EFFECT OF PROTEIN SUPPLEMENTS ON GROWTH AND FAT DEPOSITION IN THE LIVERS OF RATS FED POLISHED RICE WITH PEANUT OIL

by

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INTRODUCTION

The world population is increasing about 1.9% annually, but the demand of food is increasing by 3 to 5% annually in the developing and most rapidly growing regions of the world (1). Food supplies must expand to keep up with a growing population. In the totality of the problem of overcoming the world food shortage, providing sufficient proteins occupies a special place. The long term view, taking account of expected population increases to 1975 and beyond, opens up a truly immense demand for protein (2). To eliminate malnutrition would mean a 45% increase in total protein supplies between 1960 to 1975 (2). This problem of increasing protein supply is especially pressing in countries of low protein diets, such as those subsisting mainly on cereal grains.

The most important cereal grain in China and other countries in Asia is rice. The diet of those people consists of a large proportion of rice (60% or higher) which is usually supplemented with a small quantity of protein in form of vegetables, fish and meat prepared with a little oil, usually peanut oil (3). However, the shortage is protein quality rather than quantity. Among the adverse effects of these protein-deficient high-rice diets, fat deposition in the liver and impaired growth are noted. It has been demonstrated that the relative proportions as well as the actual amounts of amino acids in the diet are important factors affecting the deposition of liver fat in rats fed low-protein diets containing choline (4, 5, 6, 7).

This investigation is an attempt to observe and relate the fatty liver development and impaired growth of rats to diets designed to represent those of the orient. Evaluation of the qualities of proteins in those diets may lead to useful supplement formulation for alleviating fatty liver and other nutritional defects in the Far East.

REVIEW OF LITERATURE

General Dietary Habit in China

The general dietary pattern of the Chinese people is given by Krause (8). The average Chinese eats about 60% or more of his meal in bulk weight as rice with the rest as meats, fish, vegetable and others. According to the FAO 1964 report (2), in the Republic of China (Taiwan) the protein supplied in grams per day per capita is as follows:

Total protein	57
Animal protein	14
Grain	31
Starch roots	3
Pulses, oil seeds, nuts	7
Vegetables	2
Fruit	-
Meats and poultry	6
Eggs	-
Fish	7
Milk and products	1

Due to advances in agriculture and economy these figures now should be higher; however, they still reflect the distribution in the diet.

Woods (3) also summarized the rice diet of China per capita per day as follows:

Rice	359 g
Other cereals	69
Pulses	28
Vegetables	218
Fat and oil	27
Fish	30
Meat	31
Milk, egg	5
Fruit	30
Sugar	4
Total calories	2020
Total protein	55

These figures show a relatively low percent of rice consumed, about 45%.

The greatest rice-eating areas are concentrated in the south and east of

India, Southern China, the Malay Peninsula, Java, the Philippines, most of the East Indies and Japan. About two-thirds of the world's rice-growing area is in India and China. Ninety percent of the world's rice crop is produced in Eastern Asia.

The protein content of rice is lower than that of most other cereals. Compared to wheat, for example, rice provides about 35.5 grams of protein per pound as against 53.5 grams per pound. The biological value of protein in rice, however, is generally considered to be superior to that of wheat, corn or oats (3). Table 1 lists the amino acid composition of rice, corn and wheat proteins. These compositions, of course, vary with varieties. Juliano

TABLE 1

Amino acid composition of wheat, corn and rice[1].

Amino acid	% of sample		
	Rice	Wheat	Corn
Lysine	0.242	0.322	0.259
Histidine	0.158	0.270	0.343
Ammonia	0.193	0.385	0.307
Arginine	0.517	0.575	0.428
Aspartic acid	0.615	0.601	0.775
Threonine	0.222	0.366	0.424
Serine	0.306	0.564	0.592
Glutamic acid	1.135	3.814	2.712
Proline	0.270	1.169	1.277
Glycine	0.286	0.471	0.384
Alanine	0.349	0.438	0.957
Half cystine	0.095	0.471	0.351
Valine	0.361	0.478	0.577
Methionine	0.117	0.128	0.204
Isoleucine	0.253	0.423	0.443
Leucine	0.512	0.808	1.630
Tyrosine	0.253	0.368	0.521
Phenylalanine	0.327	0.555	0.641
Protein	7.0	12.2	11.4

[1]Data from Dr. Charles W. Deyoe, Department of Grain Science and Industry, Kansas State University. Tryptophan was not determined.

et al. (9) have listed the composition of five varieties of brown rice and twenty varieties of polished rice. The number of varieties of rice used by the Asian people is indeed large.

Vitamins in the husk will be lost through milling of the raw rice. Loss of nutrients varies with the degree of milling. The milling industry in Asia is on a small scale and there is no set standard process. However, lightly milled rice is generally less acceptable to the majority of consumers, white rice being preferred both as to taste and as a mark of "social prestige." Another factor against the use of husked rice or "brown rice" is its poor keeping qualities. Such rice is more susceptible to rancidity and spoilage and to insect infestation than is highly milled white rice. Parboiled rice is generally unacceptable in the Orient, aside from India, because of the accompanying changes in color and taste (3). The process of cooking also may affect the nutritive quality of rice, although Woods (3) and Juliano et al. (9) failed to find conclusive evidence.

In the Asian diet the fat content is generally low, 3-4% (3). Vegetable oil, especially peanut oil, is the main source of fat for the most of the Chinese population. The fatty acid contents of peanut oil are given in table 2.

To sum it up, the Oriental diet is generally high in carbohydrate with rice as the main source, low in protein in the forms of fish, meat and plant proteins, including of rice, and low in fat, which is usually from vegetable sources--peanut oil in particular.

Importance of Protein in the Diet

Growth and Maintenance. Protein occupies a key position in the structure and functioning of living matter (10). It is the basis of protoplasm

TABLE 2

Fatty acid contents of corn oil and peanut oil.

Saturated acids	Corn oil[1]	Peanut oil[2]
Palmitic	7.0%	9.7%
Stearic	2.4%	5.6%
Total saturated acids	9.4%	15.3%
Unsaturated acids	**Corn oil**	**Peanut oil**
Oleic	45.6%	40.8%
Linoleic	45.0%	35.9%
Iodine No.	105-125	102
Arachidic (or higher)		8.0

[1]These data were taken from a compilation prepared by Verz R. Goddard and Louise Goodall, issued by the Agricultural Research Service, USDA, May, 1959.

[2]"The Chemical Constitution of Natural Fats," T. P. Hilditch and P. N. Williams, 4th ed. John Wiley & Sons, Inc. New York (1964), p. 394.

and a component of every body cell (11). Since proteins cannot be synthesized by the animal organisms from atmospheric nitrogen or inorganic nitrogenous compounds, protein foods from animal and/or vegetable sources are needed to build living tissue. Osborne and Mendel (12) indicated that the first function of protein is maintenance and the second, growth. Protein ingested above the amount needed for the repair of the normal breakdown of the body may be used for growth.

After protein is consumed by an animal, most of it is broken down to the constituent amino acids and the amino acids are absorbed (10). The absorbed amino acids are the building blocks with which the animal synthesizes its own protein molecules. Proteins are made up of about 20 amino acids, 8 of which are considered essential (indispensable) for maximal growth for human beings and 10, for rats. The essential amino acids must be ingested for they cannot be synthesized from other constituents of the diet at a rate adequate for maximal growth by the animal. They must be present in the body in proper proportions to build body protein.

The Food and Agriculture Organization Committee on Protein has suggested an ideal essential amino acid pattern based largely upon the data available on requirements for growth and maintenance in adult human beings (13). Essential amino acid patterns required for growth and maintenance in rats and man also have been studied and compiled by Allison (14, 15). For the last half-century emphasis on the relationship of essential amino acid content and nutritional quality has been prevalent. Recent evidence indicates that a certain level of "unessential" nitrogen also must be considered; a broader term, "non-specific"[1] nitrogen might be more meaningful in defining human protein requirement (16, 17). A considerable amount of data from studies with the chick has suggested that the requirement for individual amino acids is not independent of total protein supply. For one, Grau (18) showed that the lysine requirement of chicks increased as the total protein content of the diet was raised. Snyderman et al. (19) fed young infants milk to the point that satisfactory nitrogen retention and no growth occurred. The addition of glycine to the diet at this point resulted in prompt resumption of growth and retention of a normal amount of nitrogen. Thus they concluded that the limiting factor in milk protein was the total nitrogen rather than a particular essential amino acid. However, data from a similar study

[1]Nitrogen from any metabolically usable, non-toxic source, including excess essential amino acid nitrogen not utilized in meeting essential amino acid needs.

conducted on rats (20) were contrary to the conclusion of Snyderman et al.

More investigation is needed in estimating protein and amino acid requirements for growth and maintenance (21).

Labile Protein Reserves and Liver Protein. It is recognized that the body does not store protein to create reserves in the same way that fat and carbohydrate are metabolized, but it metabolizes in a dynamic state. The dynamic state was characterized by Whipple (22) as a type of metabolic pool in and out of which amino acids are shifted as they are required. The protein pool permits rapid and continuous interchange of nitrogenous compounds between synthesis and breakdown of amino acids (15). The tissue proteins that can be depleted to contribute amino acids to the metabolic pool are called "protein reserves" (23). Some definitions of protein reserves include all body proteins that can be depleted and replaced. The labile reserves represent only a small fraction of the total body protein, but the debate concerning the significance and the role of protein reserves is often restricted to these very labile tissue proteins (24).

Several workers disagree with the concept of protein storage. Halac (25, 26) found that rats fed a high-protein (64% casein) diet had less resistance to stress conditions of protein deprivation than those on a medium-protein diet (27% casein). Observations on growing rats indicated that 15% protein diets were optimum for resistance to the stress of protein deprivation. Intakes below this figure might be regarded as in the range of protein malnutrition; diets over 15% protein increased weight gain but failed to improve resistance to the stress of protein deprivation, since the extra protein was not stored. Halac stated that his studies failed to reveal any benefit from the additional protein ingested to the stress of protein deprivation. Presumably the body had maintained its constancy of nitrogen composition in the face of an elevated protein intake by increasing the protein turnover rate (27, 28). Holt et al. (29) pointed out two misconceptions in interpreting nitrogen retention as "protein reserves" on the basis of (a) when an individual's protein intake is below his minimum requirement, he subsists on his own tissue, and (b) nitrogen loss is not the loss of protein that has been stored, but rather the loss of all the components of the tissue. Therefore, he suggested the term "tissue protein" instead of "reserve protein."

Addie et al. (30, 31, 32) observed that the various organs of the body exhibited different rates of decrease in protein when dietary protein decreased. Livers of rats fasted for seven days lost 40% of their original protein; whereas the kidneys lost 20%, the heart, 18% and all the other organs, 4%. Each organ also had its own rate and degree of rebuilding of protein. Evidence has been obtained indicating that the loss of protein reserves will be accompanied by changes in the metabolism of ribonucleic acid and phospholipid in the liver cells (30). Some of the enzyme proteins in the tissues may be considered as "labile protein reserve." The studies of Wainio et al. (33, 34, 35) showed that the total protein and certain enzymes of the liver were the most labile in protein depletion, while the total protein and the enzyme systems in the brain were resistant. The reduction in activity of some enzyme systems, particularly those associated with "protein reserves" of the liver, were inversely correlated with nutritive value of dietary proteins as determined by the standard methods for nutritive value (36, 37, 38, 39). Allison et al. (40) stated that the rise and fall of liver proteins were influenced by increase or decrease of dietary proteins. Since the protein content of the liver correlates with protein in the diet, Henry et al. (41)

suggested the use of a liver protein method for the assay of nutritive value of proteins. When their method was tested, the relative values for several types of proteins agreed with the values found by nitrogen balance and growth methods.

Amino Acid or Protein Supplementation

The shortage of protein among the cereal-eating populations of the world is frequently one of quality rather than quantity. In most cereal and vegetable proteins, one or more essential amino acids usually is deficient, and those proteins are called "low quality proteins" (42). Protein of particular foods can be improved through amino acid fortification (43) and, from a nutritional viewpoint, it is equally effective to supplement with specific amino acids or with food proteins. Harper (44) however, emphasized that (1) the successful amino acid supplementation depended upon an accurate knowledge of the type of diet being consumed by the subject, and (2) under some circumstances, amino acid supplements may not only have no benefit but actually cause adverse effects. It is important to ensure that the diet is adequate in all other respects when amino acid fortification is being considered. Normally an increase in growth or improvement in nitrogen retention in response to amino acid supplementation is taken as evidence of general improvement. In certain cases, however, it has been demonstrated that, although growth has been stimulated by amino acid supplement, accumulation of fat in the liver increased (43, 45).

Cost of amino acid supplementation should be considered especially for developing countries. Flodin (46) cited an example of wheat protein supplemented with fish flour or lysine, to obtain a protein value similar to that of casein. The calculations indicated that the lysine cost more than the fish flour. Therefore, ideally, the solution of the protein-deficiency problem will involve the greatest practicable use of available proteins jointly with amino acid fortification to develop maximum biological value (46).

There are several reports on supplementation of rice diets with protein-rich vegetables, eggs and fish protein concentrate (FPC) (45, 47, 48, 49, 50) and by fortification with limiting amino acids (51, 4, 52, 53) to improve the nutritive value of rice protein.

Supplementing the protein of FPC by rice in rat diets has been investigated by De et al. (50). The partial replacement of FPC by rice protein in the ratio of 4:1 considerably improved the Protein Efficiency Ratio (PER) of FPC protein. In a reverse manner, the substitution of two parts of 84 parts of rice by FPC caused a 40% improvement in the PER value in a 5.8% rice protein diet. Also, the ratio of liver fat/protein-nitrogen decreased as the FPC fortification increased. Table 3 shows supply of lysine, S-amino acids and tryptophan in rice, fish and egg as compared to the FAO provisional requirement pattern (50).

TABLE 3

Supply of lysine, S-amino acids and tryptophan from egg, rice and fish.

Amino acid	FAO provisional pattern requirement mg/g N	Supply in mg/g N from protein sources		
		Egg	Rice	Fish
Lysine	270	396	236	549
S-Amino acids including methionine	270	342	222	262
Tryptophan	90	106	65	62

Egg, having an assigned Biological Value of 100, is a high quality protein. Kik (49) reported that the protein of whole and milled rice can be supplemented effectively by defatted whole eggs. By the addition of a small amount (1%) of defatted whole egg to white polished rice or whole brown rice diets of rats, higher weight gain and PER were obtained than for either the polished whole rice or whole egg controls. This indicated a true and real supplementation. Tryptophan, lysine, threonine and the combination of cystine and methionine are much lower in rice proteins than in egg protein. It might be assumed that providing these amino acids was mainly responsible for the high supplementary effect of egg observed in the growth experiments, especially as lysine is considered to be the main limiting amino acid in rice proteins.

Soybean contains up to 40% protein, the nutritive value of which is among the highest of all protein sources of vegetable origin. In comparison with other plant protein, soybean protein is unusually high in lysine and is, therefore, useful as a supplement to the cereals. The limitation of soybean in terms of protein nutritive quality may be primarily a moderate deficiency in methionine (54). Harper et al. (4) fed the rats a large portion of rice (84%) supplemented with 3% protein from soybean flour for two weeks to test the effect on growth rate and liver fat deposition. The result showed that soybean flour was the least effective supplement among the following supplements: casein fibrin, pork, and fish meal (to supply 3% protein). Panemangalore et al. (48) studied the effect of supplementing a poor rice diet commonly used in India with soybean flour, methionine-fortified soybean flour or skim milk powder which was fed to girls ages 8 to 9 years. The results showed that methionine-fortified soy flour was almost as good as skim milk powder and significantly superior to soy flour as a protein supplement to a rice diet.

As for rice supplemented with amino acids, Pecora (51) and Harper (4) reported that a significant improvement in the nutritive value of white rice was accomplished by the addition of two essential amino acids, lysine and threonine. This combination produced a growth response in rats three times that obtained with an unsupplemented rice diet. When rice was supplemented with all the deficient essential amino acids simultaneously, growth was improved considerably more than when supplemented only with lysine and threonine; but optimum growth was still not obtained.

In the above studies no beneficial effect was observed when methionine was added to the rice diet fortified with lysine and threonine. In other animal experiments, Sure (55), however, found an increase in the PER of rice proteins when methionine was added in addition to lysine and threonine. Parthasarthy et al. (53) found that the addition of methionine to rice containing lysine and threonine resulted in a further improvement in nitrogen retention for girls 8 to 9 years old.

It might be more desirable, however, to improve the quality of cereal proteins by genetic means than by supplementation. A high-lysine variety of corn had been developed (56). Similar improvement in the amino acid content of rice protein would help solve the "poor quality protein" conditions in the rice-eating population.

Correlation of Liver Fat Deposition with Dietary Protein and Fat

Protein. The extensive literature on nutritional factors that affect the deposition of liver fat has been reviewed (57). Channon and Wilkinson

(58) demonstrated that the lipotropic effect of protein was proportional to the level of protein in the diet. Winje et al. (7) stated that the fat which accumulated in the liver of rats fed low-protein diets containing choline was reduced when dietary protein was increased. They suggested that the maintenance of normal fat deposition in the livers of rats fed low-protein diets containing choline depended upon the presence of a specific ratio of amino acids in the diet, especially lysine, threonine and methionine (7). The Food and Nutrition Board (43) mentioned that deficiency of at least three amino acids, lysine, threonine and tryptophan, led to fatty infiltration of the liver. The degree of such alteration in liver lipid content depended upon the degree of deficiency; partial restriction produced more extensive changes than did total deprivation. Harper (57) reported that amino acid imbalance may cause an accumulation of liver fat, particularly in the periportal area of the liver lobules. Kosterlitz (59) found that the first few days on protein deficient diets, rat liver cells contained more fat than normal.

Williams (60) reported that when rats were fed protein deficient diets, the ratio of liver weight to body weight increased almost linearly. The nitrogen concentration per milligrams of liver fell initially at a rapid rate, leveled off, and began to increase after 30 days. It is likely that glycogen and lipids were deposited during the first 30 days, and then the glycogen and lipids were lost from the liver cells, having been used for energy, which resulted in a relative increase in nitrogen.

Sauberlich (57) suggested that normal liver fat values may be obtained in some cases when high-protein diets deficient in a single amino acid are fed. This suggests that the total nitrogen level of the diet as well as the balance of amino acids may influence the effects of amino acid deficiencies.

Indeed, it has been noted that certain non-essential amino acids in rather high levels may cause some reduction in the level of fat in the livers of rats consuming protein-deficient diets that contain choline (61, 62). Dakshinamurti (45) demonstrated that rats maintained on the poor vegetarian rice diet showed a sharp increase in liver fat, reaching 12.5% (wet weight) at the end of the fourth week. The increase in the total liver lipids was accounted for mainly by the rise in the glyceride (neutral fat) fraction. A slight increase in total cholesterol was due mainly to a rise in cholesterol esters. A significant feature of the lipid distribution in those rats fed this poor vegetarian rice diet is the fall in phospholipid. According to Harper et al. (4), accumulation of fat in the liver was one of the signs of kwashiorkor. They found that fat accumulated to the extent of 8% to 10% (net weight) in the livers of rats fed rice diets for two weeks. The inclusion of 0.2% of L-lysine, HCl and 0.24% of DL-threonine in the rice diet (87%) did not prevent the accumulation of liver fat however the fat content of liver was normal when 0.4% L-lysine HCl was included with 0.24% but the growth response was somewhat less. It seems that the specific amino acid ratio which improved growth rate may not be the same specific amino acid ratio for preventing the accumulation of fat in the liver of rats.

Effects of high rice, low protein, and low fat diets on livers of albino rats have been investigated by Vijayaraghavan and Patwardhan (63) who used a common poor-Indian diet consisting of 82% rice, 10% protein and 4.2% fat. Fatty liver condition occurred in the first four months, but fat content decreased to almost normal level in the next four months. They attributed the increased fat to combined deficiencies of choline and methionine in the diet; as body growth stopped choline and methionine were available for normal lipid metabolism in the liver.

Fat. The nature of the dietary fat effects on liver fat deposition has been noted: (1) deficiencies of essential fatty acids, which in the presence of choline cause increased liver fat deposition and, (2) of different types of fatty acids on severity of fatty infiltration induced by deficiencies of lipotropic factors (64, 65). The saturated fatty acids tend to increase severity of the infiltration and requirement of choline, and unsaturated fatty acids tend to alleviate it and lower the choline requirement (65).

Holman (68) has suggested linoleic and arachidonic acids as essential fatty acids. The requirement for essential fatty acids is usually expressed as linoleic acid. Normal weanling rats should receive 50mg/day.

Mayes (69) has shown that lipid which accumulates in the livers of essential-fatty-acid (EFA)-deficient rats is mostly triglyceride. The quantity of triglyceride present in the liver is significantly increased during starvation and the feeding of high-fat diets. The increased levels of free fatty acid in the plasma occur because the production of plasma lipoprotein does not keep pace with the influx of free fatty acid (FFA), allowing triglyceride to accumulate and causing a fatty liver.

Sinclair et al. (67) showed that rats fed the EFA-deficient diet for 20 weeks had significantly more triglycerides in their livers than the control rats; the liver phospholipids also were increased by the deficiency but not as markedly as the triglycerides. Another characteristic of the EFA-deficiency is the appearance of 5,8,11-eicosatrienoic acid in the liver lipids, especially in the liver phospholipids. In the deficient rats eicosatrienoic acid accounts for 22 to 23% of the liver phospholipids; other significant changes were large decreases in the two EFA (linoleic and arachidonic), increases in both oleic and palmitoleic acid, and decreases in stearic acid. Major changes occurred in the first three weeks the rats were fed the diets. The male rats took at least ten weeks to develop fatty livers when fed diets lacking EFA. After 10 weeks the rats had been depleted of those fatty acids in their livers. A similar observation was made by Engel (66).

In addition to studies of triglycerides and fatty acids, the accumulation of cholesterol in the liver has also been investigated. Jagannathan (70, 71) fed rats diet containing 1% cholesterol and 7.5% linoleic acid, and found that the concentration of liver cholesterol in these rats was significantly lower than those fed similar diets having lower levels of linoleic acids. The same investigator found that the presence of short- and medium-chain fatty acids in the butter fat and coconut oil blends might be partly responsible for the higher elevation in serum or liver cholesterol concentration. Hydrogenated groundnut (peanut)--fat blend, containing identical linoleic acid contents and more than 14 carbon atoms, reduced fat deposit in the liver of rats. Several investigators (71, 72, 73) have discussed the effects of the non-fatty acid portion of the fat on cholesterol levels in the serum and liver: unsaponifiable matter appears to have a marked cholesterol-lowering effect.

It appears, then, that fatty liver can be caused by deficiency and depletion of essential fatty acids, the type of fatty acids available, and lipotropic factors. Those deficiencies, especially in the quantitative aspects, however, are not well defined.

Measurement of Protein Quality

There are two general ways to evaluate protein quality. First, the quality of protein can be indirectly tested in relation to some effect in the body, such as growth or nitrogen balance. Second, it can be compared to the pattern required by the recipient for optimum growth.

Amino Acids. Proteins are made of more than 20 amino acids. All monogastric animal species including humans, under all conditions require a continuous dietary supply of isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Histidine is required for growth, and maintenance in the adult rat but is not needed for maintenance by the adult human (74, 75, 76). Arginine can be synthesized by rats, but since the rate of synthesis may be limited, a dietary supply is necessary for the optimal growth.

Reference patterns. The National Research Council (79) stated that the nutritive value of a dietary protein depended upon the pattern and quantity of essential amino acids presented to the body after absorption from the intestine. Assuming that chemical analysis of a dietary protein reveals the pattern of amino acids liberated in and absorbed from the gastro-intestinal tract, an estimate can be made of the nutritive value of the protein by comparing the amino acid pattern derived from chemical analysis with a reference pattern. Actually this assumption is not always satisfactory, since liberation of amino acids in the intestinal tract through digestion and rates of absorption may alter the pattern from the one calculated from analytical data. Amount of lysine available to the animal from a food, for example, may differ markedly from the amount of lysine determined chemically. The lack of availability is often the result of food processing (77, 20).

The reference pattern for a given age, sex, and nitrogen expenditure should be the pattern which will allow the adult subject to maintain itself in nitrogen balance with a minimum nitrogen intake, or for a growing subject, normal growth with a minimum nitrogen intake. Ideally, the pattern should be completely utilized for anabolism; that is, it should possess a net protein utilization and biological value of 100 (78). Reference patterns are expressed as amino acid ratios with tryptophan as unity or in terms of amino acid concentration per unit of nitrogen. The calculations of the ratios place much emphasis upon the value of the tryptophan requirement. Bender (20) attempted a direct determination of the relative adequacy of each of the amino acids at the levels they occurred in egg protein, by using an amino acid mixture based upon the composition of egg protein. By the test he found that tryptophan was present in egg protein at 160% of the amount required. If this is a more valid estimate of the tryptophan requirement, the ratio of the requirements of other essential amino acids to tryptophan would be markedly changed from that in egg protein.

Mitchell and Block (79) proposed a "Chemical Scoring" system based on the single essential amino acid in maximum deficit compared to whole egg protein. Bender (20), however, found that the predicted utilization of protein based on chemical score failed when most of the essential amino acids were individually limiting to a serious degree. Oser (80) pointed out that the most limiting amino acid might not determine entirely the nutritive value but that all deficits might contribute. He proposed, therefore, an "Essential Amino Acid Index" which is the geometric mean of the ten nutritional egg ratios; this is computed logarithmically:

Essential Amino Acid (EAA) Index = 10 $\frac{100a}{a_e} X \frac{100b}{b_e} X \cdots\cdots X \frac{100j}{j_e}$ where $a, b, \ldots\ldots j$ are percent of each amino acid in a food (N X 6.25); $a_e, b_e, \ldots\ldots j_e$ are percent of the respective amino acids in whole egg protein. The egg ratio is the percentage ratio of amino acids in the dietary protein relative to their content in whole egg protein. This ratio can be plotted into an egg-ratio-curve or essential amino acid spectrum. This essential amino acid spectrum can indicate the complete amino acid picture. It often is used to predict the efficiency of utilization of protein. The essential amino acid indexes and biological values are relatively quite similar; that is to say, the EAA Index can be used to estimate or predict the biological value of a protein. An interesting property of the EAA Index is that it can be used to predict the effect of supplementation of proteins with amino acids individually, in group, or in the form of intact protein. Another example of the use of EAA Index is its application to the rating of the average essential amino acid content of food combinations of whole diets, or of the average consumption of population groups.

The pattern of amino acids needed for growth may differ from the one needed for maintenance, and the optimum patterns may vary with physiological state of the individual (81). However, results from Mitchell's experiments suggest that the optimum over-all pattern for growth will be best also for maintenance and replenishment of depleted tissues (82). For that reason, whole egg protein, which has the highest nutritive value for growth, has been considered as having an ideal reference pattern of essential amino acids. The essential amino acids of human milk have also been proposed as a reference for man (83). A reference pattern for rats has been constructed by carcass analysis data (84).

The FAO provisional reference pattern (78) has been derived from experimental values representing the requirements of mature subjects and infants for individual amino acids, when each was a part of a mixture of free amino acids. The pattern was called "provisional" because the committee recognized the need to study more extensively the requirement of amino acids for growth and maintenance and the effects of various patterns on the development of different tissues under physiological states.

Imbalances and toxicities. As stated earlier in this review, the nutritive value of many proteins can be improved by the judicious use of amino acid supplements. However, the many cases in which amino acid supplements have been used with success should not be allowed to obscure the fact that there are conditions under which the addition of amino acids to a diet may cause adverse rather than beneficial effects (85). A short time ago it appeared plausible to classify all of the adverse effects resulting from addition of amino acids under the general heading of amino acid toxicities. However, as information has accumulated, there appears to be enough evidence for refinement of the classifications (5).

The additions of unbalanced proteins or mixtures of amino acids lacking a single indispensable amino acid to low-protein diets were found to cause depression in growth rate of rats. This would be prevented by supplement of the amino acid that was most limiting in the diet (86, 87).

Harper (88) explained the differences between amino acid balance, unbalance and imbalance as follows:

> A protein that provides amino acids in roughly the proportions needed by the body is termed a balanced protein and has a high biological value. A protein that is low in one or more of the indispensable amino acids is termed an unbalanced protein and has a low biological value. The term "amino acid imbalance" describes "those cases in which the addition of a relatively small amount (what might be called a supplement amount) of an indispensable amino acid, or a mixture of such amino acids, or of an unbalanced protein to a diet that is low in one or more amino acids, causes a retardation of growth or some other adverse effect that can be completely prevented by supplementing the diet with a relatively small amount of the most limiting amino acid or amino acids" (88).

The critical points that distinguish imbalance from other changes in dietary amino acid patterns that cause adverse effects are that the diet must contain a marginal amount of at least one indispensable amino acid, and that the effect of imbalance can be prevented by a small supplement of the most limiting amino acids in the diet. This excludes antagonism and toxicities, in which the adverse effects are caused by addition of a large excess of individual amino acids and are seldom completely prevented by a relatively small supplement of the most limiting amino acid in the diet, nor by a high protein intake, particularly if the dietary level of protein and the level of amino acid causing the adverse effect are increased proportionately. However, these adverse effects are less severe when either the quantity of protein in the diet or the quantity of the limiting amino acid in the diet is increased (5).

There are two types of amino acid imbalances. One results from the addition of a quantity of protein or an amino acid mixture lacking one indispensable amino acid to a diet which contains a low to a moderate amount of proteins (86, 89). The other results from addition of a small amount of amino acid or acids to a diet that is low in protein. Although such an addition does not alter the amino acid balance greatly, it may cause a substantial depression in growth rate. The amino acid causing the imbalance is usually the one that is second most limiting for growth and the imbalance occurs when the protein content of the diet is relatively low (90). The creation of imbalances by the former procedure is usually quite predictable. The creation of imbalances by the latter one is not routinely predictable, which immediately raises the question of the specificity of the effect (5).

As noted earlier in this text, amino acid deficiencies and imbalances may cause not only reduced growth but also an accumulation of liver fat, particularly in the periportal area of the lobule of the liver (43). In general, this effect is corrected by amino acid or protein supplementation. In at least one case it was induced by an amino acid imbalance, but in others it occurred only when the diet was supplemented to a certain level with the limiting amino acids (6).

Fisher et al. (91, 92) demonstrated that growth depression could be caused by excessive addition of dispensable amino acids. The toxicity of a high dietary intake of tyrosine and methionine has also been shown (93, 94). Russell et al. (95) suggested that the basis for the effect was probably dependent on the structure and metabolism of the particular amino acids. Methionine, tyrosine, tryptophan and histidine, which enter into many metabolic pathways, appeared to be the most toxic. Isoleucine and valine which are readily oxidized completely and are not metabolized by a wide variety of pathways, were less toxic (96). The others fell in between (97).

Many experiments have shown the effects of amino acid imbalances in rats. It seems likely that all animals are susceptible to such effects. The significance of amino acid imbalance in human nutrition cannot yet be appraised. Adverse effects, seen as a decrease in nitrogen retention, have been observed to follow the administration of amino acid supplements. Holt et al. (98) has cited an example in which an amino acid supplement caused a growth depression in human infants consuming diets in which the protein was provided from plant sources. Hundley et al. (99), working with four adult humans consuming largely rice, observed imbalances, although results were not conclusive. There is, therefore, no reason to assume that the effects of amino acid imbalance are unlikely to occur in man, only that the possibility remains unexplored; and there is need for extensive study of the effects of amino acid supplements in the diets of man, particularly in the low-protein diets consumed by growing children.

Utilization of Protein

Growth and Protein Efficiency Ratio. As stated earlier, the quality of a dietary protein can be measured by an analysis of utilization of that protein by the body. Mitchell (100) stated that the determination of the nutritive value of a protein was a study of the nitrogen economy of animals that were fed the protein tested. The utilization value of protein can be measured by growth or growth and nitrogen balance data may be used to evaluate PER value; such methods do not demand any knowledge of the amino acid composition of the protein.

Growth or gain in body weight has been the most general and widely used criterion for measuring protein value. However, body weight gain is not an entirely satisfactory measure of growth of new tissue protein. Gain in body weight can be the result of other constituents, fat and water (101). Although gain in body weight in the rat generally correlates well with gain in body protein, this correlation is not always good. It is especially variable in some animals, such as the dog (102). Dogs receiving diets of egg albumin, casein and wheat gluten gained weight. The former two diets developed lean, active muscular animals, but those fed the last diet were obese and inactive, exhibiting some apathy. Associated with protein depletion, the dogs fed wheat gluten developed only about one-third as much body nitrogen as those fed the egg protein or casein. The gain in body weight, therefore, is not always a good measure of protein synthesis in the body as this may occur due to fat deposition. Correlation between gain in body weights and liver protein and fat content has been established (50).

Osborne et al. (103) introduced the concept of "Protein Efficiency Ratio" (PER) as a means of evaluating protein quality by growth performance. This test recognizes the fact that animals receiving diets of low protein quality will show some loss of appetite; therefore, PER relates the gain in body weight to the amount of protein or nitrogen consumed during the period of study.

Protein Efficiency Ratio also varies with the level of protein in the diet. Middleton et al. (104) stated that the effect of increasing protein levels on PER values was dependent on protein quality. Good quality proteins showed reduced PER values as the protein content of the diet was increased from 10 to 15 per cent, whereas the reverse was true for proteins of poorer quality. Derse (105) recommended that the test protein be at a level of approximately 10% by weight. The National Research Council (78) reported that PER values were influenced by the strain and sex of the rats used, the age of the rats at the beginning of the experiment, the length of the experimental period and the level of dietary protein. It was concluded that these factors had to be standardized if comparable results were to be obtained in different laboratories. Middleton et al. (104) believed that for practical regulatory or control purposes, PER determinations, carried out under careful standardized conditions, would provide reliable estimates of the nutritional value of proteins.

Nitrogen Balance. Nitrogen balance is a direct measurement of nitrogen retention and is defined as the difference between dietary nitrogen intake and nitrogen excreted in the urine and feces plus dermal losses. Nitrogen losses from the skin are frequently neglected because it is impractical to measure them (78).

When discussing nitrogen balance, the dynamic state of nitrogen balance, the dynamic state of nitrogen metabolism (discussed on page 8), should be considered, since both diet and the body contribute to the nitrogen metabolic pool, a portion of which is excreted continually. If the body nitrogen intake is greater than the excretion, there will be a positive balance. Such a balance is characteristic of growth of new or restoration of depleted tissue. Indeed, the maintenance of an adequate positive nitrogen balance in young growing humans and other animals is a good measure of the nutritive value of the dietary protein and the diet (106).

Scrimshaw and associates (107) have successfully used the maintenance of positive nitrogen balance in children as a measure of the nutritive value of various dietary proteins and the supplementing effects of amino acids.

Nitrogen balance, however, is the algebraic sum of gains and losses from all the tissues of the body. It is possible for an individual to be in positive balance by making an over-all gain in body nitrogen and yet to be gaining more adequately in one tissue than in another. Maintenance of nitrogen equilibrium does not mean that every tissue protein is being maintained (78). Allison (106, 108) gave an exaggerated example of this: positive nitrogen balance was established in the tumor-bearing animal; the tumor was in strong positive balance, the liver also might be in positive balance but many of the other tissues, especially the muscle, might be losing nitrogen during the catabolic phase of the tumor growth. Thus, maintaining the normal physiological state of the experimental individual is one of the most important precautions.

Recently, however, the general validity of the nitrogen balance determination has been questioned by some research workers. Costa (109) observed in a series of nitrogen balance studies on dogs, rats and mice that considerable nitrogen retention was recorded over quite long periods without a corresponding gain in weight. Costa concluded that a sizeable amount of nitrogen of dietary origin might be lost by some unsuspected route. Wallace (110) pointed out that the errors in measurement of N-retention were cumulative, since intakes tended to be overestimated because of unrecognized food losses, and output to be underestimated because of incomplete collection; these errors resulted in the false N-retention values, which became more serious the higher the level of intake. Waterlow (111) gave four possible sources of these discrepancies between N-retention and weight gain: (1) they result from loss of N by routes other than urine and feces; (2) there are technical errors in the balance measurements; (3) nitrogen is stored as non-protein N; (4) the assumption of a constant protein content of the lean body mass is not correct. The National Research Council (78) stated that the nitrogen balance methods are useful, reliable and reproducible if the precautions are taken. The disadvantages of nitrogen balance methods are that they are expensive and relatively time consuming.

EXPERIMENTAL PROCEDURES

Materials

Uncle Ben's long grain polished, enriched rice was used in this study. Dried egg powder (Seymour Foods, Inc., Topeka, Kansas), fish meal (Menhaden, commercial sample), and soybean meal (Kansas Soya Products, Emporia, Kansas), all were supplied by the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas. Dried egg powder and fish meal were defatted by the supplier by extracting three times with Skellysolve B. The following essential amino acids, except DL-valine (Eastman Kodak) and DL-methionine (Merck and Co., Inc.) were obtained from Nutritional Biochemical Co., Cleveland, Ohio: L-tryptophan, L-histidine•HCl, L-lysine•HCl, L-leucine, L-isoleucine, DL-phenylalanine and L-threonine.

The proximate analyses of rice, soybean meal, defatted dried egg powder, and defatted fish meal, as determined by the method of AOAC (112) are presented in table 4. The analyses for 17 amino acids and ammonia in the rice, dried egg powder, fish meal and soybean meal were performed in a Spinco (Model 120) Amino Acid Autoanalyzer. The amino acid contents of these products are shown in table 5. Tryptophan content was not determined.

Diets

Table 6 gives the content and percentage composition of the five test diets used. The rice diet without protein supplement contained 6.02% protein. The other diets supplemented from sources indicated in table 6 contained approximately 9.09% protein. In the rice diet supplemented with essential amino acids, 5.60% of the protein was from rice and the remaining protein was from the essential amino acids added to meet the minimum

TABLE 4

Proximate composition of rice and three protein supplements[1].

Constituent	Rice	Dried egg powder	Fish meal	Soybean meal
	%	%	%	%
Protein (N X 6.25)	7.0	66.4	65.4	48.0
Fat[2]	0.40	3.0	1.3	2.2
Ash	0.62	5.2	19.2	6.1
Crude fiber	0.70	0.60	1.2	2.6
Moisture	9.1	7.1	8.6	8.5
Nitrogen-free extract[3]	82.18	17.7	4.3	32.6
Carbohydrates[4]	82.88	18.3	5.5	35.2

[1]Analyses performed by the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas.

[2]Ether extractable material.

[3]Nitrogen-free extract is protein, fat, crude fiber, ash and moisture subtracted from 100.

[4]Carbohydrate is nitrogen-free extract + crude fiber.

TABLE 5

Amino acid composition of rice[1], dried egg powder[1], fish meal[1] and soybean meal.

Amino acids	Rice		Dried egg powder		Fish meal		Soybean meal	
	Sample as fed	In protein[2]	Sample as fed	In protein[2]	Sample as fed	In protein[2]	Sample as fed	In protein[2]
	g/100g	g/100g	g/100g	g/100g	g/100g	g/100g	g/100g	g/100g
Lysine	0.242	3.462	5.157	7.766	4.917	7.518	3.522	7.337
Histidine	0.158	2.261	1.651	2.487	1.780	2.722	1.494	3.112
Ammonia	0.193	2.756	1.047	1.578	0.741	1.134	1.265	2.636
Arginine	0.517	7.382	4.359	6.619	4.425	6.766	4.026	8.387
Aspartic acid	0.615	8.785	7.499	11.294	6.532	9.988	6.820	14.208
Threonine	0.222	3.174	3.374	5.081	2.781	4.252	2.262	4.712
Serine	0.306	4.378	5.365	18.080	2.020	4.465	2.873	5.984
Glutamic acid	1.135	16.217	9.751	14.685	9.764	14.930	11.099	23.123
Proline	0.270	3.854	2.513	3.784	4.615	7.057	2.814	5.864
Glycine	0.286	4.086	2.401	3.615	7.660	11.712	2.376	4.950
Alanine	0.349	4.983	4.245	6.394	5.065	7.745	2.425	5.052
Half cystine	0.095	1.360	3.108	4.681	0.492	0.752	0.695	1.449
Valine	0.361	5.158	1.439	2.167	3.401	5.201	2.695	5.615
Methionine	0.117	1.676	1.544	2.326	1.687	2.579	0.584	1.218
Isoleucine	0.253	3.617	3.634	5.473	2.628	4.019	2.493	5.195
Leucine	0.522	7.317	6.259	9.426	5.027	7.687	4.336	9.033
Tyrosine	0.253	3.609	2.895	4.360	2.049	3.133	2.044	4.258
Phenylalanine	0.327	4.678	3.817	5.749	2.621	4.008	2.901	6.044
Tryptophan	0.090[3]	0.100[3]	0.920[4]	1.400[4]	0.600[3]	0.650[3]	0.600[3]	0.670[3]
Protein (of sample)	7.00		66.40		65.40		48.00	

[1]Analyses performed by the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas.

[2]Gram of amino acid/100g of protein (Kjeldahl nitrogen x 6.25) in the foodstuff.

[3]From Joint United States Canadian Table of Feed Composition, 1959.

[4]Ref. No. 49 table II.

TABLE 6

Percentage composition of experimental diets.

Ingredients	Diets				
	I R+EAA[1]	II R+DEP	III R+FM	IV R+SBM	V R
	%	%	%	%	%
Rice	80.00	80.00	80.00	80.00	86.00
Protein supplement	EAA[2]	5.26	5.33	7.27	-
Peanut oil[3]	7.68	7.52	7.61	7.52	7.66
U.S.P.XI V salt mix.	4.50	4.23	3.48	4.01	4.47
Vitamin mixture[4]	1.00	1.00	1.00	1.00	1.00
Cellulose (Alphacel)	0.44	0.41	0.38	0.25	0.40
Water	0.72	0.35	0.26	0.10	0.17
Cornstarch	2.13	1.23	1.94	-	0.30

[1] R+EAA = Rice + essential amino acids
R+DEP = Rice + dried egg powder
R+FM = Rice + fish meal
R+SBM = Rice + soybean meal
R = Rice only.

[2] Percentage composition of individual essential amino acids added is shown in table 7.

[3] This peanut oil is from Planters Oil Company, Suffolk, Virginia.

[4] As listed by AOAC (115).

TABLE 7

Comparison of requirement of essential amino acids in diet for minimum growth of rats and quantities supplied by diets containing 80% rice.

Essential amino acid	Minimum requirement for rat growth[1] (air dry basis)	Supplied by 80% rice in diet	Amount needed to supplement diet
	%	%	%
L-tryptophan	0.15	0.07	0.08
L-histidine	0.30	0.13	0.17
L-lysine	0.90	0.19	0.71
L-leucine	0.80	0.41	0.39
L-isoleucine	0.50	0.20	0.30
L-phenylalanine[2]	0.90	0.26	0.64
L-methionine[2]	0.60	0.09	0.51
L-threonine	0.50	0.18	0.32
L-valine[2]	0.70	0.29	0.41
L-arginine	0.20	0.41	----[3]

[1]Reference No. 113, table 2.

[2]DL-form was used; the amount used was twice of the L-form.

[3]Percent of arginine in 80 g of rice is more than the minimum requirement for rat growth.

requirement for rat growth (table 7), as calculated from the nitrogen content. The other rice diets contained 5.6% protein from rice and 3.49% protein from one of the following protein supplements: dried egg powder, fish meal or soybean meal. They all contained: fat 8%, ash 5%, fiber 1%, moisture 8%, vitamin mixture 1% and cornstarch to make 100%. These diets were planned as a modification of that in the method of the AOAC (112).

The rice was ground to pass through a 40-mesh sieve. The other supplemented protein sources--essential amino acids, dried egg powder, fish meal, soybean meal were received in granular or powdered form. All the ingredients, except vitamins, were weighed on a tension balance. The supplementary vitamins as listed by AOAC (112) were weighed on a Mettler analytical balance (type B6), combined and mixed before being in turn mixed in the diets. The diets were mixed in a 20-quart Hobart electric mixer (Model A200-D) for a half-hour at low speed then a half-hour at medium speed. Diets were stored in jars in a household refrigerator. Percentage composition of the experimental diets is presented in table 6. Analysis of the experimental diets for total nitrogen were as follows: rice only, 9.75% nitrogen; rice plus essential amino acids, 15.3%; rice plus dried egg powder, 15.1%; rice plus fish meal, 15.0%; rice plus soybean meal, 15.1%.

Animals and Their Care

Twenty-five weanling male albino rats (53-62 g) of the Sprague-Dawley strain were used. The animals were distributed into individual metabolic cages (8 1/2" X 4 1/2" X 2 1/4") (fig. 1) in a room maintained between 24-25°C. The experimental diets were assigned randomly, so that each experimental diet had a homogeneous group of rats in relation to total body weight (table 14, Appendix). Front and back views of the metabolic cage unit (Acme Metal

Products, Inc.) are shown in fig. 1. Water bottles and feeders were provided at the back of each cage. The arrangement of baffle and feces cup in the removable collection funnel provided at the bottom of each cage separated the urine from the feces. Urine was collected in 500-ml Erlenmeyer flasks kept below the funnel. Since the metabolic cage unit consisted of twenty-four cages, one animal was housed in a circular individual metabolic cage, slightly different in construction.

Food and water were given ad libitum. The feed cups were filled every morning and evening, and weight records were kept of food used. The feeders were adjusted to animal size to minimize feed spillage. Water bottles were washed and refilled every two or three days or whenever found to be dirty or low in supply.

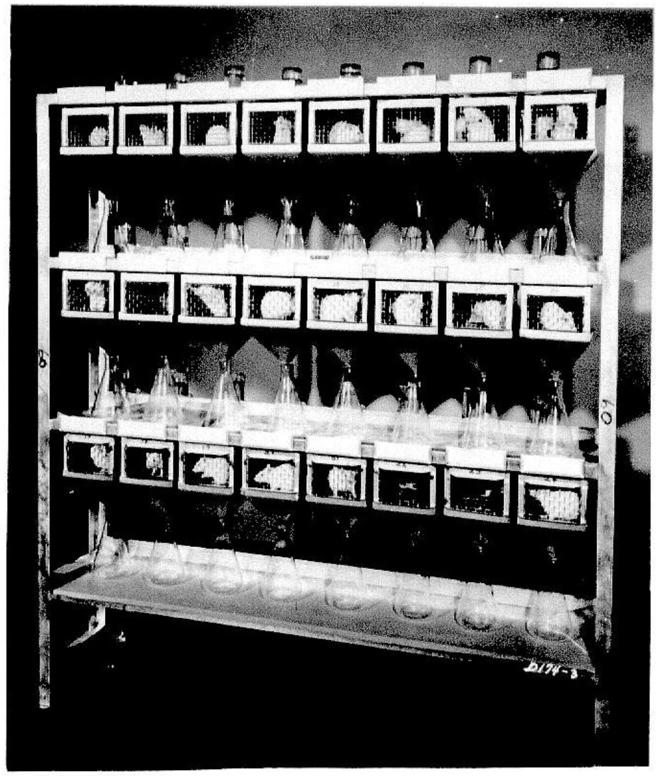
Urine and feces were collected daily; the baffle and funnel washed, dried and replaced, and the Erlenmeyer flask changed.

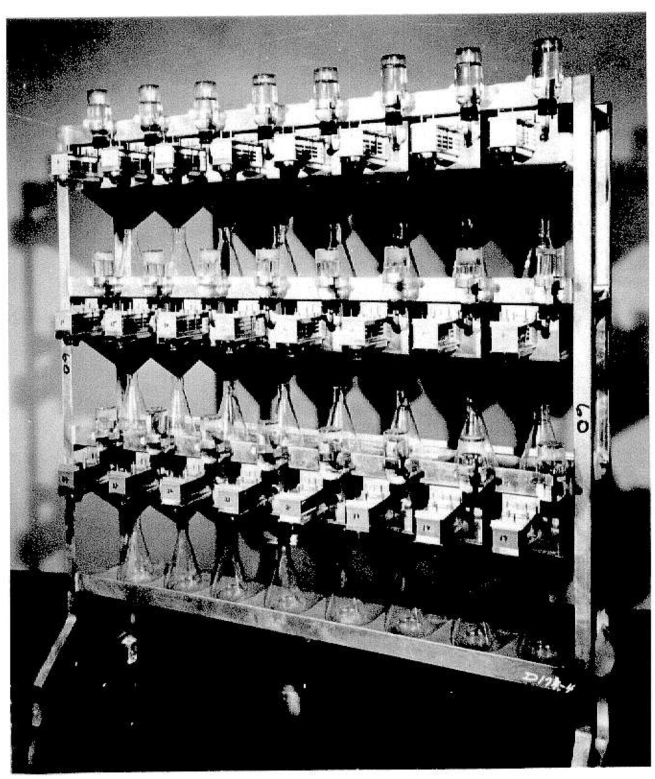
The animals were weighed to the nearest gram on a Toledo balance at the beginning of the experiment and at the end of each adjustment and collection period. Weight gain was recorded on the basis of the following periods:

Adjustment period	2 days
Collection period I	7 days
Collection period II	7 days
Collection period III	7 days
Collection period IV	7 days.

Obtaining, Preserving and Analyzing Samples

Urine. Each day of the experimental period, the urine in the collecting flasks was transferred to pharmaceutical bottles containing 3 ml of toluene as a preservative. The flasks were rinsed with distilled water and the





rinsings added to the bottles. The urine storage bottles were placed in the refrigerator. At the end of each experimental period, the urine composite of each animal was filtered through glass wool into a 250 ml volumetric flask. Three ml of concentrated hydrochloric acid was added to each flask as a further preservative and the composites were diluted to volume with distilled water. The flasks were inverted 50 times to mix the contents and the composites were poured into the clean dry pharmaceutical bottles. The bottles were tightly closed and stored in the refrigerator until the urine was to be analyzed. Prior to analysis the urine samples were brought to room temperature. Duplicate 50-ml aliquot samples of each urine composite were analyzed for total nitrogen by the macro-Kjeldahl method with boric acid modification (112). The procedure is given on page 70 (Appendix I).

Feces. Carmine (0.05g/5g test diet) was mixed with portions of each diet which were fed to the rats. The colored feces marked the beginning and the end of each collection period. Fecal material was collected from the feces cups daily, separated from hair and food sticking to it and placed in small wide-mouthed jars covered with filter paper and stored in a hood. At the end of each experimental period, the feces were dried in an oven at 65°C for 6 days to constant weight, cooled in a desiccator, weighed and ground to pass a 40-mesh screen in a Wiley laboratory mill (intermediate model). Duplicate samples were analyzed by the macro-Kjeldahl method for total nitrogen.

Livers. The animals were without food for one day before being weighed and then sacrificed. Each animal was stunned by a blow on the head with a club, and injected with sodium pentobarbital. The entire liver was removed rapidly, gently blotted with Kleenex^{R}, placed on a weighed square of aluminum foil and tightly wrapped[1]. The wrapped livers were weighed on a Mettler analytical balance, frozen and stored in a freezer at -10°C.

Prior to analysis the frozen liver was cut into two halves. Duplicate samples were prepared by slicing with a scalpel into pieces of approximately 1/6 to 1/32 inch thick as rapidly as possible. The slices of liver were put between layers of an opened cotton pad which previously had been ether-extracted dried and weighed. The liver samples in the cotton pads were put into weighing bottles, dried in vacuum oven at 110°C for 72 hours to constant weight. The dried samples were extracted with ether for 20 to 24 hours on low heat in a Goldfisch extractor. The method of extraction is given on page 72 (Appendix II).

The duplicate fat-free liver samples were analyzed for total nitrogen by the macro-Kjeldahl method (Appendix I, p. 70).

Calculation and Statistical Analysis

From the data obtained, the following parameters of protein quality were calculated:

$$\text{Percent weight gain} = \frac{\text{wt. end of period} - \text{wt. beginning of period}}{\text{wt. beginning of period}} \times 100.$$

$$\text{Protein efficiency ratio} = \frac{\text{gain in body weight}}{\text{wt. protein ingested}}.$$

Nitrogen retention = wt. nitrogen intake - wt. nitrogen excretion, where wt. nitrogen excretion = wt. urinary nitrogen + fecal nitrogen.

Analysis of variance was calculated for all measurements of protein

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[1]Thanks are due to Mr. Bill Chou, Department of Physiology, for help in sacrificing the animals and removing the livers.

quality and liver lipids to determine whether there were differences attributed to diet group or period (114). Fisher's least significant difference at the 5% level was used to note significant differences attributable to specific diets or periods. Correlation between percent liver fat and nitrogen content and correlation between percent liver fat and moisture of rats fed the five test diets were calculated.

RESULTS

The food consumption, weight and percent weight gain during the 4 collection periods for each of the animals are presented in tables 15 and 16 (Appendix). The protein efficiency ratio and nitrogen balance data of the diets during each collection period for each animal are found in tables 17 and 18 (pp. 78-81). Total liver weight, liver as percent of body weight, and the fat, nitrogen and moisture contents of the liver of each animal is given in table 19 (p. 82). The results of analysis of these are given in the following paragraphs:

Food Consumption

The mean food consumptions of rats in various diets during four collection periods, F-values and the least significant differences at the 5% level are given in table 8. For the average amount of food consumed in one period, animals fed rice only (group V) consumed significantly less food than animals fed any of the other 4 diets. There were no significant differences in food consumption between diet groups IV (R+SBM) and II (R+DEP), but the amounts of food consumed by these rats were significantly greater ($P<0.01$) than that of any of the other diet groups. Rats fed of diet group III (R+FM) had significantly ($P<0.01$) higher food consumption than those of diet group I (R+EAA).

Food consumption during Periods 1 and 2 was significantly ($P<0.01$) lower than during the other 2 periods, but were not significantly different from each other. During Period 4, food consumption was significantly higher than any of the other 3 periods. In general, there was an increasing trend in food consumption between Periods 1 and 2 for all 4 diet groups; but the diet group V (rice only) had a sharp decrease in food consumption between Periods 1 and 2.

TABLE 8

Mean food consumption of rats fed various diets during four collection periods, F-values, and least significant differences.

Groups of diets	Periods 1	2	3	4	grand means
	g	g	g	g	g
I, Rice + essential amino acids	55.2	64.4	66.6	76.4	65.6 *
II, Rice + dried egg powder	89.2	98.8	110.0	116.4	103.5 *
III, Rice + fish meal	82.0	88.4	85.6	96.6	88.1 *
IV, Rice + soybean meal	89.2	95.2	113.6	121.0	104.7 *
V, Rice only	61.4	50.0	52.0	54.2	54.4
Grand means	75.4	79.4 *	85.6 *	92.9	
F-values[1]					
Diets	142.62**				
Periods	20.53**				
Diets x periods	3.80**				
Lsd*[2]					
Diets	5.32				
Periods	4.74				

[1]** Significant at 0.01 level

[2]Lsd* Least significant difference at .05 level

Growth

Weight Gain. The cumulative average percentage weight gain of the rats fed various diets during each 7-day period is shown in fig. 2. Animals in diet groups II (R+DEP) and IV (R+SBM) had similar growth rates and the highest weight gain, followed by groups III (R+FM) and I (R+EAA) which also had similar growth rates. Growth rate of the animals in diet group V (R) was sharply depressed.

The mean percent weight gains of rats in various diets during four collection periods, F-values and least significant differences at 5 percent level are given in table 9. The mean percent weight gain per period varied significantly ($P<0.01$) among the diet groups. Weight gain of diet group V (rice only) was significantly lower than those of the other four diet groups. There was no significant difference in weight gain between diet groups II (R+DEP), IV (R+SBM) and between diet groups III (R+FM), I (R+EAA), but the weight gains of animals fed the former two diets were both significantly greater ($P<0.01$) than that of the latter two. Significantly less ($P<0.01$) growth in terms of mean percent weight gain of the animals of all five groups occurred during Periods 3 and 4 than during Period 2. Growth during Period 1 was significantly greater ($P<0.01$) than that of any other 3 periods.

Protein Efficiency Ratio. The mean protein efficiency ratio of rats fed various diets during four collection periods, F-values and least significant differences at the 5% level are given in table 10. Significant differences ($P<0.01$) for PER's among diet groups were found. In four experimental periods the highest average PER per period was obtained for diet group II (R+DEP) and the lowest for diet group V (R). Rats fed rice only (group V) had a significantly lower PER than rats receiving the other 4 diets. No

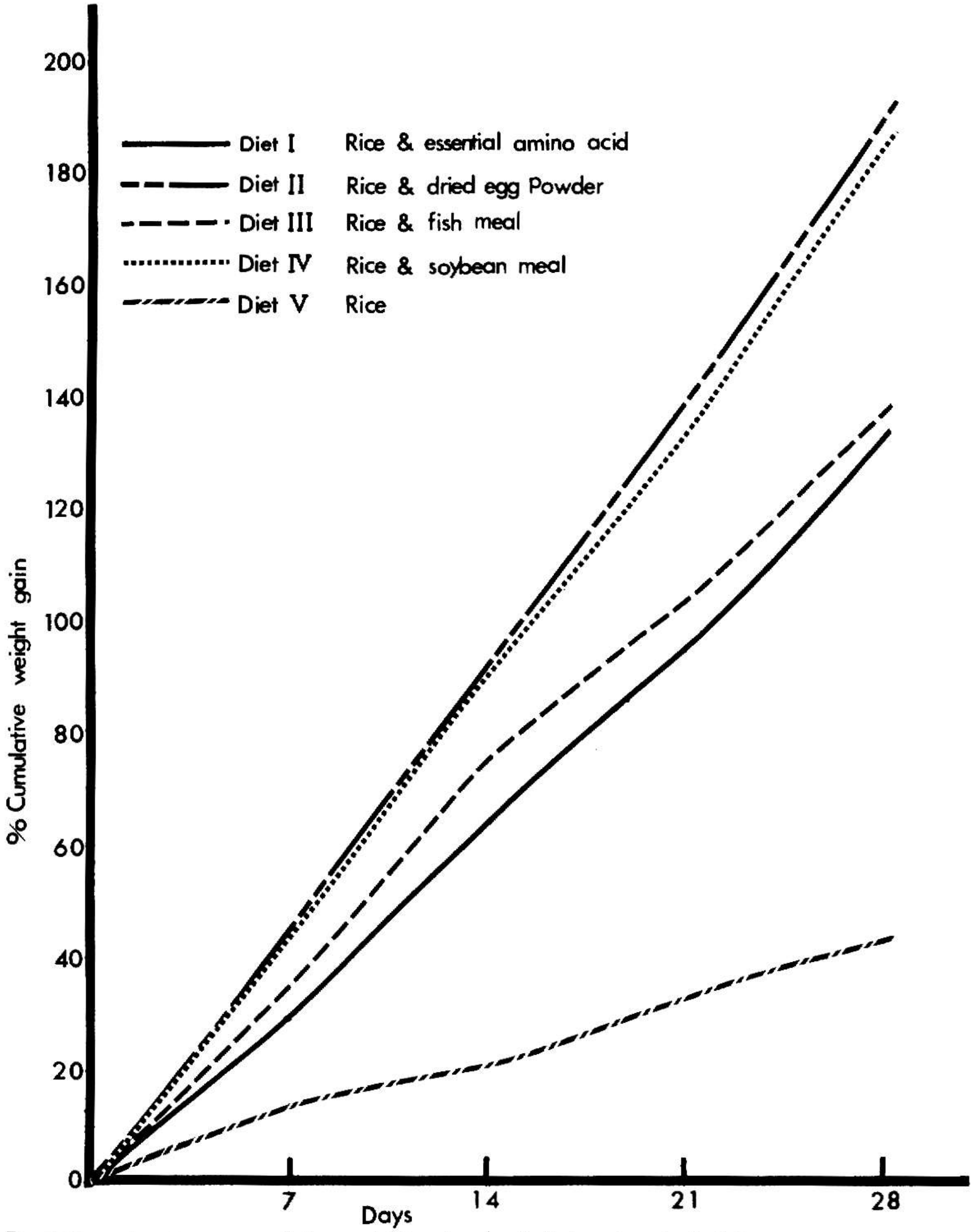

Fig. 2 Percent average cumulative weight gain of rats fed various test diets

TABLE 9

Mean percent weight gain of rats fed various diets during four collection periods, F-values, and least significant differences.

Groups of diets	Periods 1	2	3	4	Grand means
	%	%	%	%	
I, Rice + essential amino acids	29.4	26.6	18.0	19.6	23.4
II, Rice + dried egg powder	45.6	33.6	24.4	21.0	31.2
III, Rice + fish meal	35.8	29.4	16.0	16.8	24.5
IV, Rice + soybean meal	44.2	32.0	23.4	21.6	30.4
V, Rice only	13.2	7.0	11.2	6.8	9.6
Grand means	33.6 *	25.7 *	18.6	17.2	
F-values[1]					
Diets	56.22**				
Periods	50.30**				
Diets x periods	3.26**				
Lsd*[2]					
Diets	3.29				
Periods	2.94				

[1] ** Significant at 0.01 level

[2] Lsd* Least significant difference at 0.05 level

TABLE 10

Mean protein efficiency ratio of rats fed various diets during four collection periods, F-values and least significant differences.

Groups of diets	Periods 1	2	3	4	Grand means
I, Rice + essential amino acids	3.28	3.26	2.69	2.86	3.02
II, Rice + dried egg powder	3.52	3.29	2.87	2.92	3.12
III, Rice + fish meal	3.09	3.18	2.28	2.48	2.75
IV, Rice + soybean meal	3.31	3.16	2.60	2.74	2.95
V, Rice only	2.02	1.12	2.40	1.63	1.93
Grand mean	3.02	2.91 *	2.56	2.53	

F-values[1]	
Diets	19.50**
Periods	6.49**
Diets x periods	1.53^{ns}
Lsd*[2]	
Diets	0.306
Periods	0.272

[1]** Significant at 0.01 level, ns--not significant

[2]Lsd* Least significant difference at 0.05 level

significant difference in PER was found among diet groups I (R+EAA, II (R+DEP) and IV (R+SBM). The PER for diet group III (R+FM) was significantly lower than that of diet group II (R+DEP).

There was no significant difference in PER between Periods 1 and 2 and between Periods 3 and 4, but there was a significant decrease in PER beginning with the Period 3. No significant interaction was observed between diets and periods.

Nitrogen Balance

The mean nitrogen retention of rats fed various diets during four collection periods, F-values and least significant differences at the 5% level are given in table 11. There was a significant difference ($P<0.01$) in mean nitrogen retention per period among diet groups. Nitrogen retention was significantly less for rats fed rice only (group V) than for rats on the other 4 diets. In addition, nitrogen retention was significantly less for diet group I (R+EAA) than for diet groups II (R+DEP), III (R+FM), and IV (R+SBM). There was no significant difference between diet group II (R+DEP) and IV (R+SBM), but they were individually significantly greater than diet group III (R+FM).

Nitrogen retention during Periods 1 and 2 were significantly lower than during the other two periods; there was no significant difference between Periods 1 and 2. During Period 4 the nitrogen retention was significantly higher than in any of the other 3 periods.

Livers

Table 12 gives the means, F-values and least significant differences at 5% level for percent fat, nitrogen and moisture in the livers of rats in each

TABLE 11

Mean nitrogen retention of rats fed various diets during four collection periods, F-values and least significant differences.

Groups of diets	Periods 1	2	3	4	Grand means
	g	g	g	g	g
I, Rice + essential amino acids	0.626	0.709	0.698	0.812	0.711
					*
II, Rice + dried egg powder	1.032	1.134	1.230	1.285	1.170
					*
III, Rice + fish meal	0.797	0.838	0.812	0.943	0.847
					*
IV, Rice + soybean meal	0.977	1.044	1.224	1.219	1.116
					*
V, Rice only	0.395	0.343	0.369	0.369	0.376
Grand means	0.765	0.813 *	0.867 *	0.931	

F-values[1]	
Diets	244.10**
Periods	14.82**
Diets x periods	1.97*
Lsd*[2]	
Diets	0.058
Periods	0.052

[1] ** Significant at 0.01 level, * significant at 0.05 level

[2] Lsd* Least significant difference at 0.05 level

TABLE 12

Means, F-values and least significant differences for percent fat, nitrogen and moisture in livers of rats fed various diets.

Diet group	Fat	Nitrogen	Moisture
	%	%	%
I, Rice + essential amino acids	4.44	3.41	71.46
	*	*	
II, Rice + dried egg powder	11.93*	2.95*	68.11*
	*		
III, Rice + fish meal	17.84	2.76	65.09
	*		*
IV, Rice + soybean meal	10.11	*2.91	69.95
	*	*	*
V, Rice only	19.89	2.42	65.29
F-values[1]			
Diets	16.69**	20.10**	5.32**
Lsd*[2]			
Diets	4.47	0.23	3.62

[1]** Significant at 0.01 level

[2]Lsd* Least significant difference at 0.05 level

diet group. Significant differences ($P<0.01$) for fat, nitrogen and moisture were found among diet groups. The liver fat content of rats fed rice supplemented with essential amino acids (group I) was significantly lower than that of the other 4 diet groups. There was no significant differences between diet groups II (R+DEP) and IV (R+SBM), nor between groups III and V. The latter are significantly higher than the former two groups.

A negative correlation (-0.8503**) was observed between percent fat and nitrogen content in the liver; that is, as the percent fat in the liver increased, the nitrogen content decreased. Diet group I (R+EAA) had a significantly higher nitrogen content than those of the other four diet groups; while the nitrogen content of diet group V (R) was significantly lower than those of the other four diet groups. The pattern for the liver nitrogen content is almost the inverse of that of liver fat. There was no significant difference in the nitrogen content among diet groups II (R+DEP), III (R+FM), and IV (R+SBM).

The moisture content of the normal rat livers has been reported to be around 70% (45). Dakshinamurti (45) found that rats fed poor vegetarian rice diet had high fat content in the liver accompanied by low moisture content. In my experiment livers from rats of groups III (R+FM) and V (R) had significantly lower moisture contents than those from groups I (R+EAA) and IV (R+SBM). The same relationship is true in fat content of the livers of these rats (table 12). However, other significant differences in fat content of livers of rats fed these diet groups are observed without significant changes in water content.

**Significant at 0.01 level.

DISCUSSION

In this study an unsupplemented rice diet (86% rice) provided 6.02% rice-protein which resulted in poor growth, low PER, low nitrogen retention and high liver fat. Accumulation of liver fat is one symptom of the nutritional disease Kwashiorkor which is observed primarily among children consuming low-protein diets of plant origin. Also, comparing the percent amino acids present in this rice diet (table 13) with data by Harper (4), seems to indicate that the poor growth and high deposition of liver fat among the rats might be due to an unbalance of the dietary amino acids. This unsupplemented rice diet contained only 0.21% L-lysine and 0.19% L-threonine which were not only below 0.37% L-lysine and 0.31% L-threonine for normal growth but also much less than 0.53% L-lysine and 0.31% L-threonine which were required for normal fat deposition in the rat livers (4). (See Appendix III, p. 73).

When rice was supplemented with all the deficient essential amino acids simultaneously in the amount suggested by the National Research Council (113), our data showed that the growth response and nitrogen retention were improved over the unsupplemented rice diet; but this was still a sub-optimum response (weight gain of 77.0 g per rat for a four-week period) similar to that reported by Pecoa and Hundley (51). Since the L-lysine and L-threonine contents of the diet for group I were 0.90% and 0.50% respectively, the improved growth of rats over those fed unsupplemented rice diet was expected. Rats fed essential-amino-acids supplemented diet were significantly lower in growth rate than rats fed diet groups II (R+DEP) and IV (R+SBM); and their nitrogen retention and food consumption were significantly lower than the other three protein supplemented diets (groups II, III and IV). This finding shows the importance of the non-essential amino acids

TABLE 13

Amino acid composition of five experimental diets as compared with whole egg (at 9.09 protein level).

Amino acids	Whole egg	R(80%)+ EAA	R(80%)+ DEP(5.26%)	R(80%)+ FM(5.33%)	R(80%)+ SBM(7.27%)	R(86%) only
Alanine	0.58	0.28	0.50	0.55	0.46	0.30
Arginine	0.60	0.41	0.64	0.65	0.70	0.44
Aspartic acid	1.03	0.49	0.88	0.84	0.99	0.53
Glutamic acid	1.33	0.91	1.42	1.43	1.72	0.98
Glycine	0.33	0.23	0.36	0.64	0.40	0.25
Half cystine	0.43	0.08	0.24	0.11	0.13	0.08
Histidine	0.23	0.30	0.22	0.22	0.24	0.14
Isoleucine*	0.50	0.50	0.39	0.34	0.38	0.22
Leucine*	0.86	0.80	0.74	0.68	0.73	0.44
Lysine*	0.71	0.90	0.46	0.45	0.45	0.21
Methionine*	0.21	0.60	0.17	0.18	0.13	0.10
Phenylalanine*	0.52	0.90	0.46	0.40	0.47	0.28
Proline	0.34	0.22	0.35	0.47	0.42	0.23
Serine	0.73	0.24	0.52	0.40	0.45	0.26
Threonine*	0.46	0.50	0.36	0.33	0.34	0.19
Tyrosine	0.40	0.20	0.35	0.31	0.35	0.22
Valine*	0.20	0.70	0.37	0.47	0.49	0.31
Tryptophan*	0.13	0.15	0.12	0.10	0.11	0.08
Ammonia	0.14	0.15	0.21	0.19	0.24	0.17

*Essential amino acids.

which fulfilled part of the requirement of non-specific nitrogen (16, 17).

Hundley and his co-worker (99) studied the effect of amino acid supplementation on young adult human males consuming a rice diet which afforded a daily protein intake of 26 to 32 g. They found that under those conditions the primary deficiency in rice was that of non-specific, available nitrogen. A mixture of non-essential amino acids was as effective as a mixture of those which were essential. In other words, rice supplemented with only deficient essential amino acids without non-essential ones could give improved growth but not the optimum level.

As compared with the three protein-supplemented diets in this study, a significantly lower food consumption was noted for group I (R+EAA). This might be due to the less palatable nature of the rice-essential amino acid mixture relative to rice with fish meal, soybean meal or dried egg powder. The possibility of lower growth response of the rats because of the decreased food consumption should not be overlooked. The PER of the diet was not significantly different from those of the three protein supplemented diets, implying that this diet was as efficient as the other three. The high level of lysine and threonine, however, caused substantial reduction of the deposition of the liver fat to the normal level of 4.4%.

The rate of growth, protein efficiency ratio and nitrogen retention of rats fed the rice diet supplemented with three different proteins--egg powder (group II), menhaden fish meal (group III) and soybean meal (group IV)--were significantly greater than for rats fed the unsupplemented rice diet, the deposition of liver fat in diet groups II and IV was significantly decreased as compared to that of rats fed unsupplemented rice diet. However, their liver fat contents, 11.93% (group II) and 10.11% (group IV), were still in the abnormal level. The rats fed the fish meal-fortified rice diet accumulated an even higher content of fat in their livers (17.84%), which was not significantly different from that of rats fed unsupplemented rice diet (19.86%).

The availability of some essential and non-essential amino acids in the three protein-supplemented diets, of course, made them significantly better able to support growth of the rats as compared to the rice diets containing no supplement. As for the fat accumulation in the liver, the amino acid composition of the three protein-supplemented diets reveals a deficiency of L-lysine (below 0.53%) which was likely to be primarily responsible for fatty liver. The other essential amino acids might have had some influence, although no studies were made of them in this experiment. The Food and Nutrition Board (43) reported that deficiency of at least of three amino acids, lysine, threonine and tryptophan led to fatty infiltration of the liver. Harper (57) reported liver fat accumulation due to amino acid imbalance. Harper (5) also indicated that the addition of unbalanced mixtures of amino acids or proteins lacking a single indispensible amino acid to low protein diets caused depression of growth and fatty liver, adverse effects which could be prevented by a supplement of the limiting amino acid. In my study the most limiting amino acid for growth appeared to be L-lysine; although it markedly improved growth in the three protein-supplemented diets, it was not adequate for preventing fat accumulation in the rat liver. The threonine content in diet groups II, III, IV, and V was lower than that of whole egg at the 9.09 percent protein level (table 13); but, in the cases of diet groups II, III, and IV the threonine level exceeds that reported to improve growth and to prevent fat deposition in the rat liver (4). The lack of adequate threonine in group V has been discussed (see p. 50).

Although soybean meal is a less effective protein supplement than dried egg powder (80), in this study, growth response, nitrogen retention and even the deposition in the liver were not significantly different when the diets were supplemented to the same protein levels. The explanation may lie in their amino acid contents. As can be seen in table 13, the essential amino acid contents of the two diets are similar.

Fish meal is known to be a good quality protein supplement for cereals. De (50) fortified 82 parts of rice with 2 parts fish protein concentrate and obtained improvement in PER from 1.5 to 2.1. My experiment indicated that weight gain, PER and nitrogen retention from the rice diet (80 parts) supplemented with 5.33 parts of fish meal for a total of 9.09% protein (group III) also showed significant improvements over those of the unsupplemented rice diet. However, the growth response was less than the rice diet supplemented with soybean meal, and the fat deposition in the liver was higher than that of the soybean meal-rice diet. In fact, all results showed that fish meal was a poorer supplement than soybean meal, in contrast to observations of Harper (4) who supplemented 84% rice with 6.6% soybean meal and 4.3% fish meal at an 8.5% dietary protein level. However, observations by Harper were after a two-week feeding period as compared to a four-week period in the present study. The kind of fish meal used might be important; it was not specified in Harper's report.

The essential amino acid compositions (table 13) of the fish meal- and soybean meal-supplemented diets were similar. As for the non-essential amino acids, the highest glycine (0.64%) content was found in the fish meal-rice diet; at the same total dietary protein level it contained twice the glycine as the dried egg-rice diet and more than 1 1/2 times that of the soybean meal-rice diet. Salmon (117) suggested that depressed growth rate might result from biochemical response to amino acid imbalance. The imbalance stimulated amino acid catabolism and excretion resulting in loss of an amino acid that was already limiting or marginal for growth. Fisher et al. (92) suggested that growth depression might also be caused by addition of dispensable amino acids, which is one type of imbalance, but until these have been studied in more detail they are included as toxicities. Harper (5) reported that the growth depression due to an excess of an individual amino acid was greater when the diet was low in protein. Although amino acid toxicities might be reduced in severity by improving the quality of the low-protein diet with supplements of the limiting amino acid, growth depression was not prevented; whereas, in conditions classified as imbalances, improving the quality of the low-protein diet with a supplement of the most limiting amino acids completely prevented the growth depression and usually stimulated growth.

Results of the present study are in agreement with Harper's observation (4) that the specific amino acid ratio which improved growth rate might not be the specific ratio for preventing the accumulation of fat in the liver of rats.

Peanut oil was used as a source of fat in the experimental diets. It is an important cooking fat for the Oriental people. The literature gives no specific report on the effect of peanut oil on liver fat accumulation. Comparative studies of this oil with others as to their effect on liver fat accumulation were not made.

The rice used in this experiment was a local product and may not have exactly the same amino acid content as Oriental rice. However, rice used in the Orient is of many varieties. A comparison of their amino acid contents is not available, and choosing any one brand still will not be representative.

The present data have illustrated that the unsupplemented rice diet causes fatty liver, poor growth response, low nitrogen retention and low protein efficiency ratio in rats. Growth was improved by supplementing the rice diet with dried egg powder, fish meal and soybean meal, but the fat deposition in the liver of the rats was still high enough to be considered abnormal. The problem appeared to be the content of L-lysine and L-threonine in the diet, whose adequacy decreased fatty liver symptom as observed in rats fed essential amino acid supplemented rice-diet.

Extrapolation of the findings suggests that growth of Orientals who live on a high-rice low-protein diet can be improved and the fatty liver cases decreased by supplementing the diet with higher quality proteins such as whole egg, fish and soybean, plus fortification with lysine. Of course, supplementation must be made such that the diet is palatable, acceptable, and economical. Since rice constitutes 60% or higher of the Oriental diet, the problem might be solved by developing varieties of high-lysine and high-threonine rice to phase out existing varieties. Considering the generally small land-ownership and lack of government-subsidized agricultural programs in some of these countries, this is a difficult and yet vital approach.

SUMMARY

The effect of protein and essential amino acid supplements on the rate of growth and fat deposition in the livers of rats fed rice diets designed to represent those of the Orient has been studied.

Twenty-five weanling male albino rats were randomly distributed in individual metabolic cages and fed 9.09% protein diets based on (1) rice plus essential amino acids to meet the minimum requirement for rat growth, (2) rice plus dried egg powder, (3) rice plus fish meal, (4) rice plus soybean meal, and (5) rice only (6.02% protein). The rats were kept for a period of 28 days, comprised of four 7-day collection periods. During each period, urine and feces were collected. Weight and food intake of each animal were determined at the end of each period. Nitrogen in the rice, protein supplements, diets, urine and feces was determined. Fat, nitrogen and moisture contents in livers were determined at the end of the experiment. Food consumption, percent weight gain, protein efficiency ratio, nitrogen retention, percent liver fat, nitrogen, and moisture were calculated. The data were treated by analysis of variance; least significant differences and correlations were obtained.

Rats fed the unsupplemented rice diet (86% rice), providing 6.02% protein, had lower food consumption, percent weight gain, protein efficiency ratio, nitrogen retention and higher liver fat than those fed the other four diets. Rats fed the rice diet supplemented with the deficient essential amino acids had improved growth response and nitrogen retention over those fed the unsupplemented rice diet; but, as compared with those fed the other three protein-supplemented diets (whole egg protein, soybean meal and fish meal), the percent weight gain, nitrogen retention and food consumption were

lower. The significant finding on rats fed the rice diet supplemented with essential amino acids was the substantial reduction of the deposition of the liver fat; liver fat was at a normal level (4.55%). The deposition of fat in livers of rats receiving the diets containing dried egg powder or soybean meal was less than that in livers of rats fed unsupplemented rice diet. However, liver fat contents (11.9% and 10.1%, respectively) were still at an abnormal level. The rats fed the fish meal-fortified rice diet accumulated 17.8% fat in their livers, which was not significantly different from that in livers of rats fed the unsupplemented rice diet (19.9%).

This experiment has demonstrated that the unsupplemented rice diet caused fatty liver, poor growth, low nitrogen retention and low protein efficiency ratio in rats. Growth was improved by supplementing rice with whole egg powder, fish meal and soybean meal, but the levels of fat depositions in the liver of the rats were still considered to be abnormal. The problem might be related to the content of L-lysine and L-threonine in the diet, since decreases in the fatty liver symptoms were observed in rats fed the essential-amino-acid-supplemented rice diet.

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APPENDICES

APPENDIX I

Procedure for Macro-Kjeldahl Method

I. Sampling: Liquid samples--invert bottle several times to allow a reasonably representative sample. Use 20 or 25 ml sample for each Kjeldahl flask.

For solid samples weigh up approximately 1 gram on a piece of Saran wrap. Enclose sample in Saran and place unit in Kjeldahl flask.

II. Digestion:

a. Add one Kjel-pak to each sample, and several glass beads.

b. Add 25 ml concentrated H_2SO_4.

c. Place on digestion racks, turn on exhaust fan, and heat until the mixture has turned light green, and then heat approximately 15 minutes more.

1. Undigested portions that adhere to the flask should be washed into the digestion mixture by gentle rotation of the flasks.

The Kjeldahl procedure may be stopped at this point, if the flasks are removed from the digestion rack and corked.

d. After the flasks have cooled (can be handled with bare hands), add approximately 200 ml distilled water and allow to cool again. The Procedure may also be stopped at this point.

III. Distillation:

1. Add 3 drops of mixed indicator and 50 ml of 4% boric acid into 500 ml Erlenmeyer flasks and place as a receiving flask in the distillation rack so that the tip of the glass tube is just below the surface of the acid.

2. Turn on the cold water through the condensers.

3. To each Kjeldahl flask add a layer of NaOH solution, approximately 100 ml without agitation.

4. Add a few zinc granules, and then immediately connect flask to the distilling bulb on the condensers.

5. Shake flask until contents are thoroughly mixed, and turn on heat. (The contents should be bluish only temporarily, then change to a dark ppt., otherwise not enough NaOH has been added.)

6. Observe distillation until most of flasks are boiling--distill 15 minutes after flasks have started boiling.

7. After 15 minutes turn off heat, remove erlenmeyer flasks and titrate immediately. Stopper the flasks if not to be titrated at once.

IV. Titration:

1. Titrate the contents of receiving flask with 0.1N H_2SO_4. Compare the end point with a reference solution if desired (50 ml of 4% boric acid, 175 ml distilled water and 3 drops of mixed indicator). The end point should be a violet-lavender color.

APPENDIX II

Goldfisch's Fat Determination

Reagent: Ether

Procedure:

Weigh into a defatted cotton pad, the weight of which is known, an accurate amount of the sample--about 1.5 gm. Record weight of cotton pad and sample.

Dry the sample in the cotton pad in the vacuum oven at 110°C till a constant weight is obtained, about 24 hours. Deposit the weighed dried sample in the ceramic (alundum) thimble for extraction, so that it may be removed easily after extraction. Place the thimble in the glass tube with the bulb at the end, and set it in the extractor. Measure 30-40 ml of anhydrous ether in the dried, previously weighed beakers. Place the beaker in the ring, attach to the extractor. Turn on the water for cooling. Turn on the heat, low. Allow extraction for 20-24 hours.

After extraction, remove tube and sample and attach reclaiming cup in its place. Slip safety cover over heating surface during the operation. Allow the ether to collect in the reclaiming cup. The fat in the beaker should not be heated to dryness, and thus burn fat.

Remove beaker containing the fat and set in a tray. Dry the beaker and contents in a vacuum oven for one hour at 65°C. Cool in the desiccator for one-half hour. Weigh the beaker and contents. Avoid fingerprints which will alter the weights.

APPENDIX III

The amount of L-lysine and L-threonine required for normal growth and liver fat content in rats is calculated in the following manner:

According to Harper (4), for normal growth of rats fed an 87% rice diet, 0.2% L-lysine·HCl and 0.24% DL-threonine were supplemented.

0.2% L-lysine·HCl = 0.16% L-lysine

0.24% DL-threonine = 0.12% L-threonine

The L-lysine and L-threonine content of rice used in the diets of this experiment (table 15) are in agreement with the data published by Kik (115). Using these figures, rice at 87% of the diet furnishes 0.21% L-lysine and 0.19% L-threonine. Therefore, the total amounts of L-lysine and L-threonine in this diet described by Harper are:

L-lysine = (0.21 + 0.16)% = 0.37%

L-threonine = (0.19 + 0.12)% = 0.31%.

For normal liver fat content in rats, according to Harper (4), 0.4% L-lysine·HCl and 0.24% DL-threonine are required; these are equivalent to 0.32% L-lysine and 0.12% L-threonine, respectively. Supplementing these amino acids to the 87% rice diet, the total content of L-lysine and L-threonine is 0.53% and 0.34%, respectively.

TABLE 14

Random distribution of animals

Rat No.	1	2	3	4	5	6	7	8
Group	II	II	I	III	II	III	I	V
Initial weight (g)	56	57	55	56	55	61	59	62
Rat No.	9	10	11	12	13	14	15	16
Group	III	IV	II	II	IV	I	IV	V
Initial weight (g)	55	55	60	58	59	57	56	53
Rat No.	17	18	19	20	21	22	23	24
Group	III	IV	I	III	V	I	V	V
Initial weight (g)	56	57	62	58	57	53	57	57
Rat No.	25							
Group	IV							
Initial weight (g)	59							

TABLE 15

Food consumption of rats fed various diets during collection periods.

Rat no.	Period 1	Period 2	Period 3	Period 4
	g	g	g	g
Group I, Rice + essential amino acids				
7	67	77	72	84
14	36	49	60	64
3	56	62	66	72
22	53	68	67	78
19	64	66	68	84
Group II, Rice + dried egg powder				
5	85	105	113	120
1	82	100	98	108
2	89	93	99	113
11	93	89	112	116
12	96	107	128	125
Group III, Rice + fish meal				
9	86	96	98	95
4	77	84	64	96
20	88	90	92	102
6	76	76	74	86
17	83	96	100	104
Group IV, Rice + soybean meal				
10	74	94	103	106
15	91	103	114	128
18	96	96	116	121
13	84	91	116	129
25	101	92	119	121
Group V, Rice only				
8	62	35	62	53
21	67	55	57	55
16	62	56	47	56
23	55	52	47	53
24	61	52	47	54

TABLE 16

Weight and percent weight gain of rats fed various diets at the end of the adjustment period and each collection period

Rat No.	Adjustment period	Period 1		Period 2		Period 3		Period 4	
	g	g	%	g	%	g	%	g	%
Group I, Rice + essential amino acids (EAA)									
7	61	83	36	107	29	126	18	149	18
14	55	67	22	83	24	96	16	114	19
3	56	74	32	93	26	109	17	127	17
22	55	71	29	90	27	109	21	135	24
19	64	82	28	104	27	123	18	148	20
Group II, Rice + dried egg powder (DEP)									
5	60	85	42	123	45	154	25	183	19
1	63	91	44	123	35	146	19	170	16
2	62	90	45	120	33	146	22	181	24
11	68	99	46	121	22	158	31	194	23
12	65	98	51	130	33	162	25	199	23
Group III, Rice + fish meal (FM)									
9	61	83	36	107	29	132	23	149	13
4	59	78	32	101	29	109	8	129	18
20	60	84	40	105	25	124	18	145	17
6	61	80	31	100	25	110	10	130	18
17	57	80	40	111	39	134	21	158	18

TABLE 16 (Concluded)

Rat No.	Adjustment period	Period 1		Period 2		Period 3		Period 4	
	g	g	%	g	%	g	%	g	%
Group IV, Rice + soybean meal (SBM)									
10	60	83	38	113	36	140	24	164	17
15	60	89	48	122	37	147	20	181	23
18	64	95	48	126	33	151	20	184	22
13	65	88	35	117	33	148	27	185	25
25	65	99	52	120	21	151	26	182	21
Group V, Rice only									
8	64	67	5	64	-4	79	23	80	1
21	59	67	14	72	7	77	7	82	6
16	53	63	19	70	11	78	11	82	5
23	58	65	12	71	9	76	7	85	12
24	57	66	16	71	8	77	8	85	10

TABLE 17

Protein efficiency ratio of various test diets fed to rats during each collection period.

Rat no.	Period 1	Period 2	Period 3	Period 4
Group I, Rice + essential amino acids				
7	3.43	3.26	2.76	2.86
14	3.48	3.42	2.27	2.24
3	3.36	3.20	2.53	2.61
22	3.16	2.92	2.97	3.49
19	2.94	3.49	2.92	3.11
Group II, Rice + dried egg powder				
5	3.12	3.83	2.91	2.56
1	3.49	3.39	2.49	2.35
2	3.33	3.42	2.78	3.28
11	3.53	2.62	3.50	3.29
12	3.64	3.17	2.65	3.14
Group III, Rice + fish meal				
9	3.03	2.96	3.02	2.12
4	2.92	3.25	1.48	2.47
20	3.23	2.77	2.45	2.44
6	2.96	3.12	1.60	2.76
17	3.28	3.83	2.84	2.62
Group IV, Rice + soybean meal				
10	3.29	3.38	2.78	2.40
15	3.38	3.40	2.32	2.65
18	3.42	3.20	2.29	2.89
13	2.90	3.38	2.83	3.04
25	3.57	2.42	2.76	2.72
Group V, Rice only				
8	0.79	-1.40	3.95	0.31
21	1.95	1.48	1.43	1.48
16	2.63	2.04	2.78	1.17
23	2.08	1.88	1.74	2.77
24	2.68	1.57	2.08	2.42

TABLE 18

Nitrogen balance of rats fed various test diets during four collection periods.

Rat no.	Collection period	Nitrogen intake	Urinary nitrogen	Fecal nitrogen	Retention
		g	g	g	g
Group I, Rice + essential amino acids					
7	I	1.025	0.156	0.058	0.811
	II	1.178	0.182	0.113	0.883
	III	1.102	0.234	0.115	0.753
	IV	1.285	0.241	0.110	0.934
14	I	0.551	0.121	0.052	0.378
	II	0.750	0.118	0.091	0.541
	III	0.918	0.132	0.137	0.649
	IV	0.979	0.167	0.094	0.718
3	I	0.857	0.164	0.111	0.582
	II	0.949	0.198	0.083	0.668
	III	1.010	0.216	0.095	0.699
	IV	1.102	0.208	0.100	0.794
22	I	0.811	0.162	0.066	0.585
	II	1.040	0.205	0.098	0.737
	III	1.025	0.215	0.087	0.723
	IV	1.193	0.248	0.107	0.838
19	I	0.979	0.138	0.068	0.773
	II	1.010	0.177	0.119	0.711
	III	1.040	0.209	0.165	0.666
	IV	1.285	0.253	0.154	0.778
Group II, Rice + dried egg powder					
5	I	1.284	0.160	0.143	0.981
	II	1.586	0.225	0.164	1.197
	III	1.706	0.275	0.189	1.242
	IV	1.812	0.313	0.204	1.295
1	I	1.238	0.126	0.142	0.970
	II	1.510	0.161	0.162	1.187
	III	1.480	0.164	0.144	1.172
	IV	1.631	0.215	0.198	1.218
2	I	1.344	0.154	0.143	1.047
	II	1.404	0.172	0.150	1.082
	III	1.495	0.159	0.185	1.151
	IV	1.706	0.230	0.173	1.303

TABLE 18 (continued)

Rat no.	Collection period	Nitrogen intake	Urinary nitrogen	Fecal nitrogen	Retention
		g	g	g	g
11	I	1.404	0.201	0.146	1.057
	II	1.344	0.226	0.136	0.982
	III	1.691	0.259	0.190	1.242
	IV	1.752	0.298	0.186	1.268
12	I	1.450	0.202	0.142	1.106
	II	1.616	0.249	0.146	1.221
	III	1.933	0.394	0.195	1.344
	IV	1.888	0.358	0.191	1.339
Group III, Rice + fish meal					
9	I	1.161	0.270	0.140	0.751
	II	1.296	0.302	0.162	0.832
	III	1.323	0.253	0.171	0.899
	IV	1.283	0.257	0.161	0.865
4	I	1.040	0.199	0.137	0.704
	II	1.134	0.183	0.161	0.790
	III	0.864	0.208	0.133	0.623
	IV	1.296	0.188	0.161	0.947
20	I	1.188	0.151	0.154	0.883
	II	1.215	0.194	0.154	0.867
	III	1.242	0.143	0.364	0.745
	IV	1.377	0.225	0.182	0.970
6	I	1.026	0.136	0.125	0.765
	II	1.026	0.162	0.126	0.738
	III	0.999	0.136	0.131	0.732
	IV	1.161	0.121	0.170	0.870
17	I	1.121	0.084	0.156	0.881
	II	1.296	0.137	0.198	0.961
	III	1.350	0.085	0.192	1.073
	IV	1.404	0.130	0.211	1.063
Group IV, Rice + soybean meal					
10	I	1.117	0.185	0.138	0.794
	II	1.419	0.219	0.175	1.025
	III	1.555	0.226	0.198	1.131
	IV	1.601	0.248	0.224	1.131
15	I	1.374	0.189	0.191	0.994
	II	1.555	0.246	0.188	1.121
	III	1.721	0.369	0.201	1.151
	IV	1.933	0.471	0.262	1.200

TABLE 18 (concluded)

Rat no.	Collection period	Nitrogen intake	Urinary nitrogen	Fecal nitrogen	Retention
		g	g	g	g
18	I	1.450	0.201	0.208	1.041
	II	1.450	0.261	0.196	0.993
	III	1.752	0.300	0.233	1.219
	IV	1.827	0.327	0.247	1.253
13	I	1.268	0.151	0.190	0.927
	II	1.374	0.214	0.197	0.963
	III	1.752	0.243	0.200	1.309
	IV	1.948	0.369	0.254	1.325
25	I	1.525	0.159	0.239	1.127
	II	1.389	0.075	0.196	1.118
	III	1.797	0.256	0.232	1.309
	IV	1.827	0.389	0.254	1.184
Group V, Rice only					
8	I	0.608	0.107	0.102	0.399
	II	0.343	0.041	0.043	0.259
	III	0.608	0.080	0.098	0.430
	IV	0.519	0.035	0.080	0.404
21	I	0.657	0.114	0.110	0.433
	II	0.539	0.062	0.089	0.388
	III	0.559	0.038	0.076	0.445
	IV	0.539	0.020	0.081	0.438
16	I	0.608	0.131	0.084	0.393
	II	0.549	0.153	0.080	0.316
	III	0.461	0.103	0.071	0.286
	IV	0.549	0.135	0.076	0.338
23	I	0.539	0.146	0.081	0.312
	II	0.510	0.050	0.075	0.385
	III	0.491	0.059	0.072	0.330
	IV	0.519	0.038	0.075	0.406
24	I	0.598	0.071	0.089	0.438
	II	0.510	0.070	0.071	0.369
	III	0.461	0.039	0.068	0.354
	IV	0.529	0.056	0.080	0.393

TABLE 19

Liver weight, liver weight/body weight, and percent liver fat, nitrogen and moisture in livers of rats fed various test diets.

Rat no.	Liver weight	Liver weight/body weight	Fat	Nitrogen	Moisture
	g	%	%	%	%
Group I, Rice + essential amino acids					
7	4.01	2.88	4.30	3.62	70.00
14	2.72	2.59	2.60	3.64	74.14
3	3.70	3.22	4.17	3.33	71.73
22	3.97	3.25	5.56	3.28	70.45
19	4.45	3.30	6.13	3.17	71.00
Group II, Rice + dried egg powder					
5	5.02	2.99	12.68	3.03	68.10
1	5.20	3.27	11.28	3.06	67.85
2	4.79	2.90	11.02	3.03	67.58
11	6.82	3.83	8.69	2.88	72.55
12	6.45	3.51	16.00	2.76	64.48
Group III, Rice + fish meal					
9	4.18	3.05	16.95	2.83	63.80
4	4.85	4.11	13.71	2.73	67.78
20	4.94	3.74	22.39	2.51	60.26
6	3.61	3.11	13.24	3.02	66.21
17	4.87	3.31	22.91	2.71	67.40
Group IV, Rice + soybean meal					
10	5.95	4.08	14.35	2.86	66.28
15	4.96	2.99	9.90	2.98	69.83
18	5.62	3.36	9.40	2.99	70.08
13	5.46	3.25	11.17	2.76	70.29
25	6.14	3.68	5.72	2.97	73.28
Group V, Rice only					
8	2.72	3.63	19.86	2.10	65.29
21	4.06	5.27	19.22	2.28	65.70
16	2.46	3.37	22.44	2.52	62.28
23	3.01	4.12	24.25	2.51	62.80
24	3.07	4.09	13.54	2.69	70.38

EFFECT OF PROTEIN SUPPLEMENTS ON GROWTH AND FAT DEPOSITION IN THE LIVERS OF RATS FED POLISHED RICE WITH PEANUT OIL

by

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The effect of protein and essential amino acid supplements on the rate of growth and fat deposition in the livers of rats fed rice diets designed to represent those of the Orient has been studied.

Twenty-five weanling male albino rats were randomly distributed in individual metabolic cages and fed 9.09% protein diets based on (1) rice plus essential amino acids to meet the minimum requirement for rat growth, (2) rice plus dried egg powder, (3) rice plus fish meal, (4) rice plus soybean meal, and (5) rice only (6.02% protein). The rats were kept for a period of 28 days, comprised of four 7-day collection periods. During each period, urine and feces were collected. Weight and food intake of each animal were determined at the end of each period. Nitrogen in the rice, protein supplements, diets, urine and feces was determined. Fat, nitrogen and moisture contents in livers were determined at the end of the experiment. Food consumption, percent weight gain, protein efficiency ratio, nitrogen retention, percent liver fat, nitrogen, and moisture were calculated. The data were treated by analysis of variance; least significant differences and correlations were obtained.

Rats fed the unsupplemented rice diet (86% rice), providing 6.02% protein, had lower food consumption, percent weight gain, protein efficiency ratio, nitrogen retention and higher liver fat than those fed the other four diets. Rats fed the rice diet supplemented with the deficient essential amino acids had improved growth response and nitrogen retention over those fed the unsupplemented rice diet; but, as compared with those fed the other three protein-supplemented diets (whole egg protein, soybean meal and fish meal), the percent weight gain, nitrogen retention and food consumption were lower. The significant finding on rats fed the rice diet supplemented with

essential amino acids was the substantial reduction of the deposition of the liver fat; liver fat was at a normal level (4.55%). The deposition of fat in livers of rats receiving the diets containing dried egg powder or soybean meal was less than that in livers of rats fed unsupplemented rice diet. However, liver fat contents (11.9% and 10.1%, respectively) were still at an abnormal level. The rats fed the fish meal-fortified rice diet accumulated 17.8% fat in their livers, which was not significantly different from that in livers of rats fed the unsupplemented rice diet (19.9%).

This experiment has demonstrated that the unsupplemented rice diet caused fatty liver, poor growth, low nitrogen retention and low protein efficiency ratio in rats. Growth was improved by supplementing rice with whole egg powder, fish meal and soybean meal, but the levels of fat depositions in the liver of the rats were still considered to be abnormal. The problem might be related to the content of L-lysine and L-threonine in the diet, since decreases in the fatty liver symptoms were observed in rats fed the essential-amino-acid-supplemented rice diet.