals was sacrificed at the beginning of the experiment and the remainder of the animals at the end of 28 days.

The animals were anesthetized by intraperitoneal injection of a pentobarbital sodium solution.[1] The animal was opened on the ventral side and blood was drawn into heparinized tubes, and the liver removed.

The blood was centrifuged to separate the plasma for methionine assay. The liver was weighed and kept for acid hydrolysis as was the fecal material.

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[1] Nembutal, Abbott Laboratories, North Chicago, Illinois.

Nitrogen Determination

All materials: diets, urine, feces, and liver, were analyzed for nitrogen content using the Kjeldahl-Cunning-Arnold method (4, 51).

Methionine Assay

Microbiological assay was employed for the determination of the methionine content of the diets and of the plasma of the rats.

Protein hydrolysates of the diets were prepared by acid hydrolysis of one gram of diet (8). The hydrolysates were adjusted to pH 6.8 with sodium hydroxide solution and diluted to contain 4-8 µg of methionine per milliliter. McLaughlan's method (52) was used in the preparation of the protein free plasma. _Leuconostoc mesenteroides_ P-60 ATCC 8042[1] was used as the test organism. The preparation of the inoculum, the basal medium,[2] and the standard stock solution of DL-methionine[3] (100 µg/ml) was based on the methods given in the Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures (27). Unknowns were assayed in test tubes (13 x 100 mm) containing two volumes, 1 ml and 3 ml of either diet hydrolysate or protein-free plasma. A standard curve

1 American Type Culture Collection, Washington, D. C.

2 Difco Laboratories, Detroit, Michigan.

3 Nutritional Biochemicals Corporation, Cleveland, Ohio.

was constructed each time an assay was made. This was accomplished by diluting the stock solution of methionine to contain 8 µg per ml. The diluted stock solution was added to duplicate tubes in the following amounts: 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 ml. One milliliter of the basal medium was added to each tube. Distilled water was added to each tube to bring the final volume to 5 ml. After the tubes were prepared and held in a rack, they were covered with a cotton cover which was lined with cheesecloth. The mixtures in the tubes were then autoclaved for 10 minutes at 15 pounds pressure. The tubes were allowed to cool slowly in the autoclave to prevent unequal loss by distillation of water from the tubes. After cooling, the tubes were inoculated aseptically and incubated at 37° C for 20 hours. At the end of the incubation period, the assay tubes were put in the refrigerator for 15 minutes to prevent any further growth. Turbidity of each tube was measured by the Bausch and Lomb Spectronic 20 Spectrophotometer, using matched tubes.

Optical density was read at 625 mµ wavelength. A methionine standard curve was plotted from the readings of the nine pairs of standard solutions. The slope of the standard curve was calculated according to the equation:

$$y = mx + b$$

y: optical density reading

m: slope of the curve

x: concentration of methionine

b: intercept

The concentration of methionine in the unknown was calculated by using the slope of the standard curve.

CHAPTER IV

RESULTS AND DISCUSSION

Effect of the Diet on Growth

Body weight, food intake of animals and protein efficiency ratio of diets are summarized in Table 4. All animals increased in weight throughout the 4-week experimental period. The growth rate of all groups, except Group A, is similar, showing an almost uniform gain in weight (Fig. 1). As expected, animals in Group A, due to the ingestion of a low protein diet, i.e., 6% rice protein, gained the least, 17.1 gm. Animals in Group E whichwere given a diet containing 12.5% protein, gained most, 83.4 gm in average. Among the other animal groups, Group D gained 81.0 gm; Group C, 75.6 gm and Group B, 71.9 gm. However, when the variation in weight gains among groups of B, C, D, and E were subjected to the F-test,[1] a significant difference was found only between Groups B and C. Equally good growth was obtained when rice protein was supplemented with either casein or soy protein. The addition of 0.15% methionine to Diet 2 (6% rice protein, 4% Toasted Soy Protein) did not produce a marked difference in weight gain. When Toasted Soy Protein was increased to 6.5% in addition to the basal rice diet, a marked increase in weight gain resulted.

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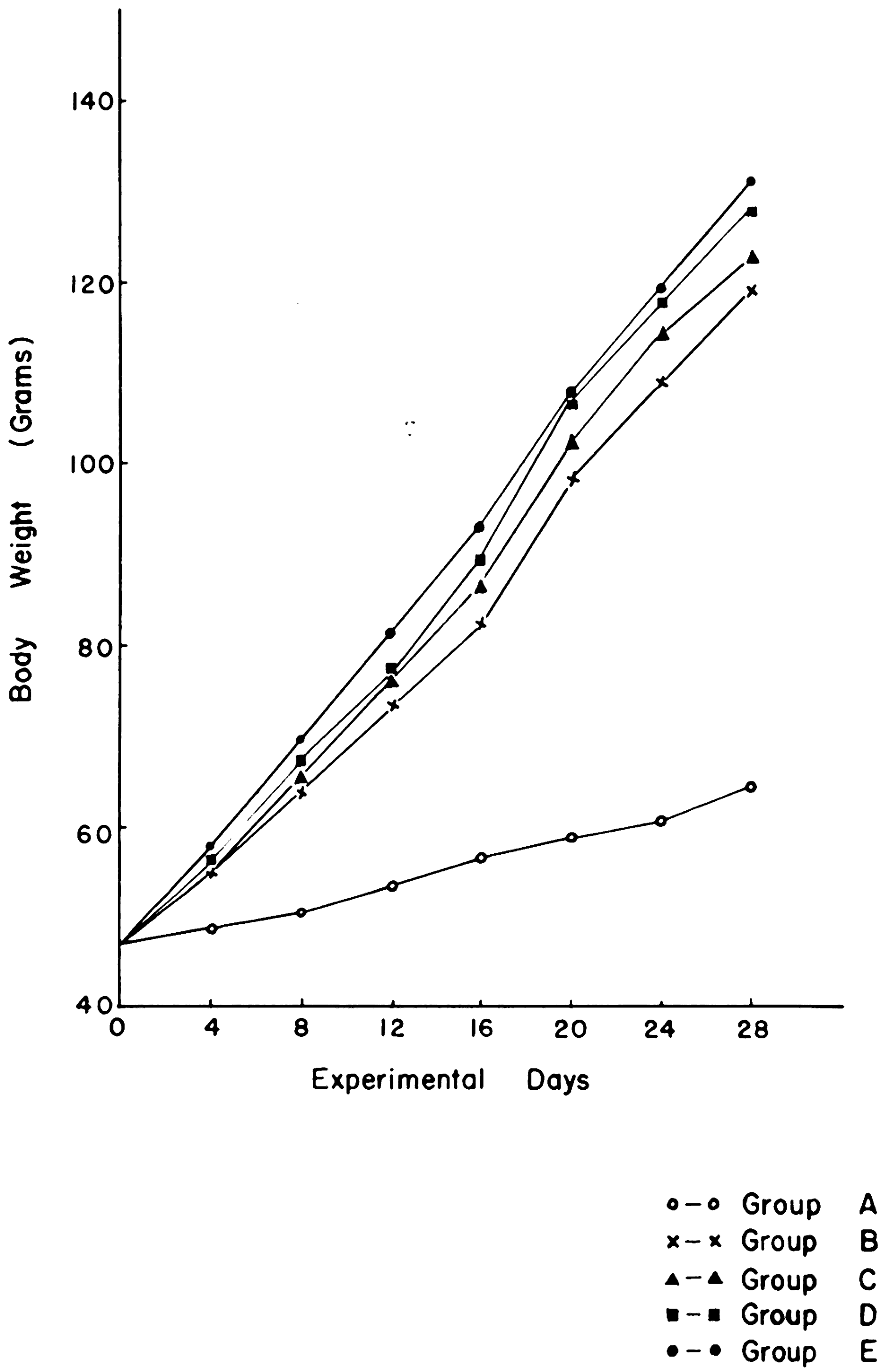
1 Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. New York: McGraw-Hill Book Company, Inc., p. 134.

Table 4

Body Weight and Food Intake of Animals and Protein Efficiency Ratio of Diets

Protein level %	Animal group	Diet designation	Source of protein	Rat no.	Initial mean weight gm	Total mean weight gain gm	Total mean food intake gm	Mean total protein intake gm	Protein efficiency ratio
6	A	1	Rice (6%)	7	47.3	17.1	173.1	10.4	1.61
10	B	2	Rice (6%) TSP (4%)	7	47.1	71.9	270.7	27.1	2.65
10	C	3	Rice (6%) TSP (4%) Methionine (0.15%)	7	47.1	75.6	278.2	27.8	2.68
10	D	4	Rice (6%) Casein (4%)	7	46.9	81.0	276.0	27.6	2.94
12.5	E	5	Rice (6%) TSP (6.5%)	7	47.0	83.4	268.6	33.5	2.50

Fig. 1. The Growth Rate of Animals
on Various Diets


Body Weight (Grams)
140
120
100
80
60
40
0
4
8
12
16
20
24
28
Experimental Days
o–o Group A
x–x Group B
▲–▲ Group C
■–■ Group D
●–● Group E

Protein Efficiency Ratio

The protein efficiency ratio (PER) is shown in Table 4. There is no significant difference (F-test) in the PER among Diets 2, 3, 4, and 5. However, the PER of Diet 4 is slightly higher than the others. The PER did not change when 0.15% methionine was added to Diet 2. An increase of Toasted Soy Protein from 4% to 6.5% reduced the PER slightly, i.e., from 2.65 to 2.50.

In studies reported by Barnes and Bosshardt (6) the PER of egg protein, peanut protein, and wheat gluten were measured. When the PER is plotted against the protein level in the diet, the shape of the curves are functions of the respective nutritive values. The researchers considered the amino acid intake and pattern of 8% egg protein in the diet to be optimum for protein anabolism. Any increase in protein intake above this level would result in lowered nitrogen utilization from the excess amino acids, thereby decreasing the PER. The broader optimum of peanut flour and wheat gluten may be correlated with the deficiency of one or more essential amino acids for growth. The ratio would continue to increase as the dietary protein is increased until the maximum amount of the limiting essential amino acid that can be utilized for protein synthesis in proportion to the other amino acids, has been reached. The addition of methionine to a diet containing 6% rice protein and 4% Toasted Soy Protein did not elevate the PER, nor did an increase of soy protein from 4% to 6.5% enhance the PER value.

It appears that the combination of 6% rice protein and 4% Toasted Soy Protein provided the amount and pattern of amino acids that may be considered adequate for the growth of young rats.

Nitrogen Retention

Table 5 shows the nitrogen balance during the 7-day period. There is no significant difference (F-test) among Groups B, C, D, and E. The slightly higher values of nitrogen retention found in Groups B and C are believed to be due to inaccurate measurement of total food intake. It was noted these animals spilled more food than did any other groups. The similar weight gain in these four groups over the 7-day period is also noted. Differences in growth rate among groups were greater during the early stage, i.e., when the animals were younger.

The effect of methionine deficiency on nitrogen intake and nitrogen balance has been studied by Denton et al. (25) employing both ad libitum and force-feed procedures. The food consumption of the animals receiving a methionine-deficient diet was much lower than that of controls when animals were fed ad libitum. There was no significant difference between the nitrogen balance values of force-fed groups which received either a complete ration or a methionine-deficient ration, but there was a marked correlation between nitrogen intake and nitrogen retention when animals were fed ad libitum.

Table 5

Nitrogen Balance of Animals on Various Diets

Animal group	Protein level %	Source of Protein	Rat no.	7-day period mean weight gain gm	Mean total protein intake gm	Mean total nitrogen intake mg	Nitrogen Excretion Urinary mg	Nitrogen Excretion Fecal mg	Nitrogen Excretion Total mg	Nitrogen Balance mg
A	6	Rice (6%)	7	5.4	10.4	387.4	81.9	72.3	154.2	+ 233.2
B	10	Rice (6%) TSP (4%)	7	19.1	27.1	1199.4	192.0	228.0	420.0	+ 779.0
C	10	Rice (6%) TSP (4%) Methionine (0.15%)	7	20.1	27.8	1231.4	252.1	183.4	435.5	+ 795.8
D	10	Rice (6%) Casein (4%)	7	20.8	27.6	1133.9	195.3	187.9	383.2	+ 750.7
E	12.5	Rice (6%) TSP (6.5%)	7	22.8	33.5	1488.0	466.2	196.0	662.2	+ 825.8

In this experiment, the addition of methionine did not increase the total food intake. The nitrogen intake of the animals receiving 4% Toasted Soy Protein as a supplement is essentially the same as that of animals receiving the same ration with additional methionine and resulted in the same nitrogen retention of these two groups. This would indicate that the diet containing 6% rice protein and 4% Toasted Soy Protein is not deficient in methionine.

Liver Nitrogen Content

As shown in Table 6, the total nitrogen content of the liver increased when the basal 6% rice protein diet was supplemented with 4% TSP, 4% TSP and 0.15% methionine, 4% casein, or 6.5% TSP. The nitrogen content of the liver usually is considered to be an index of the total nitrogen retention in the animal body. In this experiment, there is a consistent relationship between liver nitrogen and body weight gain. This may be considered an indication that the increase of the body weight is directly related to the retention of nitrogen.

The Methionine Content of Diet and Plasma

The methionine content of the diets and plasma as determined by microbioassay is shown in Table 7. The standard curve for the growth-promoting effect of methionine is shown in Fig. 2.

Table 6

Liver Nitrogen Content

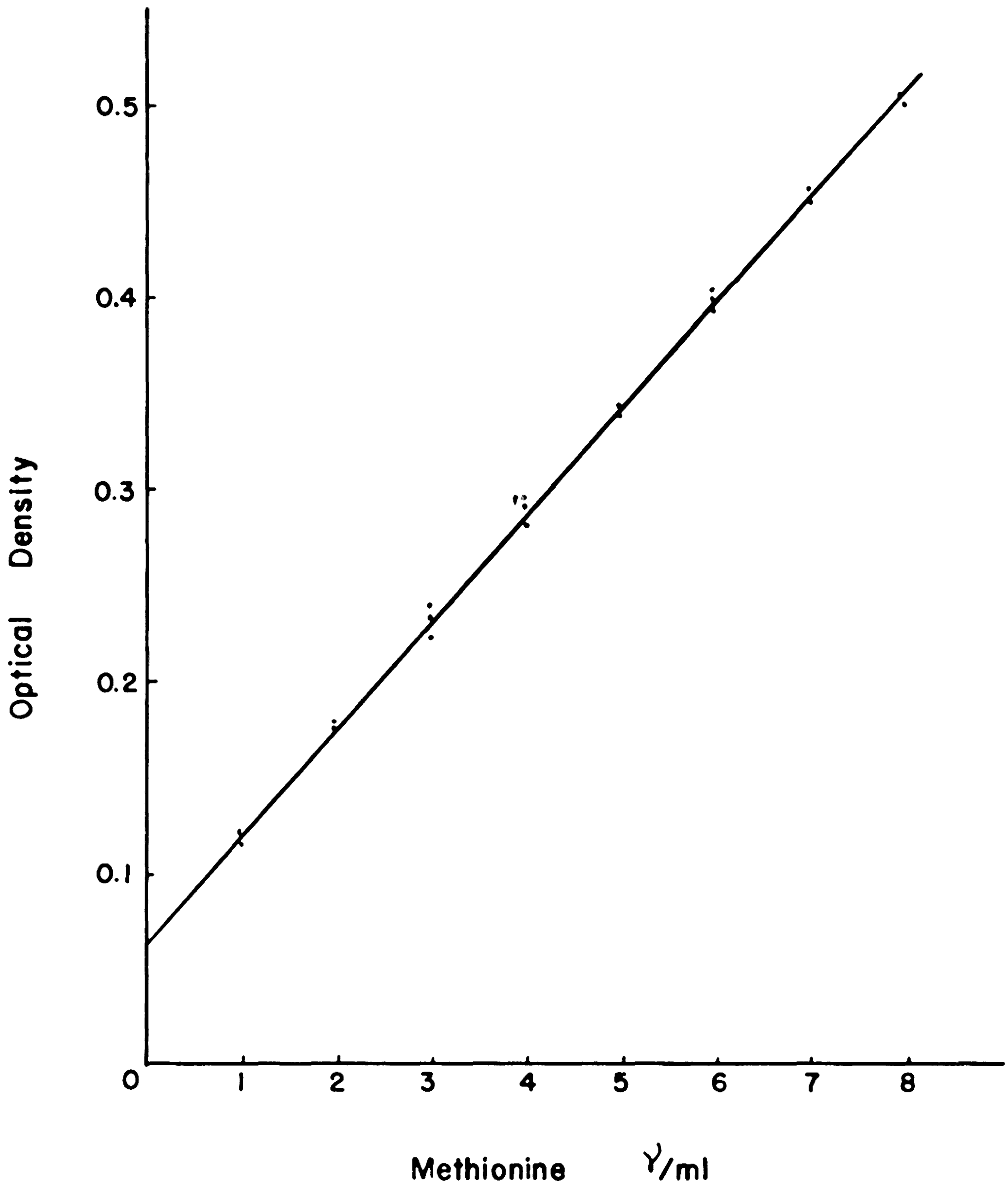
Animal group	Protein level	Source of Protein	Rat. No.	Mean total liver weight	Total liver nitrogen
	%			gm	gm
Initial	-	--	7	2.4210	0.058
A	6	Rice (6%)	7	2.8682	0.075
B	10	Rice (6%) TSP (4%)	7	4.5709	0.128
C	10	Rice (6%) TSP (4%) Methionine (0.15%)	7	4.9306	0.138
D	10	Rice (6%) casein (4%)	7	4.9599	0.146
E	12.5	Rice (6%) TSP (6.5%)	7	5.6295	0.172

Table 7

Methionine Content of Diets and Plasma

Animal group	Diet Designation	Protein level %	Source of protein	Rat. No	Dietary methionine mg/gm diet	Plasma methionine µg/ml
Initial	-	-	-	7	-	15.3
A	1	6	Rice (6%)	7	2.18	9.12
B	2	10	Rice (6%) TSP (4%)	7	2.88	13.02
C	3	10	Rice (6%) TSP (4%) Methionine (0.15%)	7	4.25	12.95
D	4	10	Rice (6%) Casein (4%)	7	3.41	8.46
E	5	12.5	Rice (6%) TSP (6.5%)	7	3.21	8.83

Fig. 2. Standard Curve of Methionine Assay


Optical Density
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
Methionine γ/ml

In this study, the methionine content of rice protein (3.63 gm/16 gm of N) as determined by microbioassay is higher than the values found in the literature, which range from 2.29 to 1.04 gm/16 gm of N (43). The methionine contents of Toasted Soy Protein and casein were calculated by substrating the methionine value of Diet 1 from Diet 2 and Diet 4, respectively. These values, 1.75 gm/16 gm of N for TSP and 3.03 gm/16 gm of N for casein are in agreement with those published in the literature (11).

Based on the work of Rackis et al. (59) and King (43) the cystine content of soybean oil meal and of rice are 1.56% and 2.14%, respectively. Thus, the 6% rice protein supplemented with 4% TSP, Diet 2, would supply a total of 0.478% of cystine+methionine. This value is very close to the requirement of 0.50% of cystine+methionine for the growing rats by Rama Rao et al (60). This may explain the fact that rats fed Diet 2 with 0.15% methionine supplement did not show much improvement in either growth or nitrogen retention when compared with animals fed Diet 2.

From the results of methionine determinations, there appears to be no direct relationship between the methionine content of the plasma and that of the diet. The plasma methionine value of the initial group is higher than Group A. This is presumably a reflection of a diet containing better quality protein ingested by the young before weaning. Free amino acids in the blood are always in a dynamic state; dietary protein is digested to free amino acids which are absorbed into the blood stream from the small intestine. At the same time, free amino acids are removed from the blood for use in tissue synthesis. The metabolism of tissue proteins also contributes free amino acids to the blood. Usually, after a meal of protein has been ingested, the free amino acid level in the blood will rise (25,66). In this experiment, food was removed at 9:00 A. M. of the last day of the experiment. The first animal was sacrificed shortly after 9:00 A. M. By the time the last animal was sacrificed, 9 hours had elapsed. Therefore, the last animal had fasted for 9 hours. This is a possible source of error in plasma methionine values.

CHAPTER V

SUMMARY AND CONCLUSION

Summary

1. Toasted Soy Protein was incorporated in a basal rice diet and tested for its nutritive value by determining growth, nitrogen balance, and plasma methionine in rats. Forty-two male weanling rats were used for a period of 28 days. A total of five diets were fed to five groups of animals having seven animals in each group. An initial group of seven was also included. The composition of the five diets is: 6% rice protein (Diet 1); 6% rice protein supplemented with 4% TSP (Diet 2); 6% rice protein supplemented with 4% TSP and 0.15% methionine (Diet 3); 6% rice protein supplemented with 4% casein (Diet 4); 6% rice protein supplemented with 6.5% TSP (Diet 5). Seven rats in each group were fed each diet for 28 days. Nitrogen balance was determined during the last seven days of the experiment. Dietary, urinary, fecal, and liver nitrogen were determined. Microbioassay was used to determine the methionine content of diets and plasma.

2. The growth rate was almost uniform for all groups except Group A. The supplementation of 6% rice protein and 4% TSP with 0.15% methionine did not result in marked increase in growth.

3. Nitrogen retention and liver nitrogen differed little among animals receiving the various diets except for Diet 1, which contained only 6% protein.

4. The protein efficiency ratio is a little higher for Diet 4 which contained 6% rice protein supplemented with 4% casein than the rest of the diets. However, no significant difference was observed when Diet 4 was compared with Diet 2, 3, and 5. An increase of TSP from 4% to 6.5% of the diet decreased the protein efficiency ratio.

5. The methionine values for rice found in this study are higher than those in the literature. The total sulfur-containing amino acids calculated for Diet 2, 0.478%, are very close to the requirement of 0.50% for growing rats.

6. No relationship was observed between dietary and plasma methionine in the animals used in this study.

Conclusion

From the observation of growth rate, nitrogen retention, liver nitrogen and PER value, it appears that a diet of 6% rice protein supplemented with 4% TSP can promote growth of young animals as well as 6% rice protein supplemented with 4% casein. An increase of the TSP level from 4% to 6.5% in the diet, i.e., an increase from 10% to 12.5% total protein, increased weight gain appreciably, but decreased the PER value. The supplementation of the diet containing 6% rice protein and 4% TSP with 0.15% of methionine did not result in improved growth or nitrogen retention. It is suggested that the total sulfur-containing amino acids in the diet containing 6% rice protein and 4% TSP were already sufficient to meet the requirements of young growing rats.

It is concluded that proper combination of Toasted Soy Protein with rice protein provides protein of good quality. This mixture of proteins of plant origins can support and promote good growth of young rats.

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APPENDIX

Table 1

Composition of Diets in Percentage

Diet	Components	Protein	Carbohydrate	Fat	Minerals	Fiber	Vitamins	Methionine	
	Basal								
	Rice flour	6	64	0.25	0.32	0.16			
1	Rice starch		12.68						
	Corn oil			9.75					
	Minerals, vitamins and fibers				4	1.84	1		
		6	76.68	10	4.32	2	1	-	100
	10% Protein								
	TSP	4	1.08	0.16	0.48	0.24			
2	Rice flour	6	64	0.25	0.32	0.16			
	Rice starch		7.12						
	Corn oil			9.6					
	Minerals, vitamins and fibers				4	1.6	1		
		10	72.2	10	4.8	2	1	-	100
	10% Protein								
	TSP	4	1.08	0.16	0.48	0.24			
3	Rice flour	6	64	0.25	0.32	0.16			
	Rice starch		6.97						
	Corn oil			9.6					
	Minerals, vitamins and fibers				4	1.6	1	0.15	
		10	72.05	10	4.8	2	1	0.15	100

(continued)

Table 1 (continued)

Composition of Diet in Percentage

Diet	Components	Protein	Carbohydrate	Fat	Minerals	Fiber	Vitamins	Methionine	
	10% Protein								
	Casein	4							
4	Rice flour	6	64	0.25	0.32	0.16			
	Rice starch		8.68						
	Corn oil			9.75					
	Minerals, vitamins and fibers				4	1.84	1		
		10	72.68	10	4.32	2	1	-	100
	12.5% Protein								
	TSP*	6.5	1.8	0.26	0.78	0.39			
5	Rice flour	6	64	0.25	0.32	0.16			
	Rice starch		3.6						
	Corn oil			9.5					
	Minerals, vitamins and fiber				4	1.5	1		
		12.5	69.4	10	5.1	2	1	-	100

* TSP = Total Soy Protein.

ABSTRACT

This study investigated the nutritive value of Toasted Soy Protein (TSP) when it is incorporated in a basal rice diet. Forty-two male weanling rats were used for a period of 28 days. A total of five diets were fed to five groups of animals having seven animals in each group. An initial group of seven was also included. The composition of the five diets is: 6% rice protein (Diet 1); 6% rice protein supplemented with 4% TSP (Diet 2); 6% rice protein supplemented with 4% TSP and 0.15% methionine (Diet 3); 6% rice protein supplemented with 4% casein (Diet 4); and 6% rice protein supplemented with 6.5% TSP.

The growth rate for animals fed Diets 2, 3, 4, and 5 is similar, all animals showing an almost uniform weight gain. Supplementation of methionine to Diet 2 did not produce marked increase in weight gain. There is no significant difference in protein efficiency ratio (PER) among animals ingesting Diets 2, 3, 4, and 5. However, an increase of the TSP level from 4% to 6.5% reduced the PER slightly. There was a direct relationship between body weight gain and nitrogen retention as measured by nitrogen balance and liver nitrogen content. The methionine content of the diets and plasma was determined by microbioassay. The total sulfur-containing amino acid content of Diet 2, i.e.: 0.478% is very close to the requirement of 0.5% for the growing rat. No direct relationship was found between methionine content of the diet and of the plasma.

A combination of 6% rice protein supplemented with 4% TSP could be a protein of high quality and this mixture of proteins of plant origins can support and promote good growth of young rats.