SUPPLEMENTAL VALUE OF SOYBEAN PROTEIN TO RICE AS MEASURED BY GROWTH RATE OF RATS

by

Yu-yan Yeh

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APPROVED:

Chairman, Dr. Genevieve P. Ho

J. C. Gorman

Dr. R. E. Webb

Dr. S. J. Ritchey (Dept. Head)

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Blacksburg, Virginia

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CHAPTER I

INTRODUCTION

Rice is one of the most important staple cereals in the world, because it is the basic daily diet for a large proportion of the world population. In the Far East, it serves as the main source of protein as well as energy. Other than rice, wheat, corn, millet, sweet potato and cassava are available for the people.

There is substantial evidence that protein malnutrition is common in many parts of the world today, particularly among young children, pregnant and lactating women. This protein deficiency is primarily one of quality (Jansen and Howe, 1964). The protein deficiency in Africa, however, is both in quality and quantity. Although rice is a good source of energy, it can not be considered as a complete food in terms of protein value. Jansen and Howe (1964), and Pollack (1956) observed independently that, in the rice-eating countries, the diet of the people is deficient in the following amino acids listed in order: lysine, threonine, and isoleucine.

Soybeans, in contrast to other leguminous seeds, contain a relatively small quantity of carbohydrate. After proper heat treatment their proteins are adequate for promoting normal growth. Properly processed soybean meal is an excellent source of protein for poultry (Altschul, 1958), and for human consumption (USDA, 1962). In view of the rapid increase in the current world population, Albanese et al. (1949)

stated that extensive use of vegetable protein diets might be necessary if future food production fails to keep pace with the population growth.

Over the years a great deal of attention has been given to providing for inexpensively produced and easily obtainable sources of all nutrients, particularly the desirable proteins, in the hope of alleviating the international food shortage and improving diet quality in general. Osborne and Mendel (1917) reported that this demand has directed attention to a renewed interest in soybeans which have long been consumed to good advantage in the Far East.

From the nutritional point of view, protein should not be differentiated as to its animal or vegetable origin, but according to its biological value. Pollack (1956) indicated that when a diet was calorically adequate, the rice protein, supplemented with small amount of various vegetable proteins and a minimal animal protein, could be nutritionally sufficient for growth. Howe et al. (1965) found similar results which showed that a diet entirely of cereal grains could be adequate both for infants and adults if the protein were of comparable quality to animal protein and if the diet satisfied protein and caloric requirements. Man can live without meat or other animal products, but he can not live without the essential amino acids. Soybean, with its protein endowment, is one of the most valuable foods in a vegetarian diet (Lager, 1945).

When compared to other vegetable protein sources, soybeans contain a large amount of total protein as well as a high proportion of essential amino acids. Some amino acids such as lysine, threonine, and the sulfur-containing amino acids, are deficient in rice (USDA, 1957). Soybean protein theoretically would be expected to have a desirable supplementary value to a rice diet because of its higher content of lysine. If a protein food of relatively high biological value could be obtained by combining rice and soybean, the problems of protein malnutrition and feeding an increasing world population might be partially relieved by using such a mixture.

Considering the above factors, the author designed an experiment to ascertain: (1) the supplementary value of soybean to a rice diet, and (2) the most efficient amount of soybean to be added.

CHAPTER II

REVIEW OF LITERATURE

Amino Acid Supplementation of Foods

Amino acid supplementation of foods and feeds has been practiced for a number of years with many beneficial results. These included the attainment of a better balanced diet, an extension or saving of the available protein supply, and an improvement in the efficiency of protein and food utilization (Albanese, 1959). In general, amino acid supplementation of foods must be not only effective and free from potential harm but also economical.

Amino acid supplementation of foods is based on the results of research on the nutritional quality of proteins and their constituent amino acids. Much of the pioneer work was carried out by Osborne and Mendel in the late 1910's. They studied the growth-promoting qualities of isolated proteins and recognized that the quantities of certain amino acids were low in those protein foods which did not support adequate growth of the animal. Consequently, there is a possibility of developing sound and practical principles for the supplementation of foods with specific amino acid(s). As a general principle for amino acid supplementation, the limiting essential amino acid should be added in such a proportion as to meet the needs of the organism. Over-all amino acid balance, however, can be achieved only when the total amount of the so-called essential amino acids in the food is in balance with the total amount of the non-essential amino acids. In many diets, especially those containing a critically low level of protein, the supplementation of a single amino acid causes nutritional imbalance resulting in an adverse physiological response such as a reduced rate of growth. In order to apply the general principle of amino acid supplementation, it is necessary to know the amino acid composition of the food to be supplemented and that of the supplement.

The available energy in a diet has been recognized to be of critical importance for the successful amino acid supplementation of animal feeds. It has long been recognized that a protein can fulfill its nutritional value only if there is sufficient energy in the diet from the non-protein sources to satisfy the energy requirements. If, however, sufficient energy is unavailable from the non-protein sources, the animal will have to use some of the protein in order to satisfy its energy need.

Supplementation of Rice with Amino Acids

In the past many works have been conducted on improving the protein value of rice by adding pure amino acid(s). Pecora and Hundley (1951) defined the most deficient amino acids in rice as lysine, histidine, methionine and threonine. They also observed that a highly significant improvement of the biological value of rice protein was accomplished by the supplementation of lysine and threonine. When rice was added with all the deficient essential amino acids simultaneously, growth was improved considerably more than with the supplement of lysine and threonine alone. Kik's (1940) investigation demonstrated similar results: when whole rice or polished rice was supplemented with lysine, methionine and cystine, its protein values were improved to a significant extent. Another experiment had been carried out by Howe _et_ _al_. (1965). They concluded that amino acid supplementation involving lysine, tryptophan and threonine increased the quality of the protein of the staple cereals, including rice, to a comparable standard of milk protein, casein. Further observations of Pecora and Hundley (1951) found that supplementation with the most deficient essential amino acid, lysine, did not improve the nutritional value of polished rice beyond that of unsupplemented rice. Deshpande _et_ _al_. (1955) investigated independently that an addition of 0.4% of L-lysine to a rice diet caused growth retardation, which was prevented by increasing the levels of lysine, isoleucine, valine and histidine. Supplementation of the rice diet with various combinations of amino acids from which one or more of lysine, isoleucine, valine and histidine had been omitted, resulted in a retardation of growth. It was evident that the addition of these four amino acids was sufficient to ameliorate the amino acid imbalance caused by the mere addition of lysine. It was suggested that the most practical way of improving the nutritive value of a rice diet would be by the supplementation with the proper combination of amino acids and/or with intact foods containing nutritionally well-balanced proteins.

Studies of the Nutritional Value of Soybean

Protein Value of Raw Soybean

In studies on the use of soybean, Osborne and Mendel (1917) found that when raw soybean meal was used as the sole source of protein in the diets, rats showed comparatively little growth. Rats, mice, and chicks showed poor growth when fed raw soybean meals or soybean products (Hayward et al. 1936a; Almquist and Merrit, 1952). Hayward et al. (1936a) noted that the poor growth resulting from feeding raw soybeans was due to some nutrient deficiency in the diets rather than to a lack of palatability. In experiments with rats, Hayward et al. (1936a) reported that raw soybeans were found to contain an insufficient amount of protein as measured by grams of growth per gram of protein consumed. Almquist et al. (1942) and Sure (1955) concluded that from the results of experiments with raw soybean, that the lack of available methionine was the chief growth-limiting factor. The impairment in utilization of the protein derived from raw soybean meals was due to an inhibition of the rate of methionine liberation but not to a reduced amount of methionine available (Melnick et al. 1946). Booth et al. (1960) postulated that the decreased growth rate caused by feeding raw soybean meal might be due to direct stimulation of the pancreas. This results in excessive loss of critical amino acids contained in pancreatic enzymes excreted in feces.

Both Mitchell and Smuts (1932) and Hayward and Hafner (1941) observed that the proteins of raw soybean are biologically deficient in available cystine as well as in methionine.

Poor growth and lower protein efficiency in rats fed raw soybean meals as a main source of dietary protein had also been investigated by Wilgus et al. (1936), Booth et al. (1960), and Rackis (1965). Raw soybeans contained sulfur and a nitrogen-containing complex, but appeared to show poor growth-promoting activity. Johnson et al. (1939) stated that the sulfur and a nitrogen-containing complex in raw soybeans were absorbable but could not be utilized for tissue building unless the raw soybean was treated properly.

Saxen et al. (1962) observed that amino acid mixtures varying from 4 to 14 amino acids added to raw soybean meal diets, failed to overcome completely the growth depression in chicks. Nevertheless, it was possible to improve the protein value of raw soybean by supplementing with synthetic amino acids. Many investigators have tried to supplement raw soybean protein with amino acid(s). Sure (1955) cited that the addition of 0.5% DL-methionine to a diet containing 10% protein derived from soybean meal resulted in trebling of the body weight and in doubling of the protein efficiency ratio. Schultze (1950) also found that rats fed purified rations containing 24% protein in the pattern of a soybean protein preparation and an addition of DL-methionine as the only source of amino acids showed satisfactory weight gains. Methionine corrected growth depression in the birds fed a raw soybean diet (Chermick et al. 1948). Borchers (1959) and Booth et al. (1960) reported that raw soybean meal supplemented with a mixture of tyrosine, methionine, threonine, and valine improved poor growth as well as food efficiency when fed to rats. Raw soybeans supplemented with cystine, one of the limiting amino acids, had a higher nutritive value than unsupplemented soybeans (Hayward et al. 1936a). The fact that cystine enhanced the protein quality of the raw soybean indicated that cystine existed in a form which was not available or was not present in adequate amounts.

Some investigations have been conducted in which antibiotics such as penicillin or aureomycin were used to correct the poor growth of animals fed raw soybeans. Higher levels of antibiotics have reversed the growth retardation of raw soybean meal on rats. It is postulated that antibiotics such as aureomycin may act to prevent utilization or destruction of the growth-promoting nutrients by intestinal micro-organisms. This saves the original amount of these nutrients available for the rats (Linksmiler et al. 1951).

Inhibitory Factors in Raw Soybean

The growth-retarding effect of raw soybean meals on animals has been known since the investigation of Osborne and Mendel in 1917. Many workers (Ham et al. 1945; Liener et al. 1949; Liener, 1951; Westfall and Hauge, 1948; Klose et al. 1946; Ham and Standstedt, 1944; and Bowman, 1944) have shown that soybean was known to contain trypsin inhibitors and a substance which inhibited the growth-promoting properties of proteins. These inhibitors were believed to be the chief causes for the poor utilization of the protein of raw or inadequately heated soybean. Ham and Standstedt (1944) substantiated that a factor causing growth retardation in chicks, and a substance which greatly retarded the activity of trypsin could both be extracted from raw soybeans with dilute acid at pH 4.2 (the isoelectric point of most soybean proteins). The similarity in properties of the growth-retarding factor and the proteolytic inhibitor indicated that the two might be identical. Ham et al. (1945) performed an experiment with chicks fed unheated soybean meal mixed with either autoclaved soybean meal or a nutritionally adequate protein from animal source. These authors demonstrated that there was a proteolytic inhibiting substance present in the raw soybean meal. Barnes et al. (1962) and Liener et al. (1949) suggested that growth depression of the animals was due to interfering with the availability of methionine. It was also suggested that the absorption of nitrogen and methionine was poorer than that of properly heated soybean. On the other hand, Desikachar and De (1947) had studied the role of the proteolytic inhibitor in relation to the nutritive value of raw soybean. They had found that it was highly probable that its action was an antigrowth factor affecting the usefulness of protein in general rather than as a factor in diminishing the degree of availability or rate of release of methionine. In an experiment with young chicks fed varying proportions of raw to cooked soybean meals, Almquist and Merrit (1952) cited that antitrypsin, a growth inhibitor in uncooked soybeans, inactivated trypsin but permitted the continued function of the remaining proteolytic system. These two theories were supported by the postulation (Liener et al. 1949) that the mode of action of the trypsin inhibitor with respect to growth might involve two mechanisms: one which interfered with the availability of methionine for growth; the other which exerted a growth-depressing effect through a mechanism unrelated to the former.

It was thought that there might be other toxic factors associated with growth-retardation in raw soybeans. Soyin, a substance isolated and purified from defatted soybean flour, was ascertained as a toxic protein, a hemagglutinating factor. Liener (1953) reported that soyin fed at a level of 1% to a group of rats on a diet containing autoclaved soybean protein or casein, inhibited growth 26% and 18% respectively. Crude trypsin was not effective in counteracting growth inhibition. Comparable experiments with raw soybean meal in the presence or absence of crude trypsin were conducted by Liener (1953). It was estimated that about half of the growth-inhibiting effect of raw soybean meal was due to its soyin content and the remainder to an effect which could be counteracted by crude trypsin. Growth impairment by soyin was due to a decrease in the quantity of food consumption.

Desikachar *et al*. (1956) investigated the improvement of the protein value of rice with 15% raw soybean added. The findings showed that the average rate of growth even in supplemented groups was far below that obtainable in animals fed a nutritionally adequate diet. Its value was found to be lower than that of rice to which autoclaved soybean was added. The expected supplementary value of soybean could have been made unavailable or off-set by the growth retarding proteolytic inhibitory factors in the untreated soybean meals.

Improvement of Raw Soybean Protein by Heating

It had been well established that the nutritive value of soybeans could be improved by heat treatment (Evans and McGinnis, 1946; Rackis, 1965; Johnson *et al*. 1939). Hayward and Hafner (1941), Almquist *et al*. (1942), and Melnick *et al*. (1946) presented evidence that an increase in the growth-promoting value of heated soybean was attributed to the general increase of the availability of methionine, and lysine.

Hayward and Hafner (1941) found that autoclaved soybean meal raised the general plane of nutrition by also increasing the availability of other amino acids. There might have been some minor limiting factors for growth in the unheated meal even in the presence of adequate amount of supplemental methionine. Several reports (Ham et al. 1945; Melnick et al. 1946; Riesen et al. 1946) showed that proper heat treatment ameliorated the release of essential amino acids by intestinal enzymatic hydrolysis of the soybean proteins. The evidence that heat treatment made cystine available had been presented by Hayward et al. (1936b). Some researchers (Klose et al. 1946; Hayward et al. 1936a) found that increasing the digestibility of the soybean protein by heating also contributed to the improvement of soybean nutrition. Klose et al. (1946) fed chicks soybean oil meals which had been autoclaved at temperatures between 100° C to 120° C for 30 minutes and found that the digestibility of these meals was greater than that of the raw meals. It has been reported also that heat treatment raises the biological value (Hayward et al. 1936a) and the protein efficiency (Westfall and Hauge, 1948) of soybeans. Another advantage of heat treatment might be increased palatability of the cooked meal, thereby inducing the rats to consume more of the food, with a resulting gain in weight (Osborne and Mendel, 1917). It was recognized that a trypsin inhibitor in raw soybean could be partially destroyed to a certain extent by moist-heat (Rackis, 1965), thereby enhancing the growth-promoting value of soybean (Hayward et al. 1936a).

Two different heat treatments, dry-heat and moist-heat, have been applied to test the nutritional value of soybeans and soybean products. Based on the report of Fritz _et al_. (1947), it was stated that dry heat was not as desirable as moist-heat. This coincided with Osborne and Mendel's (1917) finding that soybean meal heated in an electric oven at 110° C for 4 hours failed to cause any appreciable improvement in most cases. When, however, the meal was mixed with sufficient distilled water to make a thick mush and heated on a steam bath for 3 hours and subsequently dried in an air current at 80° C to 90° C, it produced growth at a normal rate. Wilgus _et al_. (1936) confirmed that the relative protein efficiency of the solvent-processed meal was good. It was shown that the proper temperature for the retention of the highest nutritive value was found to vary for different diets containing soybeans. They also demonstrated that the highest protein efficiency was found when raw soybean sample was subjected to a temperature of 140° C to 150° C in an expeller, or to a cooking temperature of 105° C in the hydraulic process. Soybean oil meal, on the other hand, gave the highest availability of methionine and cystine or organic sulfur after autoclaving at 100° C to 120° C for 30 minutes (Evans and McGinnis, 1946). Westfall and Hauge (1948) suggested that autoclaving at 108° C under 15 pound pressure, for 15 to 30 minutes produced a soybean flour of optimal protein efficiency for mice. It is apparent that the protein value of soybean is increased by moist-heat. On the other hand, dry-heat treatment of soybean tends to destroy the nutrients contained in the raw soybean.

CHAPTER III

PROCEDURE

Experimental Design of Diet

Rice flour[a] was used as the basal diet and soybean alpha protein[b] was used as the supplementary substance in all these experiments. The soybean alpha protein had been heated at 66° C for 30 minutes without any other specific treatment to destroy trypsin inhibitors. Analyses prior to the experiment showed that rice flour and soybean protein contained 8.79% and 84.44% of protein, respectively. Test diets were prepared to contain 8% protein. Animals receiving vitamin-free casein[c] were included in the experiments as an aid in establishing relative rates of growth. A protein-free diet was included as a negative control.

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[a] Rice flour: Chicago Dietetic Supply House Inc., Chicago, Illinois.

[b] Soybean alpha protein: Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

[c] Vitamin-free casein: Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

The sources of protein of the test diets were: (Values in parentheses indicate ratios of rice protein to alpha protein. These values will be used in all subsequent discussion of the diets).

Diet 1: Protein-free diet

Diet 2: 8% casein

Diet 3: 8% soybean alpha protein (0:8)

Diet 4: 8% rice flour (8:0)

Diet 5: 7% rice flour and 1% soybean alpha protein (7:1)

Diet 6: 6% rice flour and 2% soybean alpha protein (6:2)

Diet 7: 4% rice flour and 4% soybean alpha protein (4:4)

Diet 8: 2% rice flour and 6% soybean alpha protein (2:6)

Diet 9: 1% rice flour and 7% soybean alpha protein (1:7)

The length of the feeding period in all growth experiments was 28 days. The first 7 days was used as a nitrogen balance period. The composition of the diets, the vitamin mixture, and the salt mixture employed in the experiments are listed in Tables 1, 2, and 3 respectively.

Experimental Animals

Seventy-two weanling male Sprague-Dawley rats[a] weighing from 28 to 49 grams were randomly assigned into 9 groups with 8 rats in each group. They were housed individually in metabolic cages for 7 days, then in mesh wire-bottom cages for another 21 days. Diets and water were provided *ad libitum*. The animal room was kept at 22° C and relative humidity of 50% throughout the experiment.

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[a]Dublin Laboratory Animals, Dublin, Virginia

TABLE 1

Composition of Diets in Percentage

Diet	Protein: Rice flour	Protein: Alpha protein	Protein: Casein	Corn(a) starch	Corn(b) oil	Vitamin(c) mixture	Salt(d) mixture	Total percentage
1	-	-	-	93.0	2	1	4	100
2	-	-	8.4	84.6	2	1	4	100
3	-	9.5	-	83.5	2	1	4	100
4	91.0	-	-	2.0	2	1	4	100
5	79.7	1.2	-	12.1	2	1	4	100
6	68.3	2.4	-	22.3	2	1	4	100
7	45.5	4.7	-	42.8	2	1	4	100
8	22.8	7.1	-	63.1	2	1	4	100
9	11.4	8.3	-	73.3	2	1	4	100

(a) Hillco brand corn starch

(b) Mazola pure corn oil

(c) Vitamin mixture: see Table 2

(d) Salt mixture: see Table 3

TABLE 2

Composition of Vitamin Mixture[a]

Vitamin	Amount of vitamin in 1 gm vitamin mixture[b] (mg)
Vitamin A concentrate[c]	4.50
Vitamin D concentrate[d]	0.25
Alpha tocopherol	5.00
Ascorbic acid	45.00
Inositol	5.00
Choline chloride	75.00
Riboflavin	1.00
Menadione	2.25
P-aminobenzoic acid	5.00
Niacin	4.50
Pyridoxine hydrochloride	1.00
Thiamine hydrochloride	1.00
Calcium pantothenate	2.00
Biotin	0.02
Folic acid	0.09
Vitamin B_{12}	0.0014

(a) Vitamin Diet Fortification Mixture, Nutritional Biochemical Corporation, Cleveland 28, Ohio

(b) Equivalent to amount added to 100 gm of the diets

(c) 200 I.U. per mg

(d) 400 I.U. per mg

TABLE 3

Composition of Salt Mixture[a]

Salt	Mols	Grams	Element	Amount of element in 4 gm. of Salt mixture[b]
NaCl	5	292.5	Na	0.206
KH_2PO_4	6	816.6	Cl	0.319
$MgSO_4$	1	120.3	K	0.421
$CaCO_3$	8	800.8	P	0.334
$FeSO_4 \cdot 7H_2O$	0.2	56.6	Mg	0.043
KI	0.01	1.66	Ca	0.567
$MnSO_4 \cdot 2H_2O$	0.05	9.35	Fe	0.020
$ZnCl_2$	0.004	0.5452	I	0.00228
$CuSO_4 \cdot 5H_2O$	0.004	0.9988	Mn	0.00494
$CoCl_2 \cdot 6H_2O$	0.0002	0.0476	Zn	0.00047
			Cu	0.00046
			Co	0.00002

[a] Jones and Foster Salt mixture. J. Nutr., 24:245 (1942)

[b] Equivalent to amount added to 100 gm of the diets

Sample Collection and Recorded Data

The animals were weighed at the beginning of the experiment, then at approximately the same hour every 3 or 4 days during the experiment. Spilled or left-over food was weighed and discarded. The amount of food consumed was determined every other day by subtracting the spilled and uneaten food from the amount of food provided. Total nitrogen intakes were calculated on the basis of actual food consumption.

Nitrogen balance studies were conducted during the first 7 days. Daily 24-hour urine specimens were collected in test tubes (150 x 12.5 mm) containing 3 ml of 20% hydrochloric acid for each rat. The daily urine collections were pooled for each rat and stored in polyethylene bottles in a freezer. At the end of the 7-day collection period, the meshes and funnels of the metabolic cages were rinsed with 20% hydrochloric acid and warm distilled water. The washings were added into the collection bottles. Each collected urine sample was mixed homogeneously in a Waring Blender and made to a volume of 100 ml with distilled water. The feces were collected in wide mouth bottles containing 15 ml of 20% hydrochloric acid. Daily feces were stored under refrigeration for the 7-day collection period. After the collection period, the feces were mixed in the same way as urine samples to make homogeneous specimens. Aliquots of the homogeneous urine and fecal samples were taken for nitrogen analyses.

Biochemical Analysis

The micro-Kjeldahl method (AOAC, 1960a) was used to analyze the nitrogen contents of diets, urine and fecal specimens.

The fat and moisture contents of the rat carcasses were determined after killing the rats with chloroform. Incisions were made into the skull, abdomen, and body cavity before drying to a constant weight in a convection oven at 105° C for 48 hours. The dry carcasses were then ground into fine particles. Ether extraction (AOAC, 1960b) by the Goldfisch fat extractor was used for the determination of carcass fat.

Calculation Formulae for Evaluation of Protein Quality

Protein efficiency ratio (PER), nitrogen balance (NB), biological value (BV), digestibility, and net protein utilization (NPU) were computed by using the following formulae:

1. $$PER = \frac{\text{Gram of body weight gain}}{\text{Gram of protein intake}}$$

2. NB = Nitrogen intake - Fecal nitrogen - Urinary nitrogen

3. $$BV\ (\%) = \frac{\text{Retained nitrogen}}{\text{Absorbed nitrogen}} \times 100$$

$$= \frac{NI - (FN - MFN) - (UN - EN)}{NI - (FN - MFN)} \times 100$$

4. $$\text{Digestibility}\ (\%) = \frac{\text{Absorbed nitrogen}}{\text{Nitrogen intake}} \times 100$$

$$= \frac{NI - (FN - MFN)}{NI} \times 100$$

5. $$\text{NPU (\%)} = \frac{\text{Retained nitrogen}}{\text{Nitrogen intake}} \times 100$$

$$= \frac{\text{NI} - (\text{FN} - \text{MFN}) - (\text{UN} - \text{EN})}{\text{NI}} \times 100$$

Where NI is nitrogen intake; FN, fecal nitrogen; MFN, metabolic fecal nitrogen; UN, urinary nitrogen, EN, endogenous urinary nitrogen.

CHAPTER IV

RESULTS AND DISCUSSION

Growth Rate and Protein Efficiency Ratio

The data on the body weight gains, food consumptions, protein intakes of the rats and protein efficiency ratio of the diets throughout a period of 28 days are given in Table 4. Rats consuming the casein diet (Group 2) ate more food and grew faster than any other group. Rats consuming either 6% of rice flour and 2% of alpha protein (6:2) or 4% of rice flour and 4% of alpha protein (4:4) gained more weight than any of the other groups receiving the rice-soybean mixtures. There was no significant difference in food consumption between Groups 4 and 6, or Groups 4 and 7, (Table 5) indicating that the greater body gains of the animals in Groups 6 and 7 were not due to the higher food intake. Rats consuming 7% of rice flour and 1% of alpha protein (7:1) did not show better growth than those ingesting rice flour as the sole source of protein. The rats fed 8% alpha protein diet (0:8) gained very little weight during the 28-day experiment. The mean weight gain of the rats was 2.62 grams. Diet 8 (2:6) and Diet 9 (1:7), containing the lower level of rice flour protein but the higher concentration of alpha protein, gave an inferior growth response than the unsupplementated rice diet (8:0). Animals in Group 6 (6:2) and Group 7 (4:4) yielded approximately two-thirds of the growth as obtained by animals fed a casein diet, i.e. Group 2 versus Groups 6 and 7 (Figure 1). Food consumption of all animals fed the

TABLE 4

Body Weight Gain, Food Consumption, Protein Intake and Protein Efficiency Ratio of Growing Rats Fed Rice and/or Alpha-Protein for a Period of 28 Days

Group No.	Diet No.	Diet composition	No. of rat	Body weight gain (gm)	Food consumption (gm)	Protein intake (gm)	PER
1	1	protein free diet	8	-7.62 ± 2.33[a]	66.12 ± 5.30	0.27 ± 0.02	---
2	2	8% casein	8	49.88 ± 15.35	188.88 ± 45.26	15.26 ± 3.65	3.23 ± 0.24
3	3	8% alpha protein	8	2.62 ± 2.33	99.75 ± 7.44	7.87 ± 0.58	0.32 ± 0.26
4	4	8% rice protein	7	25.57 ± 8.46	158.00 ± 36.56	12.65 ± 2.19	1.97 ± 0.32
5	5	7% rice protein 1% alpha protein	8	25.38 ± 3.70	156.62 ± 19.64	11.80 ± 1.47	2.15 ± 0.18
6	6	6% rice protein 2% alpha protein	8	33.50 ± 5.78	185.88 ± 20.80	14.49 ± 1.62	2.30 ± 0.14
7	7	4% rice protein 4% alpha protein	8	33.25 ± 14.31	173.88 ± 45.50	13.36 ± 3.50	2.40 ± 0.42
8	8	2% rice protein 6% alpha protein	8	16.00 ± 4.98	125.12 ± 20.92	9.39 ± 1.57	1.67 ± 0.32
9	9	1% rice protein 7% alpha protein	8	7.38 ± 3.02	115.25 ± 7.74	8.86 ± 5.95	0.81 ± 0.29

[a] Mean ± standard deviation

TABLE 5

"T"[a] Values between Groups of Growing Rats for Body Weight Gain, Food Consumption, Protein Intake, Protein Efficiency Ratio for a Period of 28 Days

Item	Group 3 (0:8)[b] versus[c]					Group 4 (8:0) versus[d]					Group 6[e] (6:2) versus
	Group 5 (7:1)	Group 6 (6:2)	Group 7 (4:4)	Group 8 (2:6)	Group 9 (1:7)	Group 5 (7:1)	Group 6 (6:2)	Group 7 (4:4)	Group 8 (2:6)	Group 9 (1:7)	Group 7 (4:4)
Body weight gain	-13.77 (S)[f]	-13.11 (S)	- 5.59 (S)	-6.43 (S)	-3.30 (S)	0.06 (N.S.)[g]	-2.16 (S)	-1.16 (N.S.)	2.52 (S)	5.29 (S)	0.04 (N.S.)
Food consumption	- 7.16 (S)	-10.29 (S)	- 4.25 (S)	-3.02 (S)	-3.82 (S)	0.09 (N.S.)	-1.71 (N.S.)	-0.69 (N.S.)	2.02 (N.S.)	3.00 (S)	0.63 (N.S.)
Protein intake	- 6.54 (S)	-10.12 (S)	- 4.10 (S)	-2.39 (S)	-3.12 (S)	0.83 (N.S.)	1.72 (N.S.)	0.43 (N.S.)	3.12 (S)	4.38 (S)	0.77 (N.S.)
PER	-16.64 (S)	-19.80 (S)	-11.56 (S)	-9.00 (S)	-3.50 (S)	-1.38 (N.S.)	-2.75 (S)	-2.26 (S)	1.88 (N.S.)	7.73 (S)	-0.62 (N.S.)

[a] Calculated according to C. G. Paradine and B. H. P. Rivett. (1962) Statistical Methods for Technologist The English University Press Ltd., London, N. E.

[b] Values in parentheses indicate ratio of rice protein to alpha protein.

[c],[e] with degree of freedom of 14. Only those "T" values greater than +2.14 or less than -2.14 ($p < 0.05$) are considered indications of significant difference.

[d] with degree of freedom of 13. Only those "T" values greater than +2.16 or less than -2.16 ($p < 0.05$) are considered indications of significant difference.

[f],[g] S and N. S. in parentheses indicates significant difference, and non-significant difference respectively.

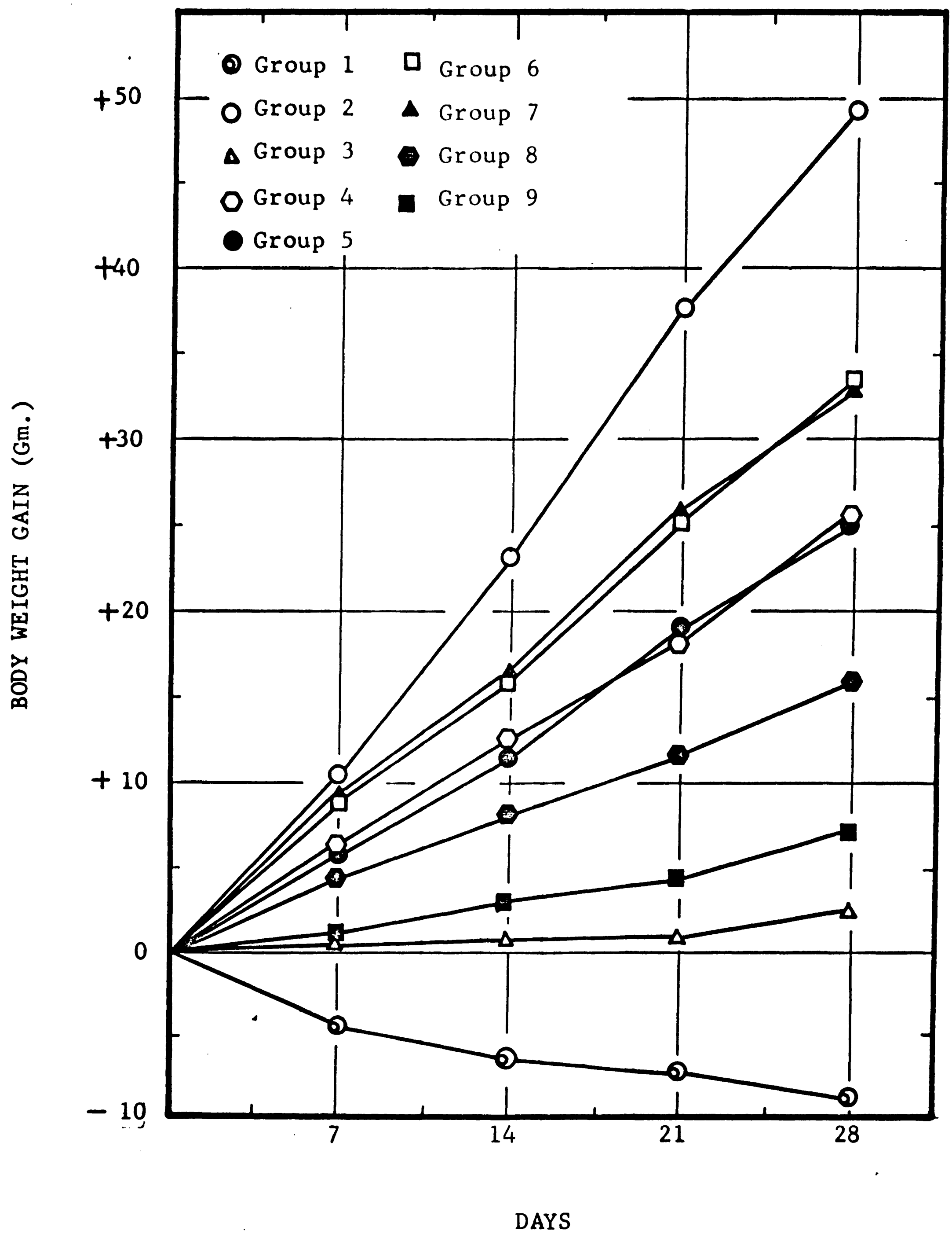

FIGURE 1. Body Weight Gain of Growing Rats

mixed diets was statistically higher than the corresponding intake of animals fed Diet 3 (0:8) (Table 5). Consequently, the total protein intakes of the mixed diets were also higher.

The protein efficiency ratios (PER) of the experimental diets 5, 6, 7, 8, and 9 were also greater than that of Diet 3 (Table 4). When compared with Diet 4, Diets 6 and 7 showed a superior quality of the mixed proteins. Statistical difference in PER values between Diet 4 and 6, and between Diets 4 and 7 were observed.

Nitrogen Balance Studies

Nitrogen balance studies were conducted throughout the first 7 days of the experiment in order to evaluate the protein quality of the rice-alpha protein mixtures. The results measured by various methods are presented in Table 6. All animals except the non-protein group maintained a positive nitrogen balance (Table 6). The highest nitrogen balance values were obtained from the animals in Groups 6 and 7 which averaged 365 mg and 364 mg of nitrogen per 7 days, respectively. These two diets contributed greater amounts of dietary nitrogen for their growth and maintenance requirements, therefore, the rats were heavier than other experimental groups (Table 4). The highest values of biological value (BV) and net protein utilization (NPU) were found in Diets 6 and 7 (Table 6). Diet 6 yielded a BV of 80 and a NPU of 68, whereas Diet 7 yielded a BV of 81 and a NPU of 70. The digestibilities of the diets showed no significant differences among the groups except Group 4 vs. Group 6 (Table 7).

TABLE 6

Nitrogen Balance, Digestibility, Biological Value, Net Protein Utilization of Growing Rats for a Period of 7 Days

Group No.	Diet No.	Diet composition	No. of rat	Nitrogen Balance (mg)	Digestibility (%)	Biological Value (%)	Net protein Utilization (%)
1	1	non-protein diet	8	- 52.11 ± 9.23[a]	---	---	---
2	2	8% casein	8	361.25 ± 74.15	92.53 ± 3.99	89.35 ± 3.69	79.73 ± 4.77
3	3	8% alpha protein	8	188.75 ± 35.63	90.95 ± 4.42	65.24 ± 9.12	55.85 ± 8.33
4	4	8% rice protein	8	305.00 ± 32.51	90.59 ± 1.96	72.67 ± 7.78	63.31 ± 7.11
5	5	7% rice protein 1% alpha protein	8	277.50 ± 86.48	87.21 ± 3.62	74.04 ± 6.77	61.70 ± 7.18
6	6	6% rice protein 2% alpha protein	8	365.00 ± 38.17	86.10 ± 2.18	80.38 ± 4.49	68.32 ± 3.61
7	7	4% rice protein 4% alpha protein	8	363.75 ± 76.71	88.74 ± 3.05	81.39 ± 3.69	69.69 ± 4.10
8	8	2% rice protein 6% alpha protein	8	220.00 ± 60.94	88.06 ± 3.36	70.68 ± 5.11	59.05 ± 6.99
9	9	1% rice protein 7% alpha protein	8	220.00 ± 88.16	90.86 ± 2.14	65.88 ± 13.55	57.07 ± 12.77

[a] Mean ± standard deviation

TABLE 7

"T"[a] Values between Groups of Growing Rats for Nitrogen Balance, Digestibility, Biological Value, Net Protein Utilization for a Period of 7 Days

Item	Group 3 (0:8)[b] versus[c]					Group 4 (8:0) versus[d]					Group 6[e] (6:2) versus
	Group 5 (7:1)	Group 6 (6:2)	Group 7 (4:4)	Group 8 (2:6)	Group 9 (1:7)	Group 5 (7:1)	Group 6 (6:2)	Group 7 (4:4)	Group 8 (2:6)	Group 9 (1:7)	Group 7 (4:4)
Nitrogen Balance	-2.44 (S)[f]	-8.93 (S)	-5.47 (S)	-1.17 (N.S.)[g]	-0.87 (N.S.)	0.79 (N.S.)	-3.17 (S)	-1.87 (N.S.)	3.26 (S)	2.40 (S)	0.04 (N.S.)
Digestibility	0.93 (N.S.)	1.39 (N.S.)	0.66 (N.S.)	0.74 (N.S.)	-0.03 (N.S.)	-0.37 (N.S.)	2.34 (S)	1.00 (N.S.)	1.05 (N.S.)	-0.13 (N.S.)	-1.33 (N.S.)
Biological value	-1.10 (N.S.)	-2.16 (S)	-2.32 (S)	-0.74 (N.S.)	-0.55 (N.S.)	-0.19 (N.S.)	-1.21 (N.S.)	-1.43 (N.S.)	0.30 (N.S.)	0.61 (N.S.)	-0.25 (N.S.)
Net protein utilization	-1.41 (N.S.)	-3.63 (S)	-3.94 (S)	-0.78 (N.S.)	-0.22 (N.S.)	0.40 (N.S.)	-1.70 (N.S.)	-2.09 (N.S.)	1.10 (N.S.)	1.11 (N.S.)	-0.66 (N.S.)

(a)Calculated according to C. G. Paradine and B. H. P. Rivett. (1962) Statistical Methods for Technologist. The English University Press Ltd. London, N. E.

(b)Values in parentheses indicate ratio of rice protein to alpha protein.

(c),(d),(e)With degree of freedom of 14. Only those "T" values greater than +2.14 or less than -2.14 ($p < 0.05$) are considered indications of significant difference.

(f),(g)S and N. S. in parentheses indicates significant difference, and non-significant difference respectively.

Moisture and Fat Content of the Carcasses

The examination of the body composition is important in the determination of protein quality because various groups of animals fed different types of proteins may have identical weight gains but have considerably different body compositions (Albanese, 1959). The analyses of the carcasses for water as well as fat were accomplished in this study to observe whether an increase in body weight was a definite index of physiological growth. Results of moisture and fat analyses are presented in Table 8. Significant differences in both fat and water contents were found between Group 3 and Groups 6 and 7, respectively. On the other hand, such differences were not found between Group 4 and Group 6; Group 4 and Group 7 (Table 9). The greater increase in body weight of the rats in Group 6 and Group 7 was not due to a change in water or fat content. As a general rule, if the protein content of a diet is at a suboptimal level or if the essential amino acids of the protein are imbalanced, the water, nitrogen, and fat concentrations of the body will be different from the normal values (Albanese, 1959). It has also been reported that the water and fat contents of the body are inversely correlated. If these characteristics existed in this experiment, the relatively higher moisture content and lower fat percentage determined in Groups 1, 3, and 9 would have been attributed to the poor protein quality of these diets (Table 8).

TABLE 8

Moisture and Fat Contents of the Carcasses of Growing Rats for a Period of 28 Days

Group No.	Diet No.	Diet composition	No. of rat	Mean Body weight (gm)	Mean moisture content (%)	Mean fat content (%)
1	1	protein free diet	8	25.75	71.15 ± 1.20[a]	2.97 ± 0.98
2	2	8% casein	8	89.00	62.25 ± 4.02	14.26 ± 4.16
3	3	8% alpha protein	8	41.25	67.56 ± 1.09	7.11 ± 1.28
4	4	8% rice protein	7	70.43	64.29 ± 2.66	14.32 ± 2.80
5	5	7% rice protein 1% alpha protein	8	66.25	64.49 ± 1.29	13.98 ± 1.66
6	6	6% rice protein 2% alpha protein	8	76.00	62.73 ± 1.64	16.36 ± 1.84
7	7	4% rice protein 4% alpha protein	8	74.12	64.67 ± 2.34	13.28 ± 2.98
8	8	2% rice protein 6% alpha protein	8	52.38	65.83 ± 2.63	11.66 ± 3.64
9	9	1% rice protein 7% alpha protein	8	46.75	67.56 ± 1.56	9.26 ± 1.73

[a] Mean ± standard deviation

TABLE 9

"T"[a] Values between Groups of Growing Rats for Moisture and Fat Contents of the Carcasses for a Period of 28 Days

Item	Group 3 (0:8)[b] versus[c]					Group 4 (8:0) versus[d]					Group 6[e] (6:2) versus
	Group 5 (7:1)	Group 6 (6:2)	Group 7 (4:4)	Group 8 (2:6)	Group 9 (1:7)	Group 5 (7:1)	Group 6 (6:2)	Group 7 (4:4)	Group 8 (2:6)	Group 9 (1:7)	Group 7 (4:4)
Moisture content	4.80 (S)[f]	6.48 (S)	2.96 (S)	1.60 (N.S.)[g]	-0.01 (N.S.)	-0.18 (N.S.)	1.29 (N.S.)	-0.28 (N.S.)	-1.05 (N.S.)	-2.74 (S)	-1.80 (N.S.)
Fat content	-8.68 (S)	-10.95 (S)	-5.03 (S)	-3.12 (S)	-2.64 (S)	0.27 (N.S.)	-1.57 (N.S.)	0.65 (N.S.)	1.46 (N.S.)	3.97 (S)	2.33 (S)

(a) Calculated according to C. G. Paradine and B. H. P. Rivett. (1962) Statistical Methods for Technologist. The English University Press Ltd. London, N. E.

(b) Values in parentheses indicate ratio of rice protein to alpha protein.

(c),(e) With degree of freedom of 14. Only those "T" values greater than +2.14 or less than -2.14 ($p < 0.005$) are considered indications of significant difference.

(d) With degree of freedom of 13. Only those "T" values greater than +2.16 or less than -2.16 ($p < 0.05$) are considered indications of significant difference.

(f),(g) S and N. S. in parentheses indicates significant difference, and non-significant difference respectively.

These results may be interpreted to indicate that alpha protein has a supplemental value to rice protein when added at levels not exceeding 50% of the total protein. However, it has an inhibitory effect rather than a supplement at levels greater than 50% of protein. One possible explanation would be that the alpha protein contained trypsin or other growth inhibitors which depressed growth rate at higher levels of intake. Supporting this suggestion might be the fact that the heat treatment of alpha protein at 66°C for 30 minutes as reported by the manufacturer removed only part but not all of the growth inhibitors.

Lower digestibilities of the diets containing 50% or more alpha protein were not observed, therefore, some other explanation may be more logical. Amino acid composition of the diets are presented in Table 10. When the amino acids are expressed as a percentage of the National Research Council's Recommended Allowances for the Growing Rat (Warner, 1962), as shown in Table 11, lysine is the most deficient amino acid in rice, sulfur-containing amino acids are the most deficient in alpha protein, and lysine is highest in alpha protein. The supplemental effect of alpha protein at low levels is probably due to the lysine supplementation to the rice diets. At higher levels of alpha protein, sulfur-containing amino acids become the most limiting amino acid and growth inhibition occurs. When the amino acids are expressed as a percentage of casein, the most limiting amino acids follow the same pattern.

TABLE 10

Amino Acid Composition of the Test Diets

(Gram Amino Acid/ 100 Gram Diet)

Amino Acid	Diet 4 (8:0)[a]	Diet 5 (7:1)	Diet 6 (6:2)	Diet 7 (4:4)	Diet 8 (2:6)	Diet 9 (1:7)	Diet 3 (0:8)	NRC[b]	Gm A.A. per 8 Gm Casein[c]
Trytophan	0.09[d]	0.09	0.09	0.08	0.08	0.08	0.08[e]	0.15	0.10
Threonine	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.50	0.34
Isoleucine	0.38	0.39	0.41	0.45	0.48	0.51	0.52	0.50	0.53
Leucine	0.69	0.68	0.67	0.64	0.62	0.61	0.60	0.80	0.81
Lysine	0.31	0.34	0.37	0.43	0.49	0.52	0.54	0.90	0.65
Total sulfur containing amino acids	0.26	0.24	0.22	0.19	0.17	0.14	0.13	0.60	0.28
Phenylalanine (including tyrosine)	0.77	0.75	0.75	0.72	0.69	0.69	0.67	0.90	0.89
Valine	0.56	0.55	0.53	0.50	0.47	0.46	0.44	0.70	0.59
Arginine	0.46	0.49	0.52	0.56	0.62	0.64	0.66	0.20	0.33
Histidine	0.14	0.15	0.15	0.17	0.19	0.20	0.21	0.30	0.24

[a] Values in parentheses indicate ratio of rice protein to alpha protein.

[b] Richard G. Warner. Nutrient Requirements of Laboratory Animals. National Academy of Science-National Research Council. Pub. No. 990, 1962.

[c],[d] Amino Acid Content of Foods. Home Economics Research Report. No. 4, USDA 1957.

[e] Based on the analyses supplied by the Nutritional Biochemicals Corporation. Cleveland 28, Ohio.

TABLE 11

Amino Acids in the Diets Expressed as a Percentage of the National Research Council's

Recommended Allowances for the Growing Rat

Amino Acid	Diet						
	4 (8:0)[a]	5 (7:1)	6 (6:2)	7 (4:4)	8 (2:6)	9 (1:7)	3 (0:8)
Tryptophan	60.00	60.00	60.00	53.34	53.34	53.34	53.34
Threonine	62.00	62.00	62.00	62.00	62.00	62.00	62.00
Isoleucine	76.00	78.00	82.00	90.00	96.00	102.00	104.00
Leucine	86.25	85.00	83.75	80.00	77.50	76.25	75.00
Lysine	34.44[b]	37.77	41.11	47.77	54.44	57.77	59.99
Total sulfur-containing amino acids	43.34	40.01	36.67	31.67	28.34	23.34	21.67
Phenylalanine (including tyrosine)	85.55	83.32	83.32	79.99	76.66	76.66	74.43
Valine	79.97	78.54	75.68	71.40	67.12	65.69	62.83
Arginine	230.00	245.00	260.00	280.00	310.00	320.00	330.00
Histidine	46.66	50.00	50.00	56.66	63.33	66.66	70.00

[a] Values in parentheses indicate ratio of rice protein to alpha protein.

[b] Underlined values are calculated to be the most limiting amino acids based on the *Nutrient Requirements of Laboratory Animals*. National Academy of Science-National Research Council. Pub. No. 990, 1962.

In order to determine if the level of methionine and/or the presence of a heat labile inhibitor(s) were factors contributing to the observed results, a further study is suggested in which the following experimental diets be used: 8% alpha protein; 8% alpha protein + methionine; 8% re-heated alpha protein; 8% re-heated alpha protein + methionine. The heat treatment should be that described by Westfall and Hauge (1948) to give optimal result. If trypsin inhibitors were partially responsible for the poor growth of rats fed the alpha protein, the heat-treated alpha protein should produce greater growth than the untreated alpha protein. However, if the problem is entirely due to a deficiency of sulfur-containing amino acids, the growth response to supplemental methionine should be large and similar for rats fed either the untreated or treated alpha protein.

CHAPTER V

SUMMARY AND CONCLUSION

Summary

(1) The value of soybean alpha protein for supplementing rice flour was investigated by determining growth rate, protein efficiency ratio, nitrogen balance, biological value, digestibility, and net protein utilization of various test diets fed to weanling male Sprague-Dawley rats. The test diets were prepared by mixing rice flour with soybean alpha protein in the following ratios to supply 8% protein: 8:0; 7:1; 6:2; 4:4; 2:6; 1:7; 0:8. In addition, a diet containing 8% protein from casein and a non-protein diet were used as controls. The length of the feeding period in all the growth experiments was 28 days and the first 7 days was taken as nitrogen balance period. The test diets, fecal and urine samples were analyzed for nitrogen. The fat and moisture contents of the carcasses were determined.

(2) The highest values of growth rate, protein efficiency ratio, nitrogen balance, biological value and net protein utilization were found in Diet 6 (6:2) and Diet 7 (4:4).

(3) Diet 6 (6:2) and Diet 7 (4:4) yielded approximately two-thirds of the growth as was obtained by a casein diet (Diet 2).

(4) Statistically significant difference in protein efficiency ratios was observed between Diet 3 (0:8) and Diet 6 (6:2); Diet 3 (0:8) and Diet 7 (4:4); Diet 4 (8:0) and Diet 6 (6:2); and Diet 4

(8:0) and Diet 7 (4:4). Statistically significant difference of body gain was found only between the animals in Groups 4 (8:0) and 6 (6:2) but not between Groups 4 (8:0) and 7 (4:4).

(5) The significant differences of both fat and moisture contents of the rat carcasses were not found either between Groups 4 (8:1) and 6 (6:2), or Groups 4 (8:0) and 7 (4:4).

(6) Diets 8 (2:6) and 9 (1:7) containing a lower level rice flour but a higher content of alpha protein gave an inferior growth response as compared to the unsupplemented rice diet (8:0).

(7) Compared with the all-alpha protein diet (0:8), all other mixed diets enhanced weight gain with statistically significant differences in food consumption, and consequently protein intake between groups.

(8) No statistically significant differences were noted between Diet 6 (6:2) and Diet 7 (4:4) in growth rate, protein efficiency ratio, nitrogen balance, biological value, net protein utilization, food consumption and protein intake.

(9) When the amino acids are expressed as a percentage of the National Research Council's Recommended Allowances for the Growing Rat, lysine is the most deficient amino acid in rice, sulfur-containing amino acids are the most deficient in alpha protein, and lysine is highest in alpha protein.

Conclusion

In this study, both Diets 6 and 7 showed the best quality of the proteins among the five mixed rice-alpha protein diets. Growth rate, PER, BV, and NPU increased with increasing proportions of alpha protein to a rice alpha protein ratio of 6:2 (Diet 6). A decrease followed from a ratio of 4:4 (Diet 7) or greater. The levels of 6:2 to 4:4 may be the ratios of which rice and alpha protein have the greatest complementary effect.

However, there are no statistically significant differences between Diets 6 (6:2) and 7 (4:4) in growth rate, protein efficiency ratio, nitrogen balance, biological value, net protein utilization, food consumption and protein intake. From a nutritional viewpoint Diet 6 (6:2) and Diet 7 (4:4) are similar, but Diet 6 would probably be the best combination to recommend because from an economic viewpoint, such a diet, having less protein, would be less expensive. Moreover Diet 6 because of its higher rice content, would undoubtedly be more acceptable to populations in which rice is the major dietary staple.

A further study is needed to determine if the methionine content and/or the presence of a heat labile inhibitor(s) are responsible for the poor response to supplements of alpha protein.

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SUPPLEMENTAL VALUE OF SOYBEAN PROTEIN TO RICE AS

MEASURED BY GROWTH RATE OF RATS

by

Yu-yan Yeh

ABSTRACT

Weanling male Sprague-Dawley rats were randomly assigned to 9 groups of 8 animals each and were given diets containing 8 per cent protein supplied from rice flour and soybean protein in the following ratios: 8:0; 7:1; 6:2; 4:4; 2:6; 1:7 and 0:8. A diet containing 8 per cent protein from casein and a non-protein diet served as controls. The supplementary value of soybean protein to rice flour was evaluated during a 28-day growth period and a 7-day nitrogen balance study.

Growth rate, protein efficiency ratio, nitrogen balance, biological value and net protein utilization increased with increasing amounts of soybean protein in a rice:soybean mixture from 8:0 to 4:4. An all soybean protein diet (0:8) supported little or no growth. Rats fed an all rice diet equalled the growth of rats fed the diet containing 7:1 of rice and soybean. It appears that a ratio of 6:2 and 4:4 of rice: soybean possess the best protein value among the other combinations. No statistically significant difference was observed between the combinations of 6:2 or 4:4 of rice and soybean. However, the diet containing 75 per cent rice flour and 25 per cent soybean in a total of 8 per cent protein, would probably be the best combination to recommend because from an economic viewpoint, such a diet, having less protein, would be

less expensive. Moreover this diet because of its higher rice content, would undoubtedly be more acceptable to populations in which rice is the major dietary staple.