12-1960

Growth of Rats on Supplemented Rich Diets

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To the Graduate Council:

I am submitting herewith a thesis written by Mandayam Ananthanpillai Nalini entitled "Growth of Rats on Supplemented Rich Diets." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Brenda B. Schofield, Major Professor

We have read this thesis and recommend its acceptance:

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(Original signatures are on file with official student records.)

December 8, 1960

To the Graduate Council:

I am submitting herewith a thesis written by Mandayam Ananthanpillai Nalini entitled "Growth of Rats on Supplemented Rice Diets." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

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GROWTH OF RATS ON SUPPLEMENTED RICE DIETS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Mandayam Ananthanpillai Nalini

December 1960

ACKNOWLEDGEMENT

The author wishes to express her deep indebtedness and sincere gratitude to Dr. Schofield for her guidance and assistance throughout, which has made this study possible. She wishes to convey her sincere appreciation to Dr. Florence MacLeod, Dr. Bernadine Meyer and Dr. Ilene Brown for their helpful criticism.

She also wishes to thank Miss Elise Morrell for her kindly help with the statistical analysis and Mrs. Rossie Mason and Mrs. Jessie Armstrong for their assistance in working with the rats.

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INTRODUCTION

Rice is the most important staple cereal of the world, since it is the basic ingredient of the daily diet for more than half the human race. It forms a major source of calories as well as an important source of proteins in the diet.

In a poor country like India, for nearly 240 out of the 400 million people, polished rice forms the bulk of the diet consumed in southern India, Orissa, Bihar, Bengal, Assam and Kashmir. Rice in a way is the national food, as more people in India live on it than all the other cereals put together. Except for the richer classes, the bulk of the southern and central Indian population lives almost exclusively on rice (Masani, '44).

A typical south Indian rice diet is one of the poorest diets in the world. It is essentially a carbohydrate diet with gross deficiencies in proteins, minerals, and vitamins (Subrahmanyan and Sur, '49). In this diet, polished rice furnishes about 60 per cent of the total calories and protein. The rest of the protein is supplied mostly by vegetable sources. Though mutton and other forms of meat are rich sources of protein, they rarely are an important article in the diet of the mass of the people as evidenced by table 1. Thus it is evident that most of the proteins in the Indian diet are supplied by the cereals and pulses, largely dhals and grams, partly due to inadequate food supplies and partly due to religious restrictions.

Rice protein is of the best quality of the cereal grains, and has higher biological value than a number of other cereal proteins for the

TABLE 1

ESTIMATED AVAILABILITY OF DIFFERENT FOOD-STUFFS AS COMPARED TO THE REQUIREMENTS OF A BALANCED DIET[1]

(ounces per adult consumption unit per day)

Food stuffs	Balanced diet	Estimated availability (1955-56)
Cereals	14.0	14.7
Pulses	3.0	2.6
Leafy vegetables	4.0)	2.6
Other vegetables	6.0)	
Ghee and vegetable oils	2.0	0.39
Milk and milk products	10.0	4.5
Meat, fish and eggs	4.0	0.4
Fruits	3.0	1.0
Sugar and jaggery	2.0	1.6
Total calories	3089	2238

[1]Swaminathan and Bhagawan, '59.

humans, although the content of several essential amino acids is below that of animal proteins. However, the level of protein in rice is so low that it is not possible to raise protein intake to adequate levels through increased consumption of rice alone. Thus malnutrition, particularly protein deficiency, forms one of the main problems in many of the rice-eating countries in south and southeast Asia.

The problem in India now is to raise the level of protein in the diet, by including in the diet other foods, with high protein content and with amino acid patterns which supplement those of rice without introducing serious imbalances; at the same time, the food included should be such that its price is within the limited resources of the people as well as acceptable to the vegetarians. At present, in India as in other parts of the world, the value of groundnut or peanut as a protein-rich food is widely recognised and various scientific studies are being undertaken by the Government to incorporate groundnut into the daily diet of the population in a variety of acceptable and economical forms. Groundnut has a high protein content, is abundantly and economically grown in India and accepted by the people as a component in their native diets. However, further information is needed regarding the supplementary value of groundnut or peanut in polished rice diets.

The purpose of the present experiment was to determine the influence on growth and protein efficiency ratio for rats, of the supplementation of polished rice diets with ground peanuts and with essential amino acids to meet the FAO reference pattern. Diets were designed in which the protein was supplied by 85 per cent polished rice alone; 5.1 per cent

of polished rice was replaced by ground peanuts; 10.5 per cent of polished rice was replaced by ground peanuts; 85 per cent polished rice was supplemented with essential amino acids to meet the FAO provisional pattern; and, each of the peanut-rice diets was supplemented with essential amino acids to meet the FAO provisional pattern proposed for human diets. Such animal studies as this afford a basis for work directed toward improvement of the nutrition of a population.

REVIEW OF LITERATURE

Rice forms a major source of calories and proteins for more than half the world's population. In India, as in other Asian countries, rice forms the staple food of the people. Polished rice furnishes more than 60 per cent of total calories and proteins, and other foods, especially those of animal origin, are eaten in relatively small amounts.

The efficiency with which dietary protein is utilized for building body protein is determined by the pattern of the indispensable amino acids absorbed into the body, and the pattern of amino acids absorbed is in turn dependent upon the chemical composition of the dietary protein and its digestion into amino acids in the gastro-intestinal tract. When we consider the proteins in rice, it is found that the deficiency is not so much in the quality of the proteins as in the quantity. The amino acid pattern of a rice diet is fairly good, but the total amount of protein consumed on this diet is only half the recommended intake for humans. The quality of proteins of polished rice seemed to be superior to that of wheat, corn, oats or rye, for the growth of rats, even though the quantity or the percentage of nitrogen was low. Net protein utilization of polished rice determined in nitrogen balance studies on rats was 75.1 per cent when fed in diets containing only 5 per cent protein (Sure and House, '48).

Many attempts have been made in different parts of the world to improve the rice diets. The use of vegetable proteins, particularly those of legumes, as supplements has been investigated. The reports reviewed in this paper deal mainly with the composition and growth promoting value of rice protein, peanut protein, and the supplementary value of peanut

proteins, together with the review of studies made on the protein and amino acid supplementation of rice diets.

Various studies have been done on the composition of polished rice. From the hydrolysis of the rice kernel, the chief protein of rice endosperm is found to be orysenin. In its general amino acid make up, the protein of the rice kernel more nearly resembles the majority of the proteins of animal tissues than do the proteins of maize or wheat (Osborne et al., '15). They suggested that this may explain the extensive use of rice as an almost exclusive diet in spite of its low protein content. Kik ('42b) found the biological value of polished rice, fed at a 5 per cent level in the diet, to be 66.7 and digestibility 98 per cent for rats. He also found that polished rice needed fortification with vitamins A and D, minerals, proteins and vitamin B complex to promote good growth in albino rats.

Hydrolysis of polished rice protein yielded a relatively large amount of each of the basic amino acids, arginine, histidine and lysine, as compared to the proteins of wheat or maize (Osborne et al., '15). Kik ('42a) analyzed polished rice for cystine, tryptophan, lysine, arginine and histidine. He found that polished rice was not lacking in cystine or lysine; the levels were low as compared to casein and wheat. But tryptophan, arginine and histidine content compared favorably with the amounts found in wheat and corn. In an earlier study, Kik ('40) showed that cystine, methionine and lysine supplemented the proteins of whole or polished rice rations in promoting rat growth to a slight but statistically significant extent. Neither tryptophan nor leucine had any beneficial effect.

Osborne and Mendel ('18) determined that when a concentrate from polished rice formed the sole source of protein in the diet, supplying 16-17 per cent of protein, rats grew well, indicating that the proteins supplied all of the essential amino acids for growth in contrast with maize and oats. In experiments with rats, Mitchell ('24b) demonstrated that in diets containing 5 per cent protein, the proteins in whole brown rice were superior to those in corn and oats, the biological values being 86.1 per cent for rice as compared to 78.6 per cent for oats and 72.0 per cent for corn.

Similar studies by Sure and House ('48) on the biological values of whole and milled cereal grains fed at a 5 per cent protein level in the diet indicated that whole rice had a higher biological value than wheat, rye and corn, the values being 85.1, 83.0, and 84.7 per cent respectively. Determinations made on milled products gave somewhat lower results, the values being 79.0, 63.5, 69.7 and 36.2 per cent respectively. On diets containing 4.5 per cent protein, Jones et al. ('48) found polished rice to be superior for rat growth to brown rice, soft wheat or yellow corn, the protein efficiency ratios being 2.22, 1.92, 1.74 and 1.42, respectively.

Sure ('46a) reported that on the same protein level in the ration, proteins in polished rice proved to be superior to those in enriched wheat flour. In experiments with rats fed rations containing 5.8 per cent protein, polished rice had a protein efficiency ratio of 1.86 compared to 0.72 for enriched wheat flour. Rats on the polished rice diet gained almost 4 times as much as those on the diet containing enriched wheat flour.

Efficiency of the proteins in polished rice was 158 per cent greater than that of the proteins of wheat flour. The superiority of the biological value of rice for humans when rice constituted the sole source of protein, as compared to mixtures of rice and wheat, or rice, wheat and pulses, was demonstrated by Mitra et al. ('48). The mean biological value of the rice diet was 66.6 per cent in contrast to 55.1 for a rice and wheat diet, 59.8 for rice, wheat and barley, 56.8 for rice, wheat and maize, 54.1 for rice, wheat and junera, 60.2 for rice, wheat and maroca, and 57.4 for rice, wheat and bajra.

Two globulins have been isolated from peanuts, arachin and conarchin. These contain a high percentage of basic nitrogen compared with other proteins used commonly. Arachin contains 4.96 and conarchin 6.55 per cent of basic nitrogen, the latter being the highest percentage of basic nitrogen recorded for any seed protein (Johns and Jones, '16-'17). Peanut kernel contains appreciable amounts of all of the nutritionally essential amino acids (Kuiken and Lyman, '48). This is in agreement with the earlier work of Johns and Jones ('17) who analyzed the globulins of the peanuts and found that they contained the basic amino acids arginine, histidine, lysine and cystine. Chemical analysis of arachin showed that it was relatively rich in lysine and tryptophan and several other amino acids essential for growth and maintenance (Holmes, '18).

But feeding experiments with rats have shown that arachin is deficient in tryptophan and methionine and also a third factor, possibly isoleucine (Kuiken and Lyman, '48). This is supported by Beach and White ('37) who analyzed arachin and found the methionine content to be as low

as 0.5 per cent of the protein. Hence, methionine appears to be the limiting amino acid in arachin. However, it has been found from experiments with laboratory animals that when peanut proteins or conarachin were employed as sole source of proteins in an otherwise adequate diet, they supported satisfactory growth (Holmes, '18; Kuiken and Lyman, '48).

The relatively high percentage of lysine in the proteins of the peanut, which approaches the lysine content of muscle of different animals, indicates that this seed might be used to advantage in supplementing diets deficient in lysine (Johns and Jones, '17). The authors suggested that peanut cake would be highly effective as a supplement for diets of cereals and other seeds deficient in the basic amino acids. In addition, the proteins of raw peanuts are of high nutritive value, nearly equal to those of milk, meat and eggs (Payne, '42). It has been confirmed by many investigators that whole defatted peanut kernel, total peanut protein or total peanut globulin is approximately equivalent to casein in promoting growth of animals (Kuiken and Lyman, '48).

Peanut proteins are well tolerated by the human body and very well digested (Holmes, '18). The coefficient of digestibility of unroasted peanuts was found to be 93 in human subjects. The average availability of the essential amino acids of peanut flour was 97.7 per cent in white rats (Kuiken and Lyman, '48).

It is now possible to manufacture peanut flour from peanuts which can be used for human consumption. Peanut flour is a defatted product of peanuts. After the whole peanuts are reduced into oil and cake, the cake is converted into peanut flour. Peanut flour is a high quality, easily

digestible, protein concentrate of comparatively low cost (Payne, '42). Studies by Jones ('44) and Jones and Divine ('44) indicate that peanut flour offers a source of a very good dietary protein for extending and partially replacing protein foods of animal origin. Its value is further stressed by Daniels and Loughlin ('18) who showed that normal growth and reproduction could be obtained in rats fed purified diets in which the sole source of protein was supplied at a 15-18 per cent level by peanut meal.

From amino acid analyses, peanut meal is found to be deficient in lysine and methionine and possibly in threonine and tryptophan. Growth studies in chicks indicated that peanut meal is primarily deficient in methionine and slightly low in lysine. Young chicks were fed diets in which all the protein was provided by peanut meal concentrates. Then, additions of various amino acids were made and effects on growth and efficiency of gain were noted. When the basal diet was supplemented with the 4 amino acids, lysine, methionine, threonine and tryptophan, growth rate was found to be 6.9 per cent. When lysine was omitted, growth rate decreased to 5.5 per cent but when methionine was omitted the growth rate came down to 2.7. Omission of threonine and tryptophan had no effect (Grau, '46). Further evidence is given by Sure ('55a) who showed that supplementation of the proteins in peanut flour with 0.5 per cent DL-methionine and 0.5 per cent DL-threonine was followed by 60.6 per cent increase in body weight and 61.5 per cent increase in protein efficiency ratio in albino rats fed a ration containing the peanut flour as the source of protein at a 9 per cent level.

Nevertheless, peanut flour is a concentrated source of high quality protein that can be used effectively to improve the protein quality of other foods, mainly cereals. Eddy and Eckman ('23) made a study to compare the protein-supplementing power of peanut flour with that of muscle protein by feeding rations so constituted as to contain only about 10 per cent of protein, 6-7 per cent of this protein being contributed by wheat flour and the rest by peanut flour or meat residue. Peanut flour promoted slightly better growth than meat and proved markedly superior for promoting reproduction in rats.

In some respects, the plant proteins of soybeans, peanuts and cottonseed compare favorably with milk proteins, which are generally regarded as mong the best (Jones and Divine, '44). The growth promoting values of soybean, peanut, and cottonseed flours were compared by the rat growth method. When peanut flour was fed at a 9 per cent protein level in the diet, average weight gain of rats was 75 gm, which was the lowest obtained with the oil-seed flours, at that protein level. However, when fed at a protein level of 15 per cent in the diet, the corresponding gain in weight was 158 gm, comparable to that produced by the other oil-seed flours. Gain in weight was very nearly that obtained with skim milk powder at the same level, 155 gm. Peanut meal has proved a valuable source of protein for supplementing the protein of corn. Zein, one of the chief proteins of corn, is deficient in lysine, tryptophan and cystine, while these amino acids are well provided in the total proteins of the peanut. A mixture of 25 parts of peanut flour and 75 parts of yellow corn meal, containing 12-15 per cent of protein, has been found to

furnish proteins adequate for the normal growth in albino rats fed an otherwise adequate diet. When the protein level was reduced to 7.2 per cent, with portions of peanuts and corn the same, this mixture promoted better growth than when corn alone furnished the same level of protein, protein efficiency being 1.23 and 0.73, respectively. Compared with the supplementary value of dried food yeasts, soybean flour, dried non-fat milk solids, and dried butter-milk, peanut meal proved the least satisfactory supplement to both milled white corn meal and milled enriched wheat flour (Sure, '48b).

As early as 1917, it was discovered that a mixture of 75 per cent of wheat flour and 25 per cent of peanut flour made an excellent bread. Such a bread had a higher protein content and contained much more lysine than bread from wheat alone (Johns and Jones, '17). Later, in 1920, a patent was issued on a bread made from 68 per cent of white wheat flour and 23 per cent of peanut flour, with butter, salt mixture and yeast. Rats fed this bread showed normal growth as against poor growth from bread made in a similar manner from white flour (Payne, '42). Bread made with 75 parts of wheat flour and 25 parts of peanut flour together with a suitable salt mixture and butterfat supplied adequate protein and water soluble vitamins for normal growth in albino rats (Johns and Finks, '20; Johns et al., '27). With just 15 parts of peanut flour and 85 parts of wheat flour, animals grew at very nearly the normal rate. Proteins in peanut bread were utilised nearly twice as well for growth as those contained in wheat bread. Excellent growth of rats was obtained on a diet containing 10 per cent proteins derived from 25 per cent peanut bread,

in contrast with poorer growth on an enriched wheat bread diet which also provided 10 per cent of proteins. A mixture of wheat and peanut flour furnished more efficient proteins for growth than those of wheat flour alone (Johns and Finks, '20).

Lal and Rajagopalan ('53) studied the effect of mutual supplementation in mixtures of several plant oil-cakes on the biological values for the rat. Two mixtures had reasonably high biological values: 70 per cent for a mixture of 55 parts groundnut and 20 of cottonseed oil-cake with 25 parts of wheat; and 72.5 per cent for a mixture containing 60 parts groundnut, 20 of soybean and 20 of sesame oil-cake. The net protein values of these mixtures, 30.13 and 33.88, compare favorably with the values for animal proteins and are considerably higher than those of commonly used cereals and pulses.

Peanut butter produced by grinding peanuts into a paste has long been used as a food in Spain, Rhodesia, and India (Rosen, '58). In Nigeria, dried biscuits made from a mixture of peanut and maize flour are given to children. In India, groundnut milk has been developed during the last decade to provide more and better food for the increasing population (Moorjani and Subrahmanyan, '50).

As early as 1917, Wallis evolved a food called "Nutramine" to be used with cereal flours for bread, biscuits, and other forms of human food. The preparation contained 84 per cent of groundnut meal, 14 per cent of dried milk and two per cent bicarbonate of soda. This flour was found to keep well and was considered a good supplement for cereal flour in bread and biscuits, and in soup or with hot milk as an invalid food

(Giri, '52). The introduction of Nutramine can be considered the stepping stone for the later developments undertaken in India towards the incorporation of groundnuts in various acceptable forms into the diet.

In India, the All India Institute of Science, Bangalore, and Central Food Technological Research Institute, Mysore, recently undertook a joint attempt to make a high-protein food with peanut flour that can be used to improve any poor diet. In this food the groundnut flour is mixed with roasted Bengal gram and roasted black gram, and the flavor is said to be excellent. The Bangalore Institute also has developed a "multipurpose food" from a groundnut-cake flour, sesame-seed-cake flour, soybean flour, wheat flour, tapioca and salt, which has proved to be an acceptable addition to the diets of children (Dean, '58). The Central Food Technological Research Institute, Mysore, has perfected a preparation which includes peanut flour, and roasted grain flour with minerals and vitamins added (Food and Agriculture Organization, '56).

Groundnuts or peanuts are being used in the manufacture of a number of highly nutritious products like groundnut butter, groundnut milk, predigested protein foods and synthetic rice grains in which one of the important ingredients used is groundnut-cake flour. The synthetio rice grains have more or less the same chemical composition and are somewhat superior to natural rice (Giri, '52).

Since all cereal grains have the double disadvantage of a relatively low protein content and a poor protein quality, due to deficiency of several of the essential amino acids, these disadvantages can be corrected only by combining cereal grains with foods of higher protein

concentration which provide essential amino acids in proper proportions to correct the deficiencies in the cereal. The inclusion of vegetable protein mixtures for human feeding involves the combination of cereal grains with such foods which will increase the quality and the quantity of the protein in the diet (Scrimshaw and Bressani, '60). Foods can be improved in protein quality either by supplementing with additional sources of proteins or with essential amino acids and the most desirable is a suitable combination of these two methods (Rosenberg, '59).

Various studies have been done on improving the protein quality of polished rice either by supplementing with protein-rich foods or with pure amino acids. Sure ('46b; '47) studied the nutritional improvement of polished rice with additions of small amounts of food yeast, brewer's yeast and soybean. In a later study ('57) he investigated the influence on growth and protein efficiency in rats of adding fish flour at one, three and 5 per cent levels to polished rice. Increases in body weight of 132.6 and 151.0 gm were obtained at the three and 5 per cent levels, respectively as compared to 90.6 with one per cent fish flour. Protein efficiency was less, however, in the diet containing 5 per cent fish meal, 2.39 as compared to 2.51 for the diets having less fish meal.

Kik ('56) used dried ocean perch fillets as supplements to milled rice at one, three and 5 per cent levels, keeping the protein content at 5.27 per cent, and studied the effect on growth of albino rats. At the one and three per cent levels body weights increased by 111 per cent and protein efficiency ratios by 52.9 and 65.7 per cent, respectively. Weight increase and efficiency of utilization were both less at the 5 per cent

level, average increases being 100 and 49.4 per cent.

Subrahmanyan et al. ('54) studied the effect on growth of rats of replacing rice to the extent of 25 per cent by tapioca flour, sweet potato flour, a mixture of sweet potato flour and groundnut-cake flour (4:1), and a mixture of tapioca flour and groundnut-cake flour (4:1). The incorporation of groundnut-cake flour together with tuber flours as a partial substitute for rice resulted in an improvement in the overall nutritive value of the diet. The incorporation of groundnut-cake flour together with tapioca flour produced an average weekly weight gain of 9.05 gm and with sweet potato flour of 9.55 gm, as compared to the plain milled rice diet which allowed an average weekly gain of 4.92 gm. When just tuber flours were substituted for rice, the gains were 7.23 and 6.22 gm for tapioca flour and sweet potato, respectively.

Many investigations have been made on the improvement of the rice diet by supplementation with amino acids. Rosenberg ('59) suggested that a protein should be supplied with its first limiting amino acid in such an amount as to obtain balance of the first limiting amino acid with the second to provide the two in the ratio in which the body needs them for protein synthesis. If this plan is followed and all other essential nutrients are provided in adequate amounts, a distinct improvement in protein quality should be expected. So proper amino acid supplementation might stretch considerably the world's protein supply.

Rosenberg and Culik ('57) suggested that the protein quality of white rice can be significantly improved by the addition of lysine, the first limiting amino acid, in amounts sufficient to bring this amino acid

in balance with the second limiting amino acid. In 5 experiments, male and female weanling rats were fed diets containing 90 per cent precooked polished rice supplemented with graded amounts of L-lysine hydrochloride. Substantial improvement in growth rate and in efficiency of food utilization were obtained with 0.05 - 0.10 per cent of L-lysine hydrochloride supplementation.

Pecora and Hundley ('51) demonstrated that highly significant improvement of natural rice protein was accomplished by the addition of two essential amino acids, lysine and threonine, to polished rice. This combination produced a growth response in white rats three times that obtained with an unsupplemented rice diet, the averages being 28.0 and 9.2 gm per week, respectively. But when the rice diet containing lysine and threonine was further supplemented with all the other essential amino acids, individually or with all the possible combinations of pairs, growth was not improved.

These results indicated that lysine and threonine are the most deficient amino acids in rice and that they are about equally limiting for rat growth. This was stressed further by the study of Kik ('56) where he fed albino rats whole and milled rice rations containing 5.59 per cent protein, with and without a supplement of 0.2 per cent L-lysine, 0.2 per cent DL-threonine and 0.1 μg vitamin B_{12} daily. Rats fed rice diets containing the amino acids and vitamin B_{12} showed better growth and utilisation of the protein than those on the unsupplemented rice diets. The protein efficiency and net utilisation of supplemented whole rice were 2.79 and 90.4 per cent against 1.91 and 84.6 per cent for

unsupplemented whole rice; the protein efficiency and net utilization of supplemented polished rice were 3.12 and 93.4 per cent compared to 1.72 and 59.5 per cent with polished rice alone. The author explained the results on the basis that there were smaller losses of nitrogen during digestion and metabolism of the supplemented diets, and hence better growth of the rats.

Similar results were obtained by Rosenberg et al. ('59) when they fed male white rats precooked rice diets supplemented with graded amounts of lysine and threonine. Fourteen different levels of lysine were tested in various combinations with 7 different levels of threonine. Best performance in the growth of rats was observed when 0.34 per cent lysine and 0.18 per cent threonine were added to the rice diet.

Sure ('55b) went a step further, and suggested from his study on rats that marked response to lysine supplementation of polished rice was found only in the presence of threonine and that growth was improved further by the addition of methionine. This is in agreement with the view of Asenjo and Goyco ('54). In another study, Sure ('55c) observed that there was no growth response on the addition of lysine alone as a supplement to the proteins in polished rice. However when 0.3 per cent DL-threonine was added along with 0.4 per cent L-lysine, there was 125.1 per cent additional growth and 51.9 per cent increase in protein efficiency ratio. The further addition of 0.5 per cent Dl-methionine was effective in producing an additional increase of 21.1 per cent in body weight and 27.0 per cent increase in protein efficiency.

Harper et al. ('55) found that growth in rats fed polished rice

was improved only when both lysine and threonine were included in the rice diet and that further improvement in growth was obtained only when a mixture of all the essential amino acids was included. Arulanantham ('54) suggested that a poor rice diet could be improved by supplementation with 10 per cent vitamin test casein after the diet was replenished with necessary amounts of vitamin A, riboflavin and calcium.

The FAO Committee on Protein Requirements (Food and Agriculture Organization, '57) has formulated a reference amino acid pattern for humans for estimating the nutritive value of the proteins in foodstuffs and to serve as a guide in the supplementation of these foodstuffs, one food with another and with amino acids. This pattern of essential amino acids was based on the requirements of healthy human adults. The FAO Committee has expressed the protein requirements in terms of "a protein of high nutritive value" defined by putting forward examples of proteins which fall into this category like the proteins of milk, eggs, and meat. The provisional amino acid pattern put forward by the FAO does not correspond precisely to that found in milk and egg protein. However, it can be assumed that the hypothetical protein containing amino acids according to the pattern would be a protein of high biological value falling into the reference category.

Experiments have been conducted to test the efficiency of the FAO amino acid reference pattern for growth of the weanling rat (Howe et al., '60). Since arginine and histidine are required for the growth of young rats, but not included in the FAO reference pattern for humans, the authors included these amino acids in the mixture in the proportions

found in casein. They observed that at a high essential amino acid content, the FAO amino acid reference pattern showed greater efficiency than an amino acid mixture patterned after casein. At a lower essential amino acid content, however, the protein efficiencies were equal, but the FAO pattern supported much more rapid growth than the mixture patterned after casein, when the diets were fed ad libitum.

Bressani et al. ('58) investigated the effect on nitrogen retention in children of supplementation of a corn-masa diet with amino acids based on the FAO reference pattern. The basic diet consisted of corn-masa as the only source of protein, supplying 20 gm of protein per kilogram of diet. This diet was supplemented stepwise with essential amino acids to match the amino acid pattern of the FAO reference standard and was found to produce good nitrogen retention by young children. Scrimshaw et al. ('58) concluded that this attempt to formulate a provisional pattern is a valuable experimental approach to the problems of amino acid supplementation, even though the reference pattern may not be ideal for all experimental conditions or physiologic states.

The FAO reference pattern of amino acids has also been successfully applied in the supplementation of wheat flour (Bressani et al., '60). Six children recently recovered from severe protein malnutrition were fed a wheat diet in which the protein was contributed by both wheat flour and wheat gluten. The nitrogen retention of young children improved considerably when the wheat flour was supplemented with essential amino acids in the proportions of the reference pattern.

PROCEDURE

The present experiment was designed to determine the influence upon growth and efficiency of protein utilization in rats of supplementation of a rice diet with raw peanuts and essential amino acids. Two series of experimental diets were used. In the first series, a diet containing 85 per cent polished rice, one in which 5 per cent of the rice was replaced by raw peanuts, and a third in which 10 per cent of the rice was replaced by peanuts, were used. In the second series, the rice and peanut-rice diets were supplemented with amino acids to provide them in proportions as nearly identical as possible with the FAO provisional amino acid pattern.

Diet I contained 85 per cent of polished rice. In diets II and III, 5.1 and 10.5 per cent of ground peanuts replaced equal weights of rice. The diets were planned to contain the peanuts at the 5.0 and 10.0 per cent level. An error in the amount of starch used in equalizing the fat content of the three diets produced small but unimportant increases in the percentage composition of diets II and III over the basic diet I. Since peanuts contain 44.4 per cent fat and polished rice a negligible amount (Taylor, '59), the latter was disregarded in equalizing the levels of fat. Peanut oil was added to diets I, II and III to provide about 7 per cent fat from peanuts and/or peanut oil. Cod liver oil (one per cent) brought the calculated fat content of each diet to approximately 8 per cent. Addition of Osborne and Mendel salt mixture and B-vitamins minimized differences in the mineral and vitamin content of the enriched

polished rice and the peanuts. The composition of the diets is shown in table 2. In the second series of diets, mixtures of amino acids were added at the expense of starch to diets I, II and III to give diets Ia, IIa and IIIa. These had practically the same percentage composition, except for essential amino acids, as the corresponding unsupplemented rice and peanut-rice diets (table 2).

Calculation of the amounts of amino acids to be added to provide the same proportions of essential amino acids as in the FAO provisional pattern was based on the work of Howe et al. ('60). These authors prepared an amino acid mixture for rat diets equivalent to the FAO pattern for man on the basis that, except for D-methionine, only the L-isomers are utilized. When racemic acids were used, the quantity incorporated was double the required amount of the L-isomer. Arginine and histidine, which are not components of the FAO pattern, were included in the proportions found in casein because of their influence upon growth of young rats. In the preparation of diets Ia, IIa and IIIa, the amounts of the 10 essential amino acids in the rice and peanut-rice diets were calculated, together with the percentages of nitrogen. Protein contents of 7.50 and 26.87 per cent for rice and peanuts (Taylor, '59) and the factor 6.25 for conversion of percentage protein to percentage nitrogen were used. The quantities of the amino acids corresponding to the FAO pattern at each level of dietary nitrogen were then determined and the appropriate amount incorporated in the particular diet (table 3). Leucine and arginine, present in the unsupplemented diets in excess of the FAO pattern were not included in the supplement. The calculated protein and total nitrogen

TABLE 2

COMPOSITION OF THE DIETS

Components	Diet I	Diet Ia	Diet II	Diet IIa	Diet III	Diet IIIa
		(gm per 100 gm of diet)				
Polished rice	85.00	85.00	81.82	81.82	78.45	78.45
Peanuts	—	—	5.11	5.11	10.46	10.46
Peanut oil	6.60	6.60	4.50	4.50	2.30	2.30
Cod liver oil	1.00	1.00	1.02	1.02	1.05	1.05
O & M salt mixture	4.00	4.00	4.08	4.09	4.18	4.18
Vitamin mixture	1.00	1.00	1.02	1.02	1.05	1.05
Total amino acids	—	1.02	—	1.20	—	1.40
Corn starch	2.40	1.38	2.45	1.24	2.51	1.11
Total	100.00	100.00	100.00	100.00	100.00	100.00

Vitamin mixture - mg per 100 gm of diet:

Thiamine	1.00	Choline chloride	120.00
Riboflavin	1.00	P-amino benzoic acid	120.00
Pyridoxine	1.00	Inositol	30.00
Calcium pantothenate	6.00	Starch	720.00
Nicotinic acid	1.00		

TABLE 3

AMINO ACID SUPPLEMENTATION OF DIETS I, II AND III

(gm per 100 gm diet)

Amino acid	Diet I Supple-ment	Diet I Nitrogen	Diet II Supple-ment	Diet II Nitrogen	Diet III Supple-ment	Diet III Nitrogen
DL - Valine	0.18	0.022	0.20	0.024	0.23	0.028
L - Leucine	—	—	—	—	—	—
L - Isoleucine	0.20	0.021	0.25	0.026	0.29	0.031
DL - Methionine	0.05	0.004	0.06	0.006	0.08	0.008
DL - Threonine	0.14	0.016	0.17	0.020	0.19	0.022
DL - Phenylalanine	0.11	0.009	0.14	0.012	0.15	0.012
DL - Tryptophan	0.08	0.011	0.09	0.012	0.11	0.015
L - Lysine. HCl	0.15	0.028	0.16	0.030	0.19	0.036
L - Histidine. HCl	0.11	0.030	0.13	0.035	0.16	0.043
L - Arginine	—	—	—	—	—	—
	1.02	0.141	1.20	0.165	1.40	0.195

content of the 6 diets is shown in table 4.

Since polished rice is used generally in India, "Comet" brand polished rice was used. Although this product is fortified with vitamins and minerals, it was considered as much like the milled rice used in the Orient as any brand locally available. The rice was purchased at a Knoxville grocery in two pound boxes and used without washing. Peanuts in the shell were bought at the same store and shelled before use. Both rice and peanuts were used without cooking.

The rice and peanuts were finely ground in an electric coffee mill. The grinding of the peanuts was accomplished without difficulty when some of the rice meal was first added to the whole peanuts. The starch, B-vitamin mixture, salt mixture and amino acids were added to the rice or rice and peanuts, followed by the peanut and cod liver oils which were worked into the dry mixture to give a homogeneous blend. Each diet was prepared in adequate quantity for the entire experiment and stored in a refrigerator.

Thirty albino rats, 4 to 5 weeks old, from the Nutrition Department stock colony at the University of Tennessee were the experimental animals. They were the young of 5 mothers, and were distributed with regard to age, sex and heredity to give 6 matched groups of 5 animals each. Each group consisted of two females and three males, with an average weight of 62.0 - 64.0 gm. The rats were housed in individual cages, with a tray below the raised bottom where a sheet of paper was placed to collect the spilt food and feces. The diet and tap water were given ad libitum.

TABLE 4

CALCULATED PROTEIN AND NITROGEN CONTENT OF THE DIETS

(gm per 100 gm diet)

Diet	Polished Rice Protein	Polished Rice Nitrogen	Peanuts Protein	Peanuts Nitrogen	Amino Acid Nitrogen	Total protein	Total nitrogen
Diet I	6.38	1.02	--	--	--	6.38	1.02
Diet Ia	6.38	1.02	--	--	0.14	6.38	1.16
Diet II	6.14	0.98	1.37	0.22	--	7.51	1.20
Diet IIa	6.14	0.98	1.37	0.22	0.16	7.51	1.36
Diet III	5.88	0.94	2.81	0.45	--	8.69	1.39
Diet IIIa	5.88	0.94	2.81	0.45	0.20	8.69	1.59

Weighed amounts of the rations in food jars were placed in the cages daily except Sunday and each rat received a double portion of diet on Saturday. Every day the left-over food in the jar was weighed and spilled food recovered to obtain the daily intake of food of each rat. The paper was replaced every day and fresh water was supplied on alternate days. The rats were weighed once a week and the weight gain was noted. The experiment was carried on for 10 weeks, at the end of which the animals were sacrificed with chloroform.

RESULTS AND DISCUSSION

The most urgent problem to be met in the improvement of the diet of rice-eating populations is to raise the level of protein. Polished rice contains less than 10 per cent protein although its biological value exceeds that of many cereals. For this reason, in the present studies on the influence of peanut and amino acid supplements upon the adequacy for rat growth of a rice diet, the peanuts were added at the expense of an equal weight of rice and the amino acids by replacement of starch. The resulting diets were made isocaloric with the rice diet, but the protein content varied with the amount of protein-rich peanuts added and the total amino acid nitrogen with the size of the essential amino acid supplement. Although variation in nitrogen content of the diets makes interpretation of growth response more difficult, this type of study seemed preferable since it parallels the kind of supplementation necessary in the improvement of cereal diets for human consumption.

In the interest of clarity, the amino acid or total nitrogen content and the growth response of rats fed rice and peanut-supplemented rice diets will be considered separately from those receiving the essential amino acid-supplemented rice and peanut-rice diets. Each group of animals has been designated by the number assigned to the diet on which the group was maintained.

Food consumption, total nitrogen intakes and weight gains of groups I, II and III are recorded in table 5. Values except food intake are those obtained for the entire 10-week period during which the animals were fed ad libitum. Initially, all rats were approximately the same size,

TABLE 5

FOOD CONSUMPTION AND WEIGHT GAIN OF RATS FED RICE AND PEANUT-SUPPLEMENTED RICE DIETS

Diet: Nitrogen source	Diet: (%)	Diet: Nitrogen content	Rat no.	Food intake gm/day	Nitrogen intake gm/10 weeks	Weight: Initial gm	Weight: Gain gm/10 weeks
Diet I							
Rice:	85.0	1.02	1	7.4	5.21	68.0	70.5
Peanut:	0.0		2	7.6	5.04	62.0	69.5
			3	7.8	5.51	64.0	81.0
			4	6.6	4.65	62.0	63.5
			5	7.1	5.02	55.0	68.5
			Average	7.3	5.08	62.2	70.6
Diet II							
Rice:	81.8	0.98	6	8.0	6.61	69.0	85.0
Peanut:	5.1	0.22	7	7.8	6.49	62.0	99.5
		1.20	8	9.2	7.63	55.0	111.5
			9	7.6	6.29	64.0	78.0
			10	8.0	6.65	63.0	83.5
			Average	8.1	6.73	62.6	91.5
Diet III							
Rice:	78.4	0.94	11	7.2	6.90	60.0	86.0
Peanut:	10.5	0.45	12	7.3	6.96	58.0	90.5
		1.39	13	8.6	8.21	64.0	114.0
			14	8.3	7.99	63.0	105.5
			15	8.4	8.07	67.0	104.5
			Average	8.0	7.63	62.4	100.1

the average weights of the three groups being 62, 63 and 62 gm. During the first week on the experimental diets food consumption and weight gains were small and similar, possibly because of adjustment to the low-protein diets. By the end of the second week the groups fed the peanut-supplemented diets II and III had begun to eat slightly more food than group I receiving the rice diet and showed the beginning of a better growth rate (fig. 1). Throughout the remainder of the experiment the same pattern persisted, animals in groups II and III growing at a greater rate than those in group I, though all growth was clearly suboptimal.

Diet I, with 85 per cent polished rice, contained 1.02 per cent protein nitrogen (table 5); diets II and III, in which 5.1 and 10.5 per cent peanuts replaced equal weights of rice, contained 1.20 and 1.39 per cent nitrogen, respectively. Since food consumption by groups II and III also exceeded that in group I, nitrogen intakes of the peanut-fed rats were significantly greater (table 6). To avoid small numbers, total nitrogen intakes over the 10-week period were recorded rather than intakes per day. Also for convenience, nitrogen instead of protein efficiency ratios were calculated for comparison of the diets to facilitate comparison with amino acid supplemented diets later. Although animals in group III ate slightly less per day, on the average, than those in group II, total nitrogen intake per rat was greater ($P < 0.05$).

Weight gains of the peanut-fed rats exceeded that of the group I animals fed the unsupplemented rice diet (table 6). Gains of the group III rats receiving the maximum amount of peanut supplement and having the highest nitrogen intake tended to be somewhat greater than those in

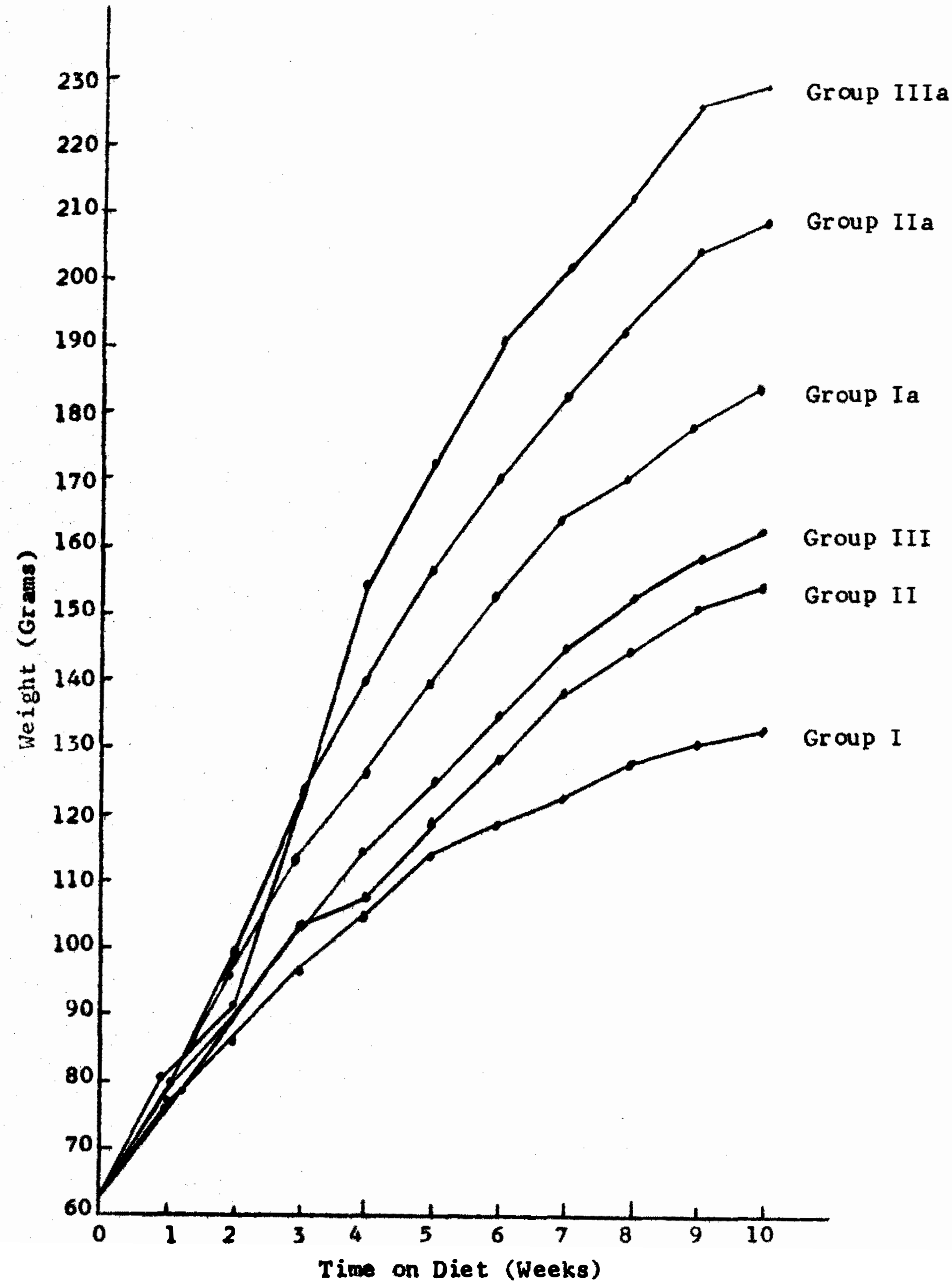

Figure 1. Growth of Rats Fed Rice, Peanut-Rice and Amino Acid Supplemented Diets. Curves Represent Average Values for 5 Rats in Each Group.

TABLE 6

EFFECT OF PEANUT SUPPLEMENTATION UPON NITROGEN INTAKE, WEIGHT GAIN AND NITROGEN EFFICIENCY IN RATS FED RICE DIETS

(10 weeks)

Diet	Nitrogen intake gm/rat	"t"[1] value	Weight gain gm/rat	"t" value	Nitrogen efficiency ratio	"t" value
I	5.08±0.31[2]		70.61±6.4		13.87±0.48	
II	6.73±0.52		91.5±13.7		13.55±1.33	
	II-I	6.07[4]		3.09[3]		0.50
III	7.63±0.64		100.1±11.5		13.10±0.52	
	III-II	2.41[3]		1.07		0.71
	III-I	7.98[4]		4.99[4]		2.44[3]

[1]Fisher "t" test of mean difference (Snedecor, '50).

[2]Standard deviation.

[3]Difference significant at 5 per cent level.

[4]Difference significant at 1 per cent level.

group II, but individual variations were large in both groups and the difference in average gains was not significant. Although growth increased with nitrogen intake, the nitrogen efficiency ratio decreased slightly with addition of peanuts to the rice diet. The efficiency of nitrogen utilization by animals fed the 10.5 per cent peanut diet was significantly less than by the rice-fed animals ($P < 0.05$) but other differences fell within the limits of individual variation. Nevertheless, a trend toward decreased efficiency of utilization was evident, suggesting that factors other than nitrogen intake alone influenced growth response on the low-protein diets. The nitrogen efficiency ratio of group I, 13.87, corresponds to a protein efficiency ratio of 2.22, which is in reasonable agreement with ratios of 1.50 to 2.01 obtained by Kik ('60) in rats fed comparable rice diets.

Sure ('48b) pointed out that protein efficiency ratio does not always express solely the weight increase per gram of protein consumed. So-called protein efficiency ratios must include as well gains in body weight produced by caloric intake. Mitchell ('24a) suggested that the gain in body weight per gram of protein consumed cannot be an absolute measure of the nutritive efficiency of the protein, since it depends upon experimental conditions, particularly the size of the animal, level of dietary proteins and amount of food consumed relative to body size. Possibly all of these factors had some influence in the studies reported in this paper.

Since the efficiency of utilization of dietary protein for building body protein is a function of the pattern of indispensable amino acids

absorbed, and the pattern of amino acids absorbed is in turn a function of protein composition and its digestion, the amino acid patterns of diets I, II and III were compared with the amino acid requirements of rats reported by Rama Rao et al. ('59). These values represent the minimum requirements necessary for maximum growth of rats on diets containing 5 per cent casein supplemented with essential amino acids and glycine to furnish a total nitrogen level of 1.6 per cent and afford a better basis for comparison with supplemented rice diets than do the data of Rose ('37), obtained for diets of pure amino acids.

The essential amino acid content of polished rice and of peanuts, together with Rama Rao's minimum requirements, were calculated as grams of amino acids per gram of dietary nitrogen for comparison (table 7). The figures indicate that in rice methionine and lysine levels are far below minimum requirements and that the threonine and phenylalanine content is inadequate. The apparently large deficit of methionine, however, fails to take into account the fairly high cystine content of rice; the tyrosine in rice supplements the phenylalanine, also. Lysine is generally assumed to be the most limiting amino acid in rice, but Pecora and Hundley ('51) found that supplementation of rice diets with both lysine and threonine was necessary to materially improve rat growth. The possibility also exists that at low dietary protein levels any unfavorable ratio of amino acids present may increase the percentage of the most limiting amino acid required for growth of the animal (Harper and Kumta, '59). In polished rice the ratio of leucine:isoleucine, 1.50, exceeds that in many high quality proteins and is greater than the ratio of 1.26

TABLE 7

COMPARISON OF THE AMINO ACID PATTERN OF POLISHED RICE, PEANUT, AND DIETS I, II AND III WITH THE RAT REQUIREMENT FOR GROWTH

(gm per gm nitrogen)

Amino acids	Requirement[1] for rats	Polished[2] rice	Peanut[3]	Diet I	Diet II	Diet III
Valine	0.35	0.39	0.50	0.39	0.41	0.42
Leucine	0.43	0.48	0.44	0.48	0.47	0.47
Isoleucine	0.34	0.32	0.26	0.32	0.31	0.30
Methionine	0.30 including cystine	0.09	0.07	0.09	0.08	0.09
Threonine	0.31	0.22	0.18	0.22	0.22	0.21
Phenylalanine	0.45 including tryosine	0.29	0.34	0.29	0.30	0.31
Tryptophan	0.07	0.08	0.12	0.08	0.08	0.09
Lysine	0.56	0.17	0.21	0.17	0.17	0.18
Histidine	0.13	0.14	0.13	0.14	0.14	0.14
Arginine	--	0.54	0.66	0.54	0.56	0.58

[1]P. B. Rama Rao et al ('59).

[2]Jones et al. ('48).

[3]C. L. Hoffpauir ('53).

found by Rama Rao to represent the requirement of leucine and isoleucine for rat growth.

In India, the interest in the possible use of peanut products as supplements in the low-protein rice diet is based on two important factors: peanuts contain about 27 per cent protein as compared to less than 8 in rice; and, peanuts, which are liked by the people, can be produced in quantity at comparatively low cost. Moreover, it has been suggested (Johns et al., '16-'17) that the relatively high percentage of lysine in peanuts might make the seed of value as a supplement to lysine-deficient diets. On the basis of grams of amino acid per gram of nitrogen, the valine and tryptophan content of peanuts is significantly higher than in rice, and lysine and phenylalanine levels are slightly better (table 7). Unfortunately, however, the content of threonine and methionine is lower than in polished rice and the leucine:isoleucine ratio is even more unfavorable, 1.69 compared to 1.50 in rice and 1.26 in the amino acid mixture shown by Rama Rao to meet the growth requirements of rats.

The essential amino acid content of diets I, II and III was expressed both as grams per gram of nitrogen (table 7) and as percentages of the diet (table 8, page 38) in an attempt to explain the growth responses of the three groups during the 10-week period. Diet I (table 7) like polished rice alone, furnished only one-third the lysine or methionine requirement, disregarding the cystine present, supplied inadequate amounts of threonine and phenylalanine, exceeded the valine requirement and had a leucine:isoleucine ratio of 1.5. Rats fed this diet grew at a retarded rate throughout the experiment. Diets II and III, containing 5.1 and 10.5

per cent peanuts, respectively, had amino acid patterns very similar to that of diet I. Valine and arginine, already high in the rice diet, were increased further by peanut supplementation. The lysine, methionine and threonine levels, per gram of nitrogen, were unaltered as was the high leucine:isoleucine ratio. The phenylalanine content of diet III was higher than that of diet I although still below the minimum requirement. Yet the weight gains of groups II and III were significantly greater than in group I (table 6, page 32). While the nitrogen intakes were higher, also, nitrogen utilization was less efficient, and animals in group III achieved only slightly larger gains than in group II in spite of significantly greater nitrogen consumption.

These results suggest that while the amino acid pattern of diets II and III was no more favorable for growth than that of diet I, the increase in the percentage of the limiting amino acids in the diets may have favored growth either in absolute terms or through lessening the influence of the unfavorable leucine:isoleucine ratio. Data in table 8 show that, per 100 gm of food, diets II and III furnished 24 and 47 per cent more lysine and 18 and 32 per cent more threonine, respectively, than diet I. Since animals in groups II and III ate more food as well, the actual intakes of the limiting amino acids were much higher. Whether the supplementary effect of peanuts was approaching its maximum at the 5 per cent dietary level or whether the slightly smaller daily food intake of the group III animals explains their failure to gain significantly more weight cannot be stated with certainty. The difference in average food intake per rat per day was exceedingly small, however, and the latter possibility seems unlikely.

TABLE 8

CALCULATED AMINO ACID CONTENT OF THE EXPERIMENTAL DIETS
AND THE FAO REFERENCE AMINO ACID MIXTURE FOR RATS

(gm per 100 gm diet)

Amino acid	Diet I	Diet II	Diet III	FAO[1] pattern	Diet Ia	Diet IIa	Diet IIIa
Valine	0.40	0.49	0.59	0.54[2]	0.58	0.69	0.82
Leucine	0.49	0.57	0.65	0.30[3]	0.49	0.57	0.65
Isoleucine	0.33	0.37	0.42	0.54[3]	0.53	0.62	0.71
Methionine	0.09	0.10	0.12	0.14[2]	0.14	0.16	0.20
Threonine	0.22	0.26	0.29	0.36[2]	0.36	0.43	0.48
Phenylalanine	0.30	0.36	0.43	0.36[2]	0.41	0.50	0.58
Tryptophan	0.08	0.10	0.13	0.18[2]	0.16	0.19	0.24
Lysine	0.17	0.21	0.25	0.35[3]	0.32	0.37	0.44
Histidine	0.14	0.17	0.19	0.27[3]	0.25	0.30	0.35
Arginine	0.55	0.67	0.80	0.32[3]	0.55	0.67	0.80

[1]Rowe et al. ('60).

[2]DL-form of the amino acid.

[3]L-form of the amino acid.

Three other groups of rats, Ia, IIa and IIIa, were maintained during the 10-week period on rice and peanut-rice diets supplemented with amino acids to give an essential amino acid composition as nearly identical as possible with the FAO provisional pattern proposed for human diets (Food and Agriculture Organization, '57). Howe et al. ('60) found such an amino acid mixture plus arginine and histidine supported more rapid growth of rats fed ad libitum than an amino acid mixture patterned after casein at the same low essential amino acid level. The percentages of amino acids in diet Ia (table 8) were essentially the same as those in the FAO reference pattern except for leucine and arginine which are present in large proportions in polished rice. In diet Ia, however, the leucine:isoleucine ratio was reduced to less than 1.0. Addition of amino acids to the peanut-rice diets II and III was made in the same proportions to give diets IIa and IIIa having the same essential amino acid composition per gram of nitrogen as diet Ia but with increasing percentages of each acid. The calculated percentage composition with respect to essential amino acids of the 6 diets used in this study is presented in table 8 together with that of a diet representing the FAO reference pattern at a level of 1.0 per cent nitrogen. The nitrogen content of diets Ia, IIa and IIIa is shown in table 9.

Growth curves of animals in groups Ia, IIa and IIIa are shown in figure 1 (page 31) along with those of groups I, II and III. During the first week on the experimental diets average gains in all 6 groups were almost identical. Following adjustment to the diets, gains of the groups receiving the three amino acid-supplemented diets began to exceed those of

TABLE 9

FOOD CONSUMPTION AND WEIGHT GAIN OF RATS FED AMINO ACID-SUPPLEMENTED DIETS

Diet: Nitrogen source	(%)	Nitrogen content	Rat no.	Food intake gm/day	Nitrogen intake gm/10 weeks	Weight: Initial gm	Weight: Gain gm/10 weeks
Diet Ia							
Rice:	85.0	1.02	16	8.9	7.13	56.0	128.5
Peanut:	0.0		17	8.3	6.66	60.0	111.0
Amino			18	9.3	7.43	68.0	134.0
acids:	1.0	0.14	19	9.3	7.47	72.0	126.0
		1.16	20	8.1	6.52	56.0	110.5
			Average	8.8	7.04	62.4	122.0
Diet IIa							
Rice:	81.8	0.98	21	7.4	6.97	57.0	96.0
Peanut:	5.1	0.22	22	8.8	8.25	64.0	137.0
Amino			23	10.5	9.92	70.0	183.0
acids:	1.2	0.16	24	9.0	8.53	67.0	144.5
		1.36	25	10.0	9.46	55.0	173.0
			Average	9.1	8.63	62.6	146.7
Diet IIIa							
Rice:	78.4	0.94	26	8.1	8.88	68.0	119.0
Peanut:	10.5	0.45	27	9.7	10.47	55.0	180.0
Amino			28	10.8	11.81	68.0	199.0
acids:	1.4	0.20	29	9.4	10.34	65.0	149.5
		1.59	30	10.1	11.02	60.0	184.5
			Average	9.6	10.50	63.2	166.4

groups I, II and III, a pattern which persisted throughout the remainder of the experiment. As would be expected, average daily food consumption by the animals growing at the more rapid rates was greater also (table 5, page 29, and table 9).

Considering first the relative adequacy for growth of diets having an identical pattern of essential amino acids but at different percentages in the diets, average total nitrogen intake, weight gains and nitrogen efficiency ratios of groups Ia, IIa and IIIa were compared (table 10). Nitrogen intakes of the groups fed the supplemented peanut-rice diets were significantly higher than that of group Ia, receiving the amino acid-supplemented rice diet. Moreover, the nitrogen intake of the group IIIa animals exceeded that of group IIa and the slightly larger food consumption resulted in a somewhat larger caloric intake, also. In spite of this, differences in growth were not significant except between groups Ia and IIIa where differences in nitrogen and caloric consumption were at a maximum. As was true in groups I, II and III, the nitrogen efficiency ratio decreased with increasing nitrogen intake, but the differences were not significant. These results suggest that the percentages of essential amino acids in diet Ia met the growth requirements of rats receiving 1.16 per cent dietary nitrogen and that the small growth accelerations found in groups IIa and IIIa may have been the result of greater nitrogen intake alone. The fact that the average final weight of the group IIIa rats compares favorably with that of animals from the Nutrition colony, of the same age, fed 18 per cent casein diets and somewhat exceeds average weight of those fed stock diets is another indication of the favorable proportions in which essential amino acids occur in the diets having the FAO

TABLE 10

EFFECT OF AMINO ACID SUPPLEMENTATION UPON NITROGEN INTAKE, WEIGHT GAIN AND NITROGEN EFFICIENCY IN RATS FED RICE AND PEANUT-RICE DIETS

(10 weeks)

Diet	Nitrogen intake gm/rat	"t"[1] value	Weight gain gm/rat	"t" value	Nitrogen efficiency ratio	"t" value
Ia	7.04±0.43[2]		122.0±10.7		17.30±0.67	
IIa	8.63±1.14		146.7±34.2		16.81±1.88	
	IIa-Ia	2.98[3]		1.54		0.55
IIIa	10.50±1.08		166.4±32.0		15.73±1.69	
	IIIa-IIa	2.67[3]		0.94		0.96
	IIIa-Ia	6.67[4]		2.94[3]		1.93

[1]Fisher "t" test of mean difference (Snedecor, '50).

[2]Standard deviation.

[3]Difference significant at 5 per cent level.

[4]Difference significant at 1 per cent level.

provisional pattern. Diet IIIa contains 1.6 per cent nitrogen, equivalent to only 10 per cent protein.

Nitrogen efficiency ratios obtained in groups Ia, IIa and IIIa were significantly greater than those for groups I, II and III. This cannot have been solely the result of a greater intake of amino acid nitrogen, since within the rice and peanut-rice series as well as in the amino acid-supplemented series of diets efficiency of nitrogen utilization tended to decrease with increasing level of dietary nitrogen. Average total nitrogen consumption of animals in group III (7.63 ± 0.64 gm) and in group Ia (7.04 ± 0.43 gm) was almost identical, yet average nitrogen efficiency ratios were 13.10 ± 0.52 and 17.30 ± 0.67, respectively. Clearly, diet Ia was better utilized for growth than diet III. Comparison of the percentage composition of the two diets with respect to essential amino acids (table 8, page 38) shows that diet III contains less of the limiting amino acids threonine and lysine per 100 gm than diet Ia and has a higher leucine: isoleucine ratio. Evidently the pattern of essential amino acids in the 10.5 per cent peanut-supplemented rice diet is less favorable for growth of rats than the rice diet supplemented with amino acids to approximate the FAO pattern.

In spite of the important influence of percentages of certain amino acids in the diets, level of dietary nitrogen was unquestionably a factor for growth. Considering the 6 diets as one series, without regard for differences in amino acid composition, weight increases during the 10-week period were significantly related to total nitrogen intake ($P < 0.01$).

If the growth response and efficiency of nitrogen utilization in rats may be taken as any indication of the value of diets for human populations, the results of the work reported in this paper suggest that supplementation of a rice diet with whole peanuts at a 10 per cent level fails to sufficiently increase the percentages of either protein or the limiting amino acids lysine and threonine. Since massive amino acid supplementation of human diets is neither economically feasible nor nutritionally desirable, use of a variety of high-protein supplements in addition to peanuts appears to be necessary if material improvement of rice diets is to be achieved.

SUMMARY

The experiment reported here was designed to study the influence upon growth and protein efficiency in rats exerted by supplementation of a rice diet with raw peanuts and essential amino acids.

Matched groups of young albino rats were maintained for 10 weeks on two series of diets. In the first series, the diets consisted of a ration containing 85 per cent polished rice, one in which 5.1 per cent of the rice was replaced by an equal weight of raw peanuts and a third in which 10.5 per cent of the rice was replaced by peanuts. The diets were made isocaloric by adjustment of the fat content with peanut oil, while the nitrogen content varied with the level of protein-rich peanuts. In the second series, the three diets in the first series were supplemented at the expense of corn starch with essential amino acids to provide a pattern equivalent at each level of dietary nitrogen to the FAO provisional pattern for man.

Growth of animals fed the rice and peanut-rice diets was sub-optimal throughout the 10-week period. Total nitrogen intake and weight gains were significantly higher on the peanut-rice diets than on the unsupplemented rice diet. Rats fed the 10.5 per cent peanut-rice diet had greater nitrogen intakes than those on the 5.1 per cent peanut-rice diet but increase in body weight was only slightly larger. Efficiency of nitrogen utilization decreased with increasing nitrogen intake in the series.

Calculation of the essential amino acid composition per gram of dietary nitrogen showed that patterns in the three diets were almost

identical but that the percentages of the essential amino acids increased with the level of peanuts in the diet. Comparison of the amino acid patterns, expressed as grams per gram of nitrogen, with the minimum requirements for rat growth in a 5 per cent casein-amino acid diet showed that the essential amino acid pattern of the three diets was not favorable for growth. Levels of several amino acids were limiting and the leucine: isoleucine ratio greatly exceeded that in the pattern meeting minimum requirements as well as in many high quality animal proteins.

The improvement in growth rate produced by peanut supplementation of the rice diet was interpreted to mean that although the amino acid pattern of the diets was the same, the greater percentage of the limiting amino acids in the peanut-rice diets favored growth either in absolute terms or through lessening the influense of the unfavorable leucine: isoleucine ratio.

In the amino acid supplemented diets the essential amino acid pattern was equivalent to the FAO provisional pattern for man except for high levels of arginine and leucine. The leucine:isoleucine ratio was significantly lower than in the rice and peanut-rice diets. Per gram of nitrogen, the essential amino acids were present in the three diets in the same amounts; as in the unsupplemented rice and peanut-rice diets the percentage of each amino acid increased with the amount of peanuts present.

Growth and nitrogen efficiency ratios of animals fed these diets were greater than on any of the unsupplemented rice and peanut-rice diets. Differences in weight gain were not significant except between the

supplemented rice and 10 per cent peanut-rice diets where difference in nitrogen intake was maximum. Growth of animals fed the supplemented 10 per cent peanut-rice diet was comparable to that of rats fed stock or 18 per cent casein diets although the dietary nitrogen was equivalent to only 10 per cent protein. Nitrogen efficiency ratios decreased slightly with increasing levels of dietary nitrogen but differences were not significant.

Weight gains were significantly related to total nitrogen intake when the effect on growth of the 6 diets was considered as a whole.

Results were interpreted to mean that, in the low-protein diets studied, the pattern as well as the percentage of the essential amino acids in the diet had a significant effect upon growth and efficiency of nitrogen utilization.

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