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BEAN PROTEIN EVALUATION AND SUPPLEMENTATION

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ABSTRACT

BEAN PROTEIN EVALUATION AND SUPPLEMENTATION

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Feeding experiments with weanling rats were conducted which showed that the protein quality of Navy beans (a poor source of sulfur-containing amino acids) can be largely improved by mixing the beans with sesame flour (a rich source of methionine).

The Protein quality of Navy beans, sesame meal, and mixtures of Navy beans:sesame flours based on protein ratios of 87.5:12.5, 75:25 and 50:50 were evaluated using Protein Efficiency Ratio (PER), Net Protein Ratios (NPR) and slope-ratio techniques. Protein scores and modified essential amino acid indices (MEAA) were also computed.

Compared to a PER of 100 for casein, the PER for beans (B), sesame (S), B:S 87.5:12.5, B:S 75:25 and B:S 50:50 were 62, 49, 72, 90 and 92%, respectively. The Relative Protein Value (RPV) and Relative Nutritive Value (RNV) were obtained from slope-ratio comparisons in which the corresponding lactalbumin values were set equal to

100. The RPV were 57, 52, 67, 71 and 74%, respectively. The RNV were 54, 52, 66, 68 and 74%, respectively. The protein scores for the tested samples were 48, 50, 60, 72 and 78, respectively. The MEAA indices were 88, 78, 89 and 88, respectively.

Linear regression equations and correlation coefficients were derived, relating the protein values obtained by the different assays for the five diets. All the bioassays correlated very well with the average correlation coefficient of 0.96. The correlation between the chemical assays was poor ($r = 0.50$) and of the two methods tested only protein scores gave a high correlation coefficient with the biological methods ($r = 0.94$).

The supplementary effect of whole egg powder on the Navy beans was also studied and the protein quality of the Navy beans was improved by the presence of egg protein in the diet.

The protein quality of the Navy beans subjected to different processing methods was estimated. The following processing methods were evaluated: Canning with or without sugar, home cooking and autoclaving. PER, methionine availability (growth assay with weanling rats) and lysine availability (reaction with 1-fluro-2, 4-dinitrophenol benzen) were used to estimate protein quality. For the canned beans, canned beans with 1.5% sugar in brine, home cooked beans and autoclaved beans

the PER values (compared to casein = 100) were 58, 56, 56 and 60%, respectively; the methionine availability values (compared to 100 for crystalline methionine) were 49, 42, 48 and 50%, respectively; the lysine availabilities were 93.3, 93.9, 97.5 and 97.4%, respectively. It was concluded that the processing methods did not seriously impair the protein quality of Navy bean.

Zinc supplementation of a 10% protein diet for the rat in which the sole source of protein was autoclaved Navy beans was carried out. Graded levels of zinc sulfate were added to the bean diets, so that the final concentration of zinc in the diet varied from 16.6 to 37.8 ppm. A very modest increase in growth, 6% in PER, over the unsupplemented diet, was observed when the zinc content of the bean diet was raised to 20 ppm. It was calculated that only 0.002% of the phytic acid of the beans could be involved in the binding of zinc. Supplementation of the same diet with 0.5% D, L-methionine resulted in a much greater increase in growth rate, 110% in PER. Furthermore, the very modest additional growth obtained by supplementing the bean diet with zinc disappeared when the bean protein quality was upgraded by adding methionine.

To

Dr. Pericles Markakis

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INTRODUCTION

Perhaps half of the world population is suffering from malnutrition. Protein malnutrition is one of the major nutritional problems in the world today. The world population consumes totally about 110 million metric tons (1966) of protein annually. Three-fourths (78%) of this quantity, 85 million metric tons, comes from plant products such as grain, legumes and potatoes. More than 26 million metric tons, or one-fourth, is represented by animal products. In terms of calories, however, nine-tenths of the human intake is derived from the plant kingdom and only one-tenth is derived from animal products (Borgstrom, 1967).

A significant proportion of the protein in the diet of large segments of the world population comes from legumes. Common beans (Phaseolus vulgaris) are among the most important legumes in human nutrition in Latin America, Far East, and parts of Africa and Asia. They contribute an important source of protein for the general population in developing countries and for some special groups in the more affluent countries.

In general, vegetable proteins lack one or more of the essential amino acids, but experiments have provided

evidence that the nutritional value of several vegetable proteins can be largely improved by appropriate supplements of amino acids or by combining them with foods containing proteins that have complementary amino acid patterns.

Two major nutritional problems have been associated with beans: the first is a deficiency in sulfur-containing amino acids and the second is the presence of anti-nutritional factors, such as trypsin inhibitors and hemagglutinins. Moderate heat treatment usually inactivates these deleterious substances and the nutritive value is improved. However, bean protein is rich in lysine while many other plant proteins are deficient in lysine.

Sesame (Sesamum indicum) is rich in sulfur-containing amino acids, particularly methionine. On the other hand, sesame is low in lysine, its first limiting amino acid.

The nutritional value or protein quality of a food protein depends not only on its content of amino acids but also on their physiological availability. This availability varies with the protein source, processing treatment (especially heating), and interaction with other diet components.

The objectives of this research were first to study the supplementary effect of sesame protein on Navy bean protein through rat feeding experiments. Protein scores and modified essential amino acid indices were

also determined. In parallel, the supplementary value of egg protein on the bean protein was also studied.

The second objective of this study was to determine if zinc supplementation can upgrade the protein quality of the beans as well as the amino acid supplementation.

The third objective was to evaluate the protein quality of beans subjected to different processing treatments, and to estimate the methionine utilization and lysine availability of the bean protein.

REVIEW OF THE LITERATURE

Amino Acids and Protein Requirements

Man, as well as other monogastric mammals, must have enough protein to provide nitrogen and certain amino acids for the synthesis of new tissue during growth and reproduction, of milk constituents during lactation, of any other nitrogen containing compounds, and for replacement of the nitrogen that is continuously lost from the body.

The credit goes to Rose (1949) for the important information as to which amino acids can be synthesized within the organism and which cannot. The latter must be provided in the diet and are termed "indispensable" or "essential." Those amino acids that can be synthesized in the body are called "dispensable" or "nonessential." Classification of amino acids in this way apply only to dietary need since all are essential for the synthesis of proteins.

Table 1 shows the classification of essential and non-essential amino acids (Rose, 1957).

Histidine is considered essential for the infant (Snyderman et al. 1963). Kopple and Swenseid (1975) recently investigated the essentiality of histidine and

TABLE 1.--Dietary Essential and Non-Essential Amino Acids for Man.

Essential	Non-Essential
Lysine	Glycine
Tryptophan	Alanine
Phenylalanine	Serine
Leucine	Cystine
Isoleucine	Tyrosine
Threonine	Aspartic Acid
Valine	Glutamic Acid
Methionine	Proline
Histidine	Hydroxyproline
	Arginine

compared the metabolic response to deletion of histidine from the diet of normal and of chronically uremic adult males. They showed some evidence of a dietary histidine requirement; subjects on a histidine-free diet had a negative N balance, but it was reversed by adding histidine.

Some amino acids may be essential for one species, but dispensable for another. Arginine is dispensable for man, even for the growing infant (Holt, 1967), but is essential for the young rat (Rose et al., 1948). Glycine is another example of an amino acid, which is essential for the chick, but not for mammals (Meister, 1965).

Some of the dispensable amino acids can be synthesized only from specific essential amino acids. If the former are provided in the diet, the need for the amino

acids from which they are synthesized is reduced. Cystine can be formed only from methionine, and when cystine is present in the diet in adequate amounts, less methionine is required (Rose and Wixom, 1955).

The other dispensable amino acids can be synthesized in the body from organic acids that are intermediates of the carbohydrate metabolism, e.g., α-ketoglutarate and pyruvate (Steele, 1952), and from the nitrogen of other amino acids or from such compounds as ammonium citrate (Rogers et al., 1970).

Nitrogen for adult humans can be supplied in large part by glycine and diammonium citrate (Terroine et al., 1930; Rose and Wixom, 1955). However, nitrogen balance in adult humans can be adversely affected if only one or two sources of nonspecific nitrogen make up a large part of the nitrogen in an amino acid diet, possibly because the rate of synthesis of the nonessential amino acids is not adequate (Swendseid et al., 1960; Anderson et al., 1969).

There is some evidence that intact proteins are superior to protein hydrolysates as a source of nitrogen for the growing rat. This is probably due, at least in part, to the absence of peptides from the hydrolysates. Also, evidence indicates that if the young rat is fed a diet containing amino acids instead of proteins from about 18 days of age, it requires another factor, not yet

identified, that is associated with proteins, but is not a component of them (Schwartz, 1970).

Because protein is the main source of nitrogen in the human diet, it is convenient to speak of the protein requirement of man; the true requirement is not for protein as such, but rather for specific amounts and proportions of essential amino acids and nonessential amino acid nitrogen.

The protein requirements for humans has been a controversial subject for many years. Voit (1876) showed that a man weighing 70 kg required 118 g protein per day. Atwater (1903) suggested 125 g per day for a sedentary man and 150 g per day for a working man. Siven (1900) and Langergren (1903) demonstrated that nitrogen equilibrium could be maintained on much lower intakes of protein than those previously suggested in the literature. Chittenden (1904), using himself as a subject, showed that it was possible to maintain nitrogen equilibrium at an intake of 36 - 40 g protein per day.

Sherman et al. (1920), reviewed the literature on the protein requirements to maintain nitrogen balance and found it to vary from 21 to 65 g per day with an average of 44 g per day for a 70 kg man (0.63 g protein/kg body weight). According to his studies, which were largely on cereal grains, he suggested a mean value of 0.5 g of protein/kg body weight/day as the requirement for nitrogen balance.

Food and Nutrition Board (1945) in its Recommended Daily Allowance adopted this value with an allowance for safety. They recommended an intake of 1.0 g protein/kg body weight/day. However, since the Recommended Allowance assumed that some of the proteins in the diet come from animal sources, the recommended intake seemed to be higher than the minimal need. Hegsted et al. (1946) suggested a value of 32.4 g protein/70 kg man/day or 0.46 g protein/kg body weight/day on a diet of mixed vegetables and 27 g protein/70 kg man/day or 0.38 g protein/kg body weight/day when the diet supplied one-third of the protein as meat.

In 1957, the FAO Committee on Protein Requirements took account of N balance studies in man to obtain an average minimum requirement for adults of 0.35 g of protein per kg of body weight, when the protein consisted of a reference protein of high nutritive value, such as whole egg protein. For optimum growth of infants, an intake of 2 g of reference protein per kg of body weight was recommended (FAO, 1957). The needs of other groups such as adolescents, and lactating women, were also estimated.

Since the nutritive value of the animal proteins of the diet is less than that of the reference protein, a further correction based on the chemical scores of the dietary protein was recommended. In a country with

a high standard of living the allowance is 0.66 g of dietary protein per kg of body weight, whereas in a country where proteins consumed have a lower chemical score, the allowance should be as high as 0.84 g per kg of body weight for adults. Further corrections were recommended when the quality of the protein in the diet is determined by NPU.

The Committee on Amino Acids of the Food and Nutrition Board (1959) estimated that an intake of 0.31 to 0.34 g/day/kg would meet the minimal adult requirement. These values were derived for a protein of high nutritional value, such as those contained in milk, eggs and meat (FAO, 1957).

The minimum nitrogen requirement of healthy adults for an ideal dietary protein has been assumed to be the sum of their urinary and fecal N losses, estimated after adaptation to an essentially N-free diet, plus integumental minimal sweat losses (FAO/WHO, 1965).

The 1968 edition of the Recommended Dietary Allowances (NAS-NRC, 1968) recommends an intake of 0.9 g of protein per kg of body weight per day for the 70 kg reference man.

The joint FAO/WHO Expert Group (FAO/WHO, 1965) established the requirement of 0.59 g of protein per kg body weight per day for an adult man.

It was estimated by the joint FAO/WHO AdHoc Expert Committee (WHO, 1973) that a 65 kg adult man would need 0.57 g of protein per kg of body weight per day to meet nitrogen requirements.

According to the latest edition of the Recommended Dietary Allowances (NAS-NRC, 1974), the allowance for the mixed proteins of the United States diet is 0.8 g/kg of body weight per day. The allowance for a 70 kg man is 56 g of protein per day.

Two approaches have been proposed for estimating the nitrogen requirements of man. The factorial method (FAO, 1957) is based on estimates of the obligatory N losses (the amount of N found in urine, feces, sweat, etc., when the diet contains no protein) and of the amounts of N needed for the formation of new tissue.

The nitrogen losses in adult men on a protein free diet (FAO/WHO, 1973) are shown in Table 2. Balance studies give direct evidence on N requirements from measurements of the lowest protein intake at which N equilibrium can be achieved in adults or satisfactory N retention and growth in children.

FAO/WHO AdHoc Expert Committee (1973) estimated that average N intake to maintain N balance in adults is 77 mg N per day when N is derived from milk, egg, cream or mixed diets containing animal protein. For subjects

TABLE 2.--Obligatory N Losses in Adult Men on a Protein-Free Diet.

Route	mg N Per kg of Body Weight	mg N Per Unit of Basal Energy (K cal)
Urine	37	1.4
Feces	12	0.4
Skin	3	0.13
Miscellaneous	2	0.08
Total	54	2.0

consuming cereals or vegetable diets the average is 93 mg of N per kg.

Table 3 summarizes some of the information on the amino acids required by adult human beings and growing rats. In general, the criterion of adequacy was to attain a positive N balance.

Evaluation of Protein Quality

Protein nutritional value of a food product is simply a reflection of the ability of that food product to meet the protein nutritional needs of the individual consuming it. In human nutrition, this might be related to growth of an infant, growth of maternal and fetal tissues, lactation, recovery from convalescence and maintenance of the body tissues. In animal nutrition, the purpose is a higher biological efficiency in terms of production of meat, milk, eggs, sweat, etc., together with maintenance of the animal.

TABLE 3.--Estimated Amino Acids Requirements of Adults and Growing Rats.

Amino Acid	Amino Acid Requirements Women[a] g/day	Men[b] g/day	Rat[c] g/day
Isoleucine	0.45	0.70	0.090
Leucine	0.62	1.10	0.138
Lysine	0.50	0.80	0.138
Methionine and Cystine	0.55	1.10	0.090
Phenylalanine and Tyrosine	1.12	1.10	0.138
Threonine	0.32	0.50	0.080
Tryptophan	0.16	0.25	0.045
Valine	0.65	0.80	0.115
Arginine	--	--	0.045
Histidine	--	--	0.080

[a]Leverton, R. M. (1959).
[b]Rose, W. C. and Wixon, R. L. (1955).
[c]Albritton, E. C. (1954).

The nutritional value or protein quality of a food protein depends on not only its content of amino acids but also on their physiological availability. This availability varies with the protein source, processing treatments (especially heating), and interaction with other diet components. The availability also depends on the condition of the consuming animal.

There are several available ways of expressing the nutritional value of dietary protein. All methods,

however, measure the capacity of proteins to supply essential nutrients for growth and maintenance of the organism.

The biological value of a protein is the proportion of absorbed protein nitrogen which is retained by the body to build, repair and maintain tissue protein. One of the principle concepts emerging from nutrition research is that the essential amino acid content of a protein determines its biological value. Even more importantly, the biological value is determined by the essential amino acid balance, that is, by the relative proportions of the amino acids present in a protein. Therefore, the more closely the essential amino acid pattern of the dietary protein conforms to the pattern used for protein synthesis, the higher the biological value of the protein. Thus, any factor inherent in the protein food itself or caused by processing which changes this pattern, has an effect on the biological value of the protein. These factors can be classfied as follows:

A. Protein Concentration

That protein concentration is an important factor in determining protein quality has been known for a long time. There is an optimum level of dietary protein for maximum efficiency of utilization (Allison, 1955, 1959; Campbell, 1963; National Academy of Sciences, National Research Council, 1963). The experimental condition

identified as "amino acid imbalance" occurs principally at low levels of dietary protein (Harper, 1959; Harper and DeMuellenaere, 1963), at which the balance or proportions of the amino acids in the protein become more critical. Likewise, decreasing levels of protein concentration reveal deficiencies in amino acids which do not appear at optimum or higher protein levels (Harper, 1957, 1958 and 1959). Another advantage to a relatively high protein concentration is that it can be diluted to an optimum concentration for maximum efficiency of utilization for the growth and maintenance processes of the organism. High protein content is also desirable in protein foods having a minor amino acid deficiency that would appear only at low levels of intake.

B. Amino Acid Content

All metabolically active tissues have about the same average amino acid composition, and thus are more or less equal as sources of good quality protein, whether they are animal (muscle), plant (young growth), or microorganism (yeasts). In contrast, the storage portions of cereals, oilseeds and legumes are very variable in amino acid composition (Bressani, 1968). Since the plant is able to synthesize amino acids from inorganic nitrogen, the storage proteins of seeds need only be suitable sources of nitrogen for the developing seedling. Therefore, the plant is indifferent to the amino acid

composition of its storage protein. Some are poor, such as the corn protein, and some are relatively good such as the soy protein.

For animal nutrition, the majority of plant proteins display deficiencies of one or more essential amino acids.

A further difference which becomes evident when one compares the essential and nonessential amino acid composition of plant and animal proteins is that the ratio of essential to total amino acids is smaller in plant than in animal proteins. This difference is also important in determining the efficiency of protein utilization and may be responsible for the lower utilization of some plant proteins, even after the essential amino acid pattern is corrected by supplementation (Harper and DeMuelenaere, 1963).

C. Amino Acid Availability

Amino acid availability of a protein depends on (1) the digestibility coefficient of the proetin, and (2) the rate of release of amino acids during digestion.

The effective essential amino acid balance, or nutritive value of a protein for animal nutrition, is not always indicated by a simple examination of its essential amino acid content. There is evidence that some of the essential amino acids present in proteins may not be fully released after digestion, and the rate

of release of different amino acids varies from protein to protein during digestion (Mauron et al., 1955). Gupta et al (1958) found that lysine availability to the weanling rat was only about 50% for corn, 70% for wheat and 85% for a roller-dried milk sample. The ten essential amino acids were all highly available from peanut flour and wheat (92-100%), whereas in cottonseed meal the availability ranged from 64.5 to 93.4%. The utilization of lysine from nineteen food proteins ranged widely from 49 to 98%, and of methionine from 48 to 83% (Schweigert and Guthneck, 1954).

There is little knowledge of the causes for the known digestibility and amino acid availability of plant proteins. Two are recognized: one is the factor inherent in the nature of the seed and seed proteins; and other is the decrease resulting from processing.

D. Heat Processing

Evidence of the effect of heat on the nutritive value of proteins was obtained as early as 1917 by Osborne and Mandel in their attempts to destroy the toxic principle in cottonseed by heat. Their experiments showed that the steaming of cottonseed for a sufficient length of time rendered it harmless to experimental animals; longer heat treatments, however, reduced the food value of the product. In their subsequent paper, Osborne and Mandel (1917) separated the protein from the raw beans and showed it to

be unable to promote the growth of rats. They reported that the normal growth obtained with cooked soybeans was quite significant, since previous investigation had shown legumes to be incapable of supporting normal growth in rats. They postulated that improvement in the palatability of the cooked beans accounted for most of the improvement, and they blamed failure of the rats, fed raw beans, to gain properly on their refusal to eat the diet.

Heat processing produces both beneficial and deleterious effects on the nutritive value of proteins. The beneficial effects are due to the inactivation by heat of the trypsin and growth inhibitors, hemagglutinins and other toxic factors present. The adverse effects are due to the decrease in availability of certain essential amino acids, such as lysine and methionine, as a result of reaction with reducing sugars and carbonyl compounds present in the food. This mechanism (Maillard reaction) has been intensively studied (Patton, 1955; Ellis, 1959). The loss of amino acids depends on the severity of heat and the moisture content of the food. In general, foods containing trypsin and growth inhibitors, such as legumes, show marked improvement in the nutritive value of their proteins on heat processing.

Severe heat processing of cereals, such as toasting, puffing and gun explosion techniques, has been

reported to cause drastic reduction in the nutritive value of various cereal proteins. Less severe heat treatment, as in cooking or baking, does not have this effect (Liener, 1960 and 1958). The proteins of most legumes show marked improvement in their nutritive value as a result of heat processing, such as pressure cooking, toasting or puffing (Kuppuswamy et al., 1958; Nenkatrao et al., 1964).

Mild heat treatment, such as in screw pressing, brings about slight improvements in the nutritive value of peanut proteins and marked improvement in that of soybean protein (Liener, 1958).

Two in vitro and three in vivo methods have been most commonly used for estimating the biological availability of amino acids in proteins.

The in vitro methods are based (a) on comparisons of the rates at which amino nitrogen or free amino acids are released from different proteins when they are incubated with proteolytic enzymes (Melnick and Oser, 1949; Riesen et al., 1947) and (b) on the measurement of the percentage of free epsilon-amino groups of lysine in different proteins by 1-fluoro-2, 4-dinitrobenzene (FDNB) (Carpenter, 1955; Carpenter et al., 1960). The former gives a relative rather than a quantitative measure of amino acid availability; the latter is a quantitative method, but only for lysine. The FDNB method is especially useful for estimating

the effects of heat processing, which lowers particularly the availability of lysine by binding of epsilon-amino groups (DeMuelenaere et al., 1967).

The in vivo methods are based (a) on the measurement of the increase in fecal excretion of a particular amino acid after feeding a test protein (Kuiken et al., 1948) (b) on the ability of a protein of known amino acid composition to replace a specific amino acid in supporting growth or repletion in animals (Schweigert et al., 1954) and (c) in maintaining nitrogen balance in a mature subject (Linkswiler et al., 1960).

The in vivo methods can be used to measure the availability of any amino acid; however, the meaning of "availability" depends on the method applied. As determined by the fecal analysis method, it is a measure of the amount of unabsorbed amino acid. It is, therefore, a measure of digestibility of a specific amino acid and depends on the digestibility of the protein and the presence of enzyme-resistant peptide bonds or enzyme-inhibiting substances in the assayed sample. The growth and nitrogen balance methods, on the other hand, assess not only digestibility but also the efficiency of utilization of the absorbed amino acid by the body (DeMuelenaere et al., 1967).

Kakade and Evans (1963) autoclaved beans at 121°C for five minutes and when these cooked samples were fed

to rats, the animals gained weight and did not die. They indicated that higher temperatures, during the same length of time, did not promote as much growth due to destruction or inactivation of some essential amino acids in the overheated beans.

Liener (1963), studying the effect of cooking, noted that when beans were autoclaved at 121°C for 30 minutes, the weight gain of chicks improved markedly compared to chicks fed raw beans; feeding the raw beans resulted in an enlargement of the pancreas.

El Nockrashy (1970) studied the effect of different carbohydrates on lysine availability of purified cottonseed protein. Available lysine was determined by reaction of ε-amino groups with fluoro-2, 4-ε-amino groups in percentage for ratio of carbohydrate to protein of 1:100, 1:10, and 1:1, respectively, were as follows: for glucose 7.6, 25 and 93.7; for sucrose 20.4, 28.6 and 36.0; for lactose 4.9, 10.6 and 44.6; for raffinose 27.3 (ratio 5:100), 56.7 and 88.2; for starch 2.2, 5.8 and 2.2, and for ratio of 2:1 of starch to protein the loss reached 43.8%.

The changes in the amino acid composition of bean proteins during culinary processing of hulled and unhulled beans were studied under various temperature regimes (Talanor, 1972). No significant decrease in the amino acid content was found after blanching (steam treatment

for two minutes at 120°C (under pressure) of hulled beans or after cooking of unhulled beans for 75 minutes at normal pressure. However, cooking of hulled beans, previously subjected to blanching for 15 minutes, led to a substantial decrease in the total amino acid contents. The highest losses occurred in the following essential amino acids: tryptophan, leucine, lysine, phenylalanine and isoleucine.

Basualdo et al. (1972) reported that the nutritional value of sunflower meal, as indicated by the essential amino acid contents, available lysine, net protein utilization and digestibility, was not significantly impaired by industrial processing.

It has been shown that excessive heating results in a decrease in fluorodinitrobenzene (FDNB)-available lysine (Carpenter et al., 1957; Lea et al., 1960; Carpenter et al., 1962); it was concluded that the low FDNB-available lysine values found in some commercial meals must reflect the heat damage. There is indirect evidence that excessive heat also decreases the level of available sulfur amino acids. Miller (1956) showed a decrease in NPU for rats on heating fish protein. Donoso et al. (1962) made a similar observation on heating prok protein. Also, the data of many investigations, reviewed by Rice and Benk (1953), showed that excessive heat results in a decrease in vitro enzymatic release of methionine and other amino acids.

Methods of Estimating the Protein Quality

The methods used for the evaluation of protein quality can be devided into the following groups:

1. Methods which are based on chemical analysis of amino acids; such as the chemical score of Mitchell and Block (1946), the protein score by the FAO/WHO procedure (FAO, 1957) and the essential amino acid index (Oser, 1951).

2. Methods based on enzymatic and microbiological procedures.

3. Animal bioassays.

Chemical Scores

In 1946, after figures of amino acid composition of proteins had become known, Mitchell and Block (1946) published a method for assessing the biological value of proteins, based on the law of minimum, a concept developed by Liebig (1840) for fertilizers; that is protein synthesis is limited by those essential amino acids which are in the smallest amount, in relation to their requirements. According to Mitchell and Block (1946) the content of each essential amino acid in a food protein is supressed as a percentage of the content of the same quantity of egg protein. The amino acid showing the lowest percentage is called the limiting amino acid and this percentage is the chemical score.

Whole egg protein was chosen as standard because it is almost perfectly utilized in digestion and in the metabolism of the growing rat (Mitchell and Carman, 1926), the mature rat (Briker and Mitchell, 1947), the dog (Allison et al., 1949) and the adult man (Hamley et al., 1948). The excellent protein quality of the whole egg was demonstrated by Mitchell (1950) when he showed that the growth-promoting value of this protein was not improved for the growing rat by supplementation with any of the essential amino acids except lysine, which induced a three percent increase in body weight in a 28-day feeding period.

Oser's Essential Amino Acid Index

Oser (1951) introduced the use of the geometric mean of the egg ratios to estimate the nutritive value of proteins. He incorporated the "egg ratio" concept of Mitchell and Block, but included all the essential amino acids, plus cystine, tyrosine, histidine and arginine. The essential amino acid index (EAA index) was defined as the geometric mean of the "egg ratio" (the ratio of the essential amino acids in a protein relative to their respective amounts in whole egg protein).

This procedure was modified by Mitchell (1954) and is known as "Modified Essential Amino Acid Index" (MEAA). The procedure is illustrated in Table 4, taking casein as an example.

TABLE 4.--Computation of the Modified Essential Amino Acid Index.

Amino Acids	Whole Egg Protein (g/16g N)	Casein (g/16g N)	Egg Ratio	Corrected Ratio	Logs of Corrected Ratios
Lysine	7.8	8.1	104	100	2.0000
Methionine and Cystine	5.3	3.3	62	62	1.7924
Phenylalanine and Tyrosine	9.3	10.7	115	100	2.0000
Leucine	8.8	9.8	111	100	2.0000
Isoleucine	5.9	5.8	98	98	1.9912
Valine	7.1	7.2	101	100	2.0000
Threonine	4.9	4.1	84	84	1.9243
Tryptophan	1.4	1.2	86	86	1.9345
Histidine	2.6	3.2	123	100	2.0000

Average Log = 1.9603

MEAA = 91

The geometric mean of the corrected egg ratio (values above 100 are corrected to this value) is computed by taking the logarithm of each egg ratio, averaging these logarithms and then obtaining the antilogarithm of this value. In the example, the average logarithm was 1.9603; and its antilogrithm, 91, is the modified EAA index for casein.

Protein Scores by the FAO/WHO Procedure

In 1963, the FAO/WHO Expert Group modified the computation of the chemical score in order to obtain a better agreement with the experimental biological value. The whole egg provides more than double the requirements of each essential amino acid necessary to maintain nitrogen equilibrium in the adult man (Allison, 1958).

The calculation of the protein score is done as follows:

a. Add the amounts of all the essential amino acids together with those of cystine and tyrosine.

b. Calculate the percentage contributions of the potentially limiting amino acids to this total.

c. Compare these percentages with the corresponding ones for the reference pattern.

The concentrations of essential amino acids in whole egg protein and the new reference pattern (FAO/WHO, 1973), which was used as the reference, in this study are given in Table 5.

TABLE 5.--Essential Amino Acid Composition of the Whole Egg Protein and the New Reference Pattern (FAO/WHO, 1973).

Amino Acids	Whole Egg (g/16g N)	FAO/WHO Reference Pattern (g/16g N)
Lysine	7.8	5.4
Methionine and Cystine	5.3	3.5
Phenylalanine and Tyrosine	9.3	6.1
Leucine	8.8	7.0
Isoleucine	5.9	4.0
Valine	7.1	4.9
Threonine	4.9	4.0
Tryptophan	1.4	1.0
Histidine	2.6	--
Total	53.1	36.0

Biological Value (BV)

Protein quality bioassays with rats are subdivided into those based on weight gain and those based on N retention in the whole body either by direct carcass analysis or by N balance techniques.

Thomas (1909) introduced the concept of biological value (BV) and the method of assessing BV with humans. Since that time, the term biological value has been synonymous with protein quality. He used adult subjects, but Mitchell (1923) adopted the method to both growing and adult rats.

Biological value is usually expressed by the following equation:

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

The second equation expresses more meaningfully the biological value:

$$BV = \frac{I-(F-F_K)-(U-U_K)}{I-(F-F_K)} \quad \text{or} \quad \frac{B-(B_K-BO)}{I-(F-F_K)}$$

A = Absorbed nitrogen $[I-(F-F_K)]$

B = Body nitrogen (at the end of test period)

B_K = Body nitrogen at zero nitrogen intake (at the end of test period on animals fed a nonprotein diet)

BO = Body nitrogen at zero time (measured on a control group of animals at the beginning of the test period)

F = Fecal nitrogen

F_K = Metabolic nitrogen (endogenous fecal nitrogen)

I = Nitrogen intake

U = Urinary nitrogen

U_K = Endogenous urinary nitrogen

Mitchell (1923) reported that the biological value was affected by the level of protein in the diet. He provided data which showed that an increase in the protein content of diets, when the protein sources were milk, corn, oat and potato, from five percent to ten percent, resulted

in a decrease in BV on the average by eleven percentage points.

Even though the customary equation for BV looks simple, the method is too involved to be used as a routine procedure for protein quality.

Net Protein Utilization (NPU)

Net protein utilization (NPU) was derived from the Thomas-Mitchell procedure (Mitchell, 1923) for the determination of biological value.

NPU is defined by the FAO/WHO Expert Group (1965) as the proportion of nitrogen intake which is retained, i.e., the product of biological value and digestibility.

$$\text{Digestibility} = \frac{A}{I} = \frac{I-(F-F_K)}{I}$$

$$\text{NPU} = \frac{B}{I} = \frac{I-(F-F_K)-(U-U_K)}{I} \text{ or } \frac{B-B_K}{I}$$

Protein Efficiency Ratio (PER)

Osborne, Mendel and Ferry (1919) defined "a method of expressing numerically the growth-promoting value of proteins involving the determination of the gain in body weight per gram of protein consumed at level of dietary protein associated with the highest protein efficiency ratio (PER)."

$$PER = \frac{\text{Gain in body weight (g)}}{\text{Protein intake (g)}}$$

Protein efficiency ratios appear to be related reasonably well to other methods of evaluating proteins. Block and Mitchell (1946) found that there was a good relation between PER's and biological values and also between PER's and chemical score based on amino acid composition. Bender (1956) reported a good correlation between PER and net protein utilization.

Since protein efficiency ratios are well correlated with several other methods of protein quality evaluation and since it is probably more frequently used than any other method and it is still the only official method for protein quality determination (AOAC, 1970), it was decided to use this method for evaluating protein quality in this work.

Determination of PER requires strict adherence to certain conditions. It has been shown that several factors influence the PER determination:

a. Strain and sex of rats (Morrison and Campbell, 1960): It appears, however that there is as much difference between strains of rats as there is between sexes.

b. Species differences: Hegsted et al. (1947) and Mitchell (1954) reported that the results of growth trials in rats could be generally applicable to the assessment of human diets. Flodin (1959) found a good correlation

between PER values in rats and biological values in the adult man. Hegsted (1957) reported that the amino acid requirements of man and of the rat are, generally speaking, the same. Allison (1957) found remarkable correlations in the nitrogen balance indices of six different proteins in man, dog and the rat.

c. Age of the rat and assay period: Chapman et al. (1959) have shown that significant differences could be obtained in PER values between rats put on test at 22, 36 or 45 days of age. In Osborne's (1919) original method, the assay period was eight weeks. According to Chapman et al. (1959) and Morrison and Campbell (1960), the coefficient of variation of PER's had a tendency to drop after the first week and would be generally lower at the end of the third or fourth week, which indicates that the assay is becoming more stable at that point.

d. Protein level: obviously comparisons will be valid if the only variable between one diet and another is the nature of the protein, i.e., all diets should have the same nitrogen content. Different levels of protein in the diet have been proposed in the literature. The standard AOAC method recommends 10% protein.

Since such discrepancies would make impossible the comparison of data obtained by different researchers, it was felt necessary to standardize the method. The official method finally adopted is the one proposed in

1960 by the Association of Official Analytical Chemists (AOAC) which is described in detail in the Materials and Methods section of this thesis.

Net Protein Ratio (NPR)

The protein efficiency ratio method has been criticized because it does not take into account the protein required for maintenance, since only gain in weight is used in calculation.

Bender and Doell (1957) introduced the net protein ratio method in which a group of animals fed a protein-free diet is included in each test.

Net Protein Ratio (NPR) is defined as the overall difference in gain (gain in weight of the test group plus loss in weight of the protein-free group) divided by the amount of protein eaten.

NPR was shown to be highly correlated with NPU: $Y = 3.3 + 15.5\ X$ (Y = NPU, X = NPR); $r = 0.986$; $P < 0.01$ (Bender and Doell, 1957).

Slope Ratio Technique

The Relative Protein Value (RPV) or slope ratio technique was proposed by Hegsted and associates (Hegsted and Chang, 1965; Hegsted and Worcester, 1966; Hegsted et al., 1968; Hegsted and Neff, 1970). In this method the slope of the regression line relating body weight or body protein or body water of young rats fed a standard

protein (egg protein or lactalbumin), assumed to have maximal nutritive value, was compared to that of rats fed the test protein at various protein levels. It was originally assumed, as in the estimation of BV and NPU, that the dose-response lines should have a common intercept at zero dose. However, using several levels of intake, this assumption can be statistically tested; and it is shown, for most proteins, the regression lines do not have a common intercept. But, when young rats are fed diets of varying protein content, up to levels that permit substantial increases in body weight, the slope of the regression line may not be greatly influenced, whether or not one forces the regression through a common zero point. However, since it is certain that the regression lines do not meet at a common point, the slope of the regression line relating dose to response should be calculated for each protein individually and then compared to that of the standard protein (Hegsted, 1974).

This method is claimed to be especially appropriate for evaluating protein quality when the protein content of the food is 10% or less (Hegsted, 1974; Elias et al., 1974). These authors evaluated protein quality in such foods as rice, corn and cassava by using regression equation between protein intake and weight gain. They considered the method to be appropriate for poor quality protein at

higher levels of intake, and in fact for any situation where the response (growth) is low whether it is due to protein quality or quantity.

Microbiological Methods

The microbiological procedures for the determination of the nutritive value of proteins and the availability of individual amino acids have passed through several stages. First, the production of lactic acid was used as a measure of growth of bacteria in a medium in which one amino acid was limiting. Next was to assay the overall value by measuring the growth response of microorganisms such as Leuconostoc mesenteroides and Streptococcus faecalis after hydrolysis of the protein. In this technique, the results were compared with those of a casein standard and termed "relative nutritive value."

The next development was to use microorganisms that would themselves hydrolyze the protein and so measure the available amino acid as distinct from total amino acid content. The organisms used included Streptococcus zymogenes, Pseudomones aeruginosa and the protozoan, Setrahymena.

In general, the results correlated well with rat PER and NPU assays, which indicated a similarity between the microbes and rat requirements.

Methionine Bioassay

Schweigert and Guthneck (1954) investigated the utilization of methionine with the young growing rat and the protein-depleted adult rat. Hydrogen peroxide-treated casein (oxidized casein), with appropriate supplements, was used as the source of amino acids in the methionine deficient ration. The rate of gain for the 14-day test period for groups receiving the graded levels of methionine was followed. They studied the methionine utilization in several animal and plant proteins. It was concluded that less variation among individual animals within each group was observed with the weanling rat than with the adult rat. The percentage of methionine utilized for weight gain varried for the different foods from 48 (unheated soybean flakes) to 83 (fresh pork) with the weanling rat. The methionine utilization from casein was high in both tests, 81% for weanling rat and 84% for the adult rat. They reported that 80% of the methionine from soybean oil meal was utilizable for the growth of weanling rats.

DeMuelenaere and Feldman's (1960), using the rat as the test animal, reported an estimate of 95% for the availability of methionine in corn.

Evans et al. (1974) using a balance study with growing rats showed that the availability of bean methionine and cystine was poor when compared to that found for the same amino acids from soybeans. They also indicated that

the degree of availability of these amino acids correlated with the PER values obtained for either beans or soybeans.

Several investigators have used chicks as the test animal for bioassays of methionine. Miller et al. (1965) showed by direct determination of available methionine by a growth procedure with chicks, that a large proportion of methionine of animal protein concentrates can be present in an unavailable form.

Legumes

Leguminosae comprise approximately 600 genera with around 13,000 species. Today, only a few species, about 20, are used for human food. Eight of these are extensively grown, but even within this group there are striking differences in the area of adaptation and use.

Roberts (1970) has grouped them into four major classes depending primarily upon climate requirements.

A. Low humid tropics
    1. Pigeon Peas (Cajanus cajan)
    2. Cow Peas (Vigna sinesis)
B. Semi-dry or seasonal tropics
    1. Ground nuts or peanuts (Arachis hypogaea)
C. Tropical intermediate elevations to temperate zones
    1. Soybeans (Glycine max)
    2. Dry Beans (Phaseolus vulgaris)

D. Cool weather, high elevation zone

1. Chick Peas (Cicer arietinum)

2. Peas (Pisum satinum)

3. Broad beans (Vicia faba)

Next to soybeans and ground nuts, peas and dry beans are the most extensively grown legume crops. Data on production and acerage of the major food legume crops and wheat, rice and corn (FAO, 1972) are given in Table 6. The data indicate that of the total area planted with legumes, which includes legume foods, soybean and peanut, close to 54% of it is in legume foods (pulses). This statistic also demonstrates that the area for cultivation of legume foods is about half of that allotted to rice and almost one-fifth of wheat and corn.

Legumes are nutritionally important among vegetable foods because of their relatively high protein content. The protein located primarily in the cotyledons and embryonic axis, with only small amounts present in the seed coat (Singh et al., 1968). The seed coat of the Navy bean seed contains 4.8% crude protein, while the cotylecons and the embryonic axis have 27.5 and 47.6%, respectively. Since the cotyledons represents the greater part of the whole seed, they contribute the major amount of protein to the whole seed (Singh et al., 1968; Varner and Schidlowsky, 1963).

TABLE 6.--Total World Acreage and Production of the Major Food Legume Crops and Wheat, Rice and Corn.[a]

Products	Area (1000 hectares)	Production (1000 metric tons)
Legume foods	67,619	43,700
Soybeans	38,489	53,024
Peanuts	19,665	16,887
Total legumes	125,773	113,611
Wheat	213,494	347,610
Rice	131,230	295,380
Corn	108,208	301,390

The essential amino acid content of beans has been the subject of numerous investigations. Two points are of particular interest. First, the lysine content of beans is rather high. Cereal products, on the other hand, tend to have a low lysine content. This fact is responsible for the complementarity of cereals and legumes. Second, beans have a low methionine and cystine content.

The amino acid content of legume grains depends on species, varieties, localities and management practices.

Landon et al. (1957) studied the factors influencing proteins, methionine, lysine and tryptophan content of 25 varieties of beans. Differences in nitrogen and tryptophan content among varieties and between localities were highly significant. The fertility of the land did not affect the content of nitrogen, methionine, lysine and tryptophan.

The essential amino acid content of selected beans is given in Table 7 and compared with the FAO pattern of amino acid requirements (FAO/WHO Energy and Protein Requirements, WHO tech. Rep. Ser. No., 522-1973).

TABLE 7.--Essential Amino Acid Content of Selected Beans Compared with the FAO/WHO Pattern (g/16g N).

Amino Acid	FAO/WHO Reference Pattern[a]	Navy Beans[b]	Kidney Beans[c]	Black Beans[c]	Red Beans[c]
Histidine	--	2.4	2.6	2.9	3.2
Lysine	5.4	5.7	6.7	6.7	7.2
Methionine and Cystine	3.5	1.7	1.9	3.0	2.2
Phenylalanine and Tyrosine	6.1	8.4	9.8	9.6	9.9
Leucine	7.0	6.7	8.1	7.7	7.7
Isoleucine	4.0	3.7	4.2	4.1	3.8
Valine	4.9	4.4	5.1	4.9	5.0
Threonine	4.0	4.1	4.2	3.1	3.5
Tryptophan	1.0	1.2	1.5	1.5	1.7

[a] FAO/WHO (1973) reference pattern.

[b] This study.

[c] Evans and Bandemer.

The amino acid differences within Phaseolus vulgaris cultivars reported by King (1964) and Lantz et al. (1958) indicate that, through selection and hybridization, improved cultivars might be developed.

According to Kelly (1971) the level of methionine in mature seeds of the common bean is determined genetically and sufficient variation exists within the species to permit improvement through hybridization and selection.

Kakade and Evans (1965) found some Navy bean varieties to vary in their protein content as well as in their methionine content.

While inorganic sulfur occurs in relative abundance in the earth, the ocean and the air, organic sulfur compounds such as methionine and cystine are in short supply (Rose et al., 1955; Borgstrom, 1964). Only plants have the necessary mechanism to reduce sulfate, an oxidized form of sulfur, and synthesize methionine and cystine (Thompson et al., 1970; Allaway and Thompson, 1966). Animals depend on plants to obtain the methionine and cystine they need. Dietary cystine provides a methionine sparing effect. It is able to replace 80 to 89% of the methionine requirement (Rose and Wixom, 1955).

Methionine is an important source of methyl groups in the mammalian metabolism being very important in transmethylation reactions. Vitamin B_{12}, epinephrine, ergosterol and lecithin are end products of transmethylation (White et al., 1968). Compounds such as S-adenosyl-methionine, lypoic acid, co-enzyme A, thiamin and biotin, have organic S-compounds as their building blocks. In bacteria, and also in the mitochondria of eucariyotic cells, the

synthesis of proteins starts with N-formylmethionyl-t-RNA, a formulated methionine t-RNA (Clark and Marcker, 1968; Lehninger, 1970).

The amount of protein nitrogen in relation to nonprotein nitrogen seems to be affected by the availability of sulfur. According to Stewart and Porter (1969), the protein nitrogen represented less than 25% of the total nitrogen found in sulfur deficient plants of wheat, corn and beans. However, 75% of the total nitrogen was protein when the sulfur content was adequate in these plants.

Methionine and cysteine are very unstable during acid hydrolysis. This causes a problem for the determination by the usual procedure for amino acid analysis which involves, as a first step, an acid hydrolysis of the proteins. These two amino acids are especially unstable during acid hydrolysis when carbohydrates are present (Blackburn, 1968). As a result of the acid hydrolysis, methionine is partially oxidized to sulfoxide, and cysteine is destroyed almost completely. Schram et al. (1954) developed a procedure which overcomes the problem of destruction of these two amino acids during acid hydrolysis. They have proposed a prior oxidation of cystine and cysteine residues with performic acid to cysteic acid which is stable to acid hydrolysis. After this step, the acid hydrolysis of the protein is carried out, and the quantitative

determination of other amino acids in the hydrolysate is done by ion-exchange chromatography.

Hirs (1956) used the same performic and oxidation procedure to oxidize both methionine and cystine in ribonuclease to methionine sulfone and cysteic acid, respectively. The oxidation is followed by hydrolysis and determination of all amino acids, including the two derivatives, methionine sulfone and cysteic acid by ion-exchange chromatography.

In cases where there is no serious interference from carbohydrates, the colorimetric method of McCarthy and Sullivan (1941) can be used with success for the determination of methionine.

The biological value--the amount of absorbed nitrogen retained in the body--has been found to be low in beans. This has been attributed to the low sulfur containing amino acids in the legumes. Jaffé (1950) and Patwardhan (1962) showed a variation in the biological value of beans from 32% to 78%. Values presented by these authors for the Black bean show a variation of 62-68% for the biological value. The beneficial effect of methionine has been demonstrated by several investigators (Patwardhan, 1962; Kakade and Evans, 1965; Russell et al., 1946), not only tested by biological value, but also through protein efficiency ratios.

The problem of essential amino acid deficiency in plant proteins is usually approached through mutual supplementation between plant proteins, by amino acid supplements and supplementation with protein rich concentrate such as animal proteins.

The supplementation of legume proteins with cereals and millets has been very successful. Since legume proteins have high amounts of lysine and threonine, they complement to a marked extent those of cereals and millets, which are characteristically low in these amino acids. On the other hand, the low methionine content of legumes is compensated by a higher content of this amino acid in cereals and millets. It has been shown that mixtures of cereals and legumes contain proteins superior to those of cereals or legumes alone. Phansalkar et al. (1957) showed a good supplementary effect of chick peas, Black gram, Green gram and Red gram proteins on those of wheat, soy beans, and pearl millet. These authors emphasize that there is a point in the combination where an optimum supplementation is achieved; in general, this maximum effect occurs when about 50% of the legume protein is replaced by cereal protein. Bressani et al. (1962) reported that the best combination of cooked Black beans and lime-treated corn was one where each component contributed 50% of the total protein of the diet. Several combinations were reported by Bressani and Elias (1969) of cooked Black beans and

Opaque-2 corn. The best results were obtained when 50% of the protein in the diet was derived from each one of the components. Improved nutritive values were obtained by Bressani and Scrimshaw (1961) and Bressani and Nalarbinte (1962) when polished rice and cooked Black beans were combined in the range of 50-80% of rice protein and 50-70% for Black beans. A combination of 19% rice, 80% legumes and 1% of green vegetables, compared well to the stock rat diet which consisted of one of the vegetable rations supplemented with milk and meat (Baptist, 1956).

Amino acid supplementation has been another approach to improve the nutritional value of plant proteins. Supplementation of wheat proteins with lysine has been found to cause significant improvement in its PER (Hutchinson et al., 1959). Whole wheat gave a PER of 0.93, (vs 2.50 for casein) but, when supplemented with 0.2%, L-lysine HCl, the PER increased to 1.45, and for wheat + 0.2% L-lysine HCl + 0.2% D, L-threonine, the PER value was improved to 2.00 (Howe et al., 1965).

Methionine supplementation markedly improved the PER of cowpeas, peas, kidney beans, chick peas, green gram, black gram and Navy beans (Russell et al., 1946; Bressani et al., 1963; Richardson, 1948; Kakade and Evans, 1965). An addition of 0.2% methionine to autoclaved Navy beans gave a PER value similar to that obtained for casein.

Another way in which plant protein can be utilized more efficiently, particularly when it is of lower nutritional quality, is by supplementation of the staple food with small amounts of vegetable-protein concentrates or isolates or with animal proteins. This method has been tested extensively for wheat but has been applied only in limited areas of the world. This method has been shown repeatedly to be very useful not only as a means of improving protein quantity and quality but also as a means of introducing other necessary nutrients as well (Bressani and Marenco, 1963).

The protein efficiency ratio (PER) of lime-treated corn was increased from about 1.0 to 2.25 by adding small amounts of egg protein, 3%, to the diet (Bressani and Marenco, 1963).

Reports of several investigators indicate that small amounts of good quality protein improve significantly the quality of wheat flour and other wheat products. Extensive reviews on this subject is written by Hegsted et al. (1954) and by Moran (1959).

Legumes, besides the described deficiency in sulfur containing amino acids, are known to contain antinutritional factors (Pusztai, 1967; Jaffé, 1968) such as trypsin inhibitors, hemagglutinins, cyanogenic glycosides, goitrogenic factors, flatulence factors and lathyric factors.

Amont the anti-nutritional factors found in legumes, the ones which are more commonly present in the common bean are the trypsin inhibitors, hemagglutinins and flatulence factors (Liener, 1962).

Perhaps the best studied of all the antinutritional factors is the trypsin inhibitor which was first isolated from the soy bean by Kunitz (1945). Trypsin inhibitors have been isolated from a variety of plant materials as well as from various animal tissues. The reaction between trypsin and an inhibitor is one of the few known cases of pure protein interaction (Green and Work, 1953). Several trypsin inhibitors have been isolated and purified. Examples are the inhibitors from soy bean (Kunitz, 1945); Rackis et al., 1959), the lima bean (Fraenkel-Conrat et al., 1952) and the Navy bean (Bowman, 1948; Wagner and Riehm, 1967).

Another factor usually associated with the low nutritional value of legumes in general and beans in particular, is their well-known low digestibility. Unfortunately, information on this particular problem is limited. It is not known whether these effects are caused by a more rapid movement of the cooked legumes through the intestinal tract or by resistance to protein hydrolysis by the gastrointestional enzymes (Bressani et al., 1973).

In 1944, Everson and Heckert found that raw Navy beans were injurious to rats when fed a diet at 10% protein

level. They also reported that heating the beans destroyed the injurious effect. This was confirmed by Kakade and Evans (1963) who observed that rats given 10% protein diet based on raw Navy beans lost weight and died during the experiment. Kakade and Evans (1965) studied the effect of heating Navy beans on the growth of rats. They autoclaved the Navy bean flour for 5, 10, 15, 30, 60 and 240 minutes and found a better growth when the flour was autoclaved for 5-10 minutes at 121°C.

Sesame

Sesame, Sesamum indicum L., a member of the Pedaliacene family, has been called the "queen of the oilseed crops" because of the high yield of oil obtained and the good qualities of the seed, oil and meal (Eckey, 1954). The plant, cultivated in India for several thousand years, is grown extensively in tropical and subtropical areas of Asia, Mediterranian countries, and South America. During the last decade, imports of sesame seed by the U.S., largely for baked goods and confectionery products, have increased from 24 to 40 million pounds (Agricultural Statistics, 1970).

The valuable components of sesame seed are the oil and protein and the contents of these have been determined for several varieties of sesame which were grown in the southern and southwestern parts of the U.S. (Kinman

et al., 1954). Oil contents varied from 45-63% and averaged 54%. Protein contents varied from 17-32% and averaged 26%. The average protein content of the oil-free meals was 57%.

Sesame protein is rich in sulfur containing amino acids, particularly methionine. It is deficient only in lysine and somewhat in isoleucine.

Being a good source of methionine, sesame meal offers great advantage as a natural supplement to many legume proteins deficient in this amino acid. The supplementary value of sesame to soya, groundnut, chick pea or mixtures of these legumes in different proportions has been demonstrated in several investigations using rats as experimental animals (Krishnamurthy et al., 1960; Tasker et al., 1960; Guttikae et al., 1965).

Evans and Bandemer (1967) demonstrated the effect of fortification on the nutritive value (relative to casein) of sesame meal. Sesame alone had a protein nutritive value of 47%. Fortification with 0.2% lysine raised this to 94%. The protein nutritive value of a 1:1 mixture of sesame and soybean protein was about the same as that of casein. Soybean protein has an abundance of lysine but is deficient in methionine.

In related studies (Joseph et al., 1962; Joseph et al., 1958), it was demonstrated that incorporation of 25% of sesame meal raised the PER of a 2:1 mixture of

peanut and bengal gram protein from 1.79 to 2.03. In human feeding tests (Gopalorn, 1961), the sesame-peanut-bengal gram protein mixture, which is somewhat deficient in lysine, was nearly as effective as skim milk in controlling the clinical manifestations or protein malnutrition but was inferior to skim milk with regard to serum albumin regeneration.

High protein vegetable mixtures for human feeding containing 35% sesame flour, were developed (Scrimshaw et al., 1961) by the Institute of Nutrition of Central America and Panama (INCAP). These low-cost mixtures were readily accepted and well-tolerated as the chief protein source of a needy population.

Zinc

Zinc was first shown to be essential for growth of the rat by Todd et al. (1934). However, the essentiality of zinc under practical conditions was not demonstrated until Tucker and Salmon (1955) showed that zinc would prevent or cure parakeratosis in swine fed diets composed of natural foodstuffs. The fact that zinc deficiency develops in animals fed diets based on soybean protein and corn whereas it is not seen when diets, containing the same amount of zinc, are based on animal protein has led to the hypothesis that zinc in certain plant proteins is not readily available to animals. The decreased availability of zinc from plant-seed was attributed to

phytate (O'Dell et al., 1960). This has been confirmed several times in many species (Likuski et al., 1914; Oberleas et al., 1966), and a detailed discussion on the mechanism of phytate action was published (Oberleas et al., 1966).

The hypothesis was tested by adding phytic acid to a casein-based diet and the growth response of chicks were compared to those fed soybean protein as the source of protein (O'Dell and Savage, 1970). Phytic acid decreased the availability of zinc in the casein diet and produced symptoms similar to that observed among animals fed soybean protein containing a comparable level of phytate.

Oberleas and Prasad (1969) studied the effect of supplementation of zinc to soya protein diets at several levels of dietary protein on growth of the rat over a 10 week feeding experiment. Chemically pure phytic acid was added to all diets to equalize the phytic acid content to one percent. They concluded that zinc supplementation of plant proteins will make them as good as that of animal proteins.

Rats fed casein or egg white as the source of protein required approximately 12 PPM of zinc in the diet, while those fed soybean protein required 18 PPM (Forbes et al., 1960). The apparent absorption of zinc by rats fed casein was 84%, compared to 44% by those fed soybean protein. Similar results were obtained in balance studies performed on growing chicks (Savage et al., 1964).

MATERIALS AND METHODS

Navy Beans

Navy beans of the Sanilac variety were grown in Michigan and obtained from the Department of Crop Science at Michigan State University.

The finely ground beans (60-mesh) were autoclaved in shallow pans at 121°C for 10 minutes. After autoclaving, the samples were placed in a ventilated hood, allowed to dry at room temperature, and reground to a fine meal (60-mesh).

Germination

Prior to germination, the dry beans were washed with soap and rinsed thoroughly, and soaked in water for five hours at room temperature. After soaking, the beans were spread in a 3 cm thick layer of vermiculite (W. R. Grace and Co., Mass.). Sufficient distilled water was added in order to keep the seed bed always moist without immersing the beans in water. Germination was continued at room temperature for approximately 60 hours. At the end of this time, almost all of the beans had sprouts varying in length from two to ten mm. They were ground in a meat grinder. The resulting flour was autoclaved in

shallow pans at 121°C for 10 minutes and then dried as described previously. They were then reground to a fine meal (60-mesh) in a Wiley mill.

Sesame Seeds

Sesame seeds were obtained from food stores at Michigan State University. They were already dehulled.

The flours were prepared by extracting the oil with commercial hexane. The seeds were ground with twice as much hexane in a fireproof blender. After blending, the slurry was filtered under vacuum in a Buchner funnel. The extraction with hexane was repeated four times and after the last extraction the flour was left as a thin layer in a ventilated hood for 24 hours at room temperature for a complete removal of the solvent.

Amino Acids Analysis

The analysis for amino acids in the Navy bean and sesame flours was performed on 22 hour hydrolysates of the protein in the flours. These analyses were carried out on a Beckman Model 120C Amino Acid Analyzer in which the amino acids were separated by column chromatography and quantitatively determined by automatically recording the intensity of the color produced by their reaction with ninhydrin (Moore and Stein, 1948, 1951, 1954; Moore et al., 1958; Spackman et al., 1958). Samples were prepared according to the method described in the Beckman manual for amino acid analysis (Toeffer, 1965).

About 25 mg portions of Navy bean flour and 8 mg of sesame flour were weighed into 10 ml ampoules. Five milliliters of glass-distilled 6 N HCl were added to the ampoules. The contents of the ampoules were frozen in a dry-ice alcohol bath, evacuated with a high vacuum pump, and allowed to melt slowly under vacuum to remove gases. After evacuation, the ampoule contents were again frozen in the dry-ice alcohol bath and then sealed with a pin-point air-propane flame. The evacuated and sealed ampoules were placed in an oil bath at 110±1°C oven. After hydrolysis for 22 hours, the ampoules were removed from the oven and cooled to room temperature.

The top of the ampoules was cut and one milliliter of 2.5 μM norleucine solution was added to each ampoule as an internal standard to measure mechanical losses during transfer. The contents of each ampoule were transferred to a pear-shaped evaporating flask and evaporated to almost dryness under vacuum on a rotary flask evaporator immersed in a 45-50°C water bath. After evaporation, a small volume of distilled water was added and the evaporation was repeated. Addition of distilled water and evaporation was repeated twice more in order to eliminate completely all remaining hydrochloric acid.

The dry hydrolysate was transferred from the pear-shaped flask into a 5 ml volumetric flask with a citrate -HCl buffer, pH 2.2. In order to separate solid

particles, the 5 ml hydrolysate was filtered and the filtrate was transferred into a small vial and stored at 4°C. An aliquot of 0.2 ml was then applied to the analyzer for amino acid analysis. The amino acid analyzer was operated at 57°C. The running time was four hours, 55 minutes for the short and 185 minutes for the long column.

The chromatograms were compared to the one obtained from a standard amino acid calibration mixture.

Sulfur Containing Amino Acids

Methionine and cysteine are known for their instability during acid hydrolysis when carbohydrates are present (Schram et al., 1953). In order to protect these amino acids during acid hydrolysis, a preliminary oxidation with performic acid was carried out, cysteine being oxidized to cysteic acid and methionine to methionine sulfone as described by Schram et al. (1954) and Lewis (1966).

The performic acid solution was prepared by mixing one volume of 30% (w/w) hydrogen peroxide with nine volumes of 8.8% (w/w) formic acid. This mixture was allowed to stand for one hour at room temperature.

Eight milligrams sesame flour and 25 milligrams of Navy bean flour were weighed into a special pear-shaped flask and cooled to 4°C. Then, 10 ml of performic acid, which was previously cooled to 0°C, was added to the samples. Oxidation was carried out at 4°C for 16 hours.

After the oxidation, the performic acid was evaporated on a rotary evaporator, with a cold finger trap for the very corrosive performic acid. After evaporation, the residues were hydrolyzed, as described previously, with 5 ml of 6 N HCl for 22 hours at 110±1°C. Following the hydrolysis, the norleucine standard was added and the sample was treated exactly the same as in the procedure described previously. The chromatograms were compared to those of the standard methionine sulfone and cysteic acid.

Tryptophan

Tryptophan is very labile during acid hydrolysis, and after prolonged hydrolysis of a protein, little or none of the amino acid is left. Therefore, it must be determined separately. Tryptophan was determined colorimetrically after hydrolysis with the enzyme pronase as described in Procedure W by Spies (1967):

a. A 10-30 mg sample was weighed directly into a 2.0 ml glass vial with a screw cap.

b. To each vial 0.1 ml (100 µl) of pronase hydrolytic solution and a drop of toluene, as a preservative, was added. Pronase hydrolytic solution was prepared daily by adding 100 mg pronase (Calbiochem, activity 45,000 PUK/g, lot 101185) to 10 ml of 0.1 M phosphate buffer, pH 7.5. The suspension was shaken gently for 15 minutes, then clarified by centrifugation for 15 minutes at 10,000 RPM.

c. The vials were closed and incubated for 24 hours at 40°C. After incubation, 0.9 ml of 0.1 M phosphate buffer, pH 7.5, were added to each vial. The uncapped vials were placed into 50 ml Erlenmeyer

flasks containing 9.0 ml of 21.2 N sulfuric acid and 30 mg of dimethylaminobenzaldehyde (DAB). The vials were tipped over and the contents were quickly mixed by rotating the Erlenmeyer flasks. Samples were cooled to room temperature and kept in the dark at 25°C for six hours.

d. 0.1 ml of 0.045% sodium nitrate solution was added to each Erlenmeyer flask. After gentle shaking, the flasks were left standing for 30 minutes for the development of the color. The absorbance was measured at 590 nm, using a Beckman DU spectroptotometer.

Duplicate blanks of the pronase hydrolytic solution were treated similarly and the tryptophan content of pronase was subtracted from the total tryptophan content.

A standard curve from zero to 120 µg of tryptophan was prepared according to the Procedure E described by Spies and Chambers (1948).

D, L-tryptophan (2.4 mg) was dissolved in 200 ml 21.2 N sulfuric acid containing 600 mg of dimethylyamino-benzaldehyde (DAB). 0, 1, 2, 4, 6, 8 and 10 ml of this solution were made up to 10 ml with solutions of 21.2 N sulfuric acid containing 600 mg DAB/200 ml, and placed in 50 ml Erlenmeyer flasks. The mixtures were kept in the dark at 25°C for six hours, then 0.1 ml of 0.045% sodium nitrite was added to each flask. The flasks were allowed to stand for 30 minutes for color development, and absorbance was measured at 590 nm, using a Beckman DU spectrophotometer. A straight line relationship was obtained between absorbance and tryptophan content.

Total Protein

The "total protein" content (N x 6.25) of the beans and defatted sesame seeds was determined according to the AOAC (1970) micro-Kjeldahl method, after grinding the samples to pass a 100-mesh sieve.

The "total protein" content (N x 6.25) of the diets was determined according to the AOAC (1970) macro-Kjeldahl method.

Crude Fiber

The crude fiber content of the beans and sesame flour was determined by the AOAC (1970) method.

Oil

The oil content of the beans and sesame flour was determined according to the AOAC (1970) method.

Ash

The ash content of all samples was determined according to the AOAC (1970) method.

Moisture

The moisture content of all samples was determined according to the AOAC (1970) method.

Biological Evaluation of Protein Quality

A. The Protein Efficiency Ratio (PER) Method

Weanling male rats of the Sprague Dawley strain, 21 days of age, 10 for each diet (unless otherwise specified) were used throughout the experiments.

Rats were housed individually in stainless steel cages in a room at 23°C. Assay diets and water were offered ad libitum. The animals were weighed twice a week and their food intake and waste measured every three days. The total experimental period for PER determination was 28 days.

Prior to the 28 days test period, the animals were fed a standard rat diet for three days in order to adapt the animals to the new environmental conditions.

The materials under test were fed as the sole source of protein at the 10% protein level. The composition of the basal diet is shown in Table 8.

The average 28 days weight gain and protein (N x 6.25) intake per rat for each group were calculated. The Protein Efficiency Ratio (weight gain/Protein intake) was, then determined for each group. The ratios x 100 of PER for each assay group to PER for ANRC casein reference group was also calculated.

B. Net Protein Ratio (NPR)

NPR values were calculated in the same way as the PER values, except the weight loss of one group of four

TABLE 8.--Compositions of Basal Diet for the PER Method.

Ingredient	Amount %
Protein source[1]	10
Corn oil	8
Salt mixture[2]	5
Vitamin mixture[3]	1
Non-nutritive fiber	1
Corn starch	To complete 100

[1]Vitamin-free casein; purchased from Tecklad Test Diets., P.O. Box 4220, Madison, Wisconsin, Protein content: 83.6 (N x 6.25). All rations contained the equivalent of 10% protein (N x 6.25).

[2]USP X IX Salt mixture (Tecklad Test Diets., P.O. Box 4220, Madison, Wisconsin). Salt mixture composition (%): Sodium chloride (Na Cl), 13.93, Potassium iodide (KI), 0.079; Potassium phosphate monobasic (KH_2PO4), 38.90; Magnesium sulfate (Mg S04), 5.73; Calcium carbonate (CaCo3), 38.14; Ferrous sulfate ($FeSo_4:7H_20$); Manganese sulfate ($MnSO_4:H_20$), 0.401; Zinc sulfate ($ZnSo4.7H_20$), 0.548; Cupric sulfate ($CuSo4.5H_20$), 0.0477; Cobalt chloride ($COCl_2.6H_20$), 0.0023.

The nitrogen sources used in the diets contained different amounts of ash. To compensate for that, salt mixture was added to the diets at a level to make the sum of the mineral mixture and the ash in the test materials equal to five percent.

[3]The vitamin mixture contained (mg/100 g diet): Vitamin A, 2000 (IU); Vitamin D, 200 IU); Vitamin E, 10 (IU); Menadione, 0.5; choline, 200; P-Aminobenzoic acid, 10; Inositol, 10; Niacin, 4; Ca-D-Pantothenate, 4; Riboflavin, 0.8; Thiamine. HCl, 0.5; Pyridoxine. HCl, 0.5; Folic acid, 0.2; Biotin, 0.04; Vitamin B 12, 0.003.

weanling rats fed the non-protein diet was taken into account.

The Net Protein Ratio (NPR) is defined as (gain in weight of test group + weight loss of non-protein group) divided by protein intake.

C. Slope Ratio Technique

Weanling male rats of the Sprague Dawley strain, 21 days of age, four for each diet were used.

Rats were housed individually in stainless steel cages in a room at 23°C. Assay diets and water were offered ad libitum. The animals were weighed twice a week and their food consumption was recorded during a 3-week experimental period. Prior to the test period, the animals were fed a standard rat diet for three days as described previously.

The lactalbumin was fed as the standard diet at the 4, 6, 8 and 10% protein level. All other test materials were fed at the 6, 8, 10 and 12% protein level. Each protein level for each test diet used four rats equally distributed by weight. Four rats were also fed the non-protein diet. The composition of basal diet is shown in Table 9.

Weight gain and protein intake were determined for each rat for the 21 days test period. For the Relative Nutritive Value (RNV) calculation a regression analysis

TABLE 9.--Composition of Basal Diet for the Slope Ratio Technique.

Ingredient	Amount %
Protein source[1]	4, 6, 8, 10
Salt mixture[2]	4
Vitamin mixture[3]	1
Cod liver oil	1
Corn oil	9
Non-nutritive fiber	1
Corn starch	To complete 100

[1]Lactoalbumin--Purchased from United States Biochemical Co., Cleveland, Ohio, protein content: 77.4% (N x 6.25).

[2]Salt mixture was the same as Table 8. The nitrogen sources used in the diets contained different amounts of ash. To compensate for that, the mineral salt was added to the diets at a level to make the sum of the mineral mixture and the ash in the test materials equal to four percent.

[3]Vitamin mixture was the same as in Table 8.

was performed between weight gain and protein intake including the data for the rats fed the nonprotein diet. Therefore, each line was calculated from 20 points (four animals per group fed one of four test levels of protein and four animals consuming nonprotein diet). The value for RNV was calculated for each test diet as the ratio of the slope of the regression line of the test diet to the slope obtained with lactalbumin in the same experiment, the value for lactalbumin being taken as 100. Relative

Protein Value (RPV) was calculated in the same way except that the zero data was omitted, the repression lines being calculated only from the 16 points i.e., four animals per group at four levels of protein.

Evaluation of the Effect of Processing on the Navy Bean Protein

This study was conducted to determine the effect of processing on the protein quality of Navy beans. Beans of Sanilac variety, obtained from the Department of Crop Science at Michigan State University, were subjected to different processing methods used at home and commercially (Bedford, C., personal communication, 1976). The processing methods evaluated were as follows:

A. Canning

Beans were washed and rinsed three times with water. They were then soaked in a 90°F water for half an hour. After soaking, the temperature of soaking water was elevated to 180°F and the beans were kept in the water for another half an hour. The beans were cooked in the same water for two minutes at 212°F. The water was discarded and approximately 7 ounces of beans were weighed in a 303 can. Plain hot water was added to fill the head space and after sealing, they were autoclaved in a retort for 45 minutes at 240°F. After retorting, the cans were water cooled to approximately 58°F in the retort.

The content of the cans was emptied into a Waring blender and blended for about 10 minutes. The slurry was dried in a cabinet drier operating at 150°F for approximately 8 hours. After drying, the samples were ground to a fine flour (60-mesh).

B. Canning with Sugar

The same procedures were followed as described previously except a 1.5% (w/w) sucrose solution was added to the cans to fill the head space before sealing.

C. Home Cooking

Beans were washed and rinsed three times with water. They were then soaked in a 90°F water for half an hour and slow-cooked at simmering temperature (about 200°F) in the same water until they were well-cooked. The water was added as needed to cover the beans during cooking. After the beans were well-cooked (orally tested), which took approximately two hours, they were blended, dried and ground as described before.

D. Autoclaving

The same procedures were followed as described in the preparation of the bean sample in the supplementation experiments.

Lysine Availability

A chemical method, using 1-fluoro-2, 4-dinitrobenzene (DNFB), to measure lysine availability was first introduced by Carpenter and Ellinger (1955). In this technique, DNFB reacts with free ε-amino groups in the protein, forming DNFB-ε-amino lysine which is stable to acid hydrolysis the sample is then acid-hydrolyzed and the unavailable lysine is determined using an amino acid analyzer. In our study a modification of this method was used as described by Couch (1975).

Approximately, one 350 mg sample was weighed and transferred to a 500 ml boiling flask along with 4-5 glass beads. Ten ml of freshly prepared 10% $NaHCO_3$ solution, 10 ml alcohol (ethanol) and 0.4 ml of a 90% dinitrofluorobenzene (DNFB) solution were added to the flask. The flask was stoppered and shaken for 73 hours using a mechanical shaker. The mixture was carefully acidified with approximately 2 ml of 6NHCl, and evaporated to oily dryness at 40°C in a vacuum rotary evaporator. The vacuum was released very slowly to avoid disturbing the residue. Then, 50-75 ml anhydrous ether was added, and re-evaporated, in a rotary evaporator at 40°C without vacuum. The washing with ether and evaporation were repeated three additional times.

Approximately, 125 ml of 6NHCl was added to the sample and a 5 ml aliquot was transferred to a 10 ml

glass vial with screw cap. From this point, the procedures were the same as the total amino acid determination, except only the short column in the Amino Acid Analyzer (Model 120C) was used to measure the unavailable lysine content of the sample. The total lysine was determined on the untreated sample. The available lysine was calculated by difference.

Methionine Bioassay

There is no established method to determine the biological availability of the methionine for the rat. The following procedure is rather novel.

The essential amino acid composition of a 7% protein diet in which casein is the only source of protein is very close to that of a 10% protein diet in which Navy beans is the only source of protein (Table 10). Cystine and tyrosine were also compared for their sparing effect on methionine and on phenylalanine, respectively.

Therefore, a 7% casein protein diet was prepared to which adequate amount of essential amino acids (including cystine) were added to match the 10% bean protein diet.

Since in the amino acid analysis of the bean protein only 82% of the total N was recovered as amino N, 1.2% dispensible amino acids (equal amount of glycine and glutamine) were added to the casein diet to compensate for the total amino N difference.

TABLE 10.--The Essential Amino Acid Content of a 7% Protein Diet Based on Casein and a 10% Protein Diet based on Beans, Expressed as g/100 g Diet (Parenthesis Shows Second Level of Methionine Supplementation).

Essential Amino Acid	Casein Diet[1] + Added Amino Acids	Bean Diet[2] + Added Amino Acids
Lysine	0.57	0.57
Leucine	0.67	0.67
Isoleucine	0.38	0.37
Threonine	0.32 + 0.09	0.41
Valine	0.48	0.44
Phenylalanine	0.39 + 0.16	0.53
Tyrosine	0.41	0.31 + 0.10
Tryptophan	0.11	0.12
Arginine	0.53	0.51
Methionine	0.2 + 0.0 (0.3)	0.1 + 0.10 (.40)
Cystine	0.02 + 0.05	0.07

[1]From FAO (Amino Acid Content of Foods, 1972).

[2]Results from this study.

This 7% protein diet from casein was used as the reference and the methionine utilization of the bean protein, relative to casein, was studied. To correct for the difference in the methionine content of the two diets, 0.1% L-methionine, assume to be 100% available, was added to the bean diet to make it equal to the casein diet (0.2% methionine).

It was also decided to check the methionine utilization of the bean diet at another dietary level of methionine. Therefore, 0.3 and 0.4% L-methionine were added to the casein and bean diets, respectively, to make the methionine level 0.5% of the diet.

To compensate for the sparing effect of cystine, 0.1% L-cystine was added to all diets.

For this experiment, weanling male rats of the Sprague-Dawley strain, 21 days of age, 5 for each diet, were utilized. They were housed individually and fed ad libitum throughout the test period which was 14 days. The animals were weighed twice a week and their food intake was recorded.

Zinc Supplementation Study

In this study weanling rats were individually housed in stainless steel cages, fed ad libitum; the rats were offered distilled water which was also demineralized by passing through a mixed ion exchange

bed. The salt mixture was the same as used before except for zinc which was excluded.

The zinc content of beans, casein, corn oil and corn starch was determined by atomic absorption spectrophotometry, using a Perkin-Elmer Atomic absorption Spectrophotometer Model 303.

Instrument Parameters

a. Lamp current: 15 ma
b. Fuel: acetylene
c. Support: air
d. Wavelength: 214 nm

Wet Ashing Procedure

Approximately one g sample was weighed into a 100 ml Kjeldahl flask. Twenty-five ml of HNO_3: perchloric acid mixture (17:3) were added and the flasks and contents were heated for approximately two hours. When the digestion was completed, the digests were transferred to volumetric flasks and appropriate dilutions were made with distilled deionized water.

Preparation of Standard Solutions

1.000 g of zinc granules (99.99% pure) was dissolved in 4.0 ml 1:1 hydrochloric acid and diluted with distilled deionized water to give 1000 mg zinc/ml solution. Further dilutions were made to prepare

solutions containing 0.2, 0.5, 1.0, 2.0 and 3.0 ppm zinc. These solutions were used to make a standard curve for zinc.

RESULTS AND DISCUSSION

Sample Materials

The autoclaved Navy bean flour, and defatted sesame flour were analyzed for moisture, oil content, total protein (N x 6.25), crude fiber, ash and zinc.

The results of these analyses are shown in Table 11.

Total Amino Acids of Sesame and Bean Flours

The total amino acid content of sesame and bean flours expressed as grams of amino acids per 16 grams of total N and as mg of amino acids per g of total N are given in Table 12.

It is evident from Table 12 that the total sulfur containing amino acids in the sesame meal (methionine + cysteine = 4.7 g per 16 g of total N) is approximately 2.8 times that of the Navy bean flour (1.7 g per 16 gm total N). On the other hand, the lysine content of the protein in the Navy bean flour is 2.5 times higher than the lysine content of the protein in the sesame meal.

Figure 1 shows the comparison of the essential amino acids composition (expressed as mg amino acid per gram of total N) of sesame meal, whole egg and Navy bean

TABLE 11.--Composition of Defatted Sesame Flour, and Navy Bean Flour.

	Sesame Flour	Navy Bean Flour
Moisture (%)	8.0	8.6
Oil (%)	1.5	1.84
Total Protein (N x 6.25) (%)	58.19	21.06
Ash (%)	3.01	3.92
Crude Fiber (%)	3.0	4.1
Zinc (PPM)	172.8	29.0
Carbohydrate (%)[1]	26.3	60.5

[1]Obtained by the difference.

flour. It is reasonable to assume at this point that sesame flour and Navy beans could complement each other since each of them is deficient in an essential amino acid that the other one has in excess; whole egg protein could also supplement the Sulfur amino acid in which the protein of beans is deficient.

Supplementation of Navy Bean Protein With Sesame Flour

The first feeding experiment was carried out to test two mixtures of autoclaved Navy bean and sesame flour as a source of protein in the diet. A 10% protein diet was prepared in which: (a) 50% of the protein came from the Navy bean flour and 50% from the sesame flour;

TABLE 12.--Total Amino Acid Composition of Sesame and Bean Flours (Expressed in Grams Amino Acid per 16 Grams of Total Nitrogen and as mg Amino Acid per Gram Total Nitrogen).

Amino Acid	Navy Bean Flour		Sesame Flour	
	g/16g Total N	mg/gN	g/16g Total N	mg/gN
Lysine	5.7	356.3	2.3	143.8
Histidine	2.4	150.0	2.2	137.5
Arginine	5.1	318.8	11.6	725.0
Aspartic Acid	10.6	662.5	6.7	418.8
Threonine	4.1	256.3	3.0	187.5
Serine	5.1	318.8	3.6	225.0
Glutamic Acid	12.8	800.0	17.1	1068.8
Proline	3.3	206.3	3.0	187.5
Glycine	3.1	193.8	3.6	225.0
Alanine	3.3	206.3	3.5	218.8
Cysteine	0.7	43.8	1.6	100.0
Valine	4.4	275.0	3.9	243.8
Methionine	0.98	61.3	3.1	193.8
Isoleucine	3.7	231.3	3.0	187.5
Leucine	6.7	418.8	5.4	337.5
Tyrosine	3.1	193.8	2.9	181.3
Phenylalanine	5.3	331.3	3.9	243.8
Tryptophan	1.2	75.0	1.5	93.8

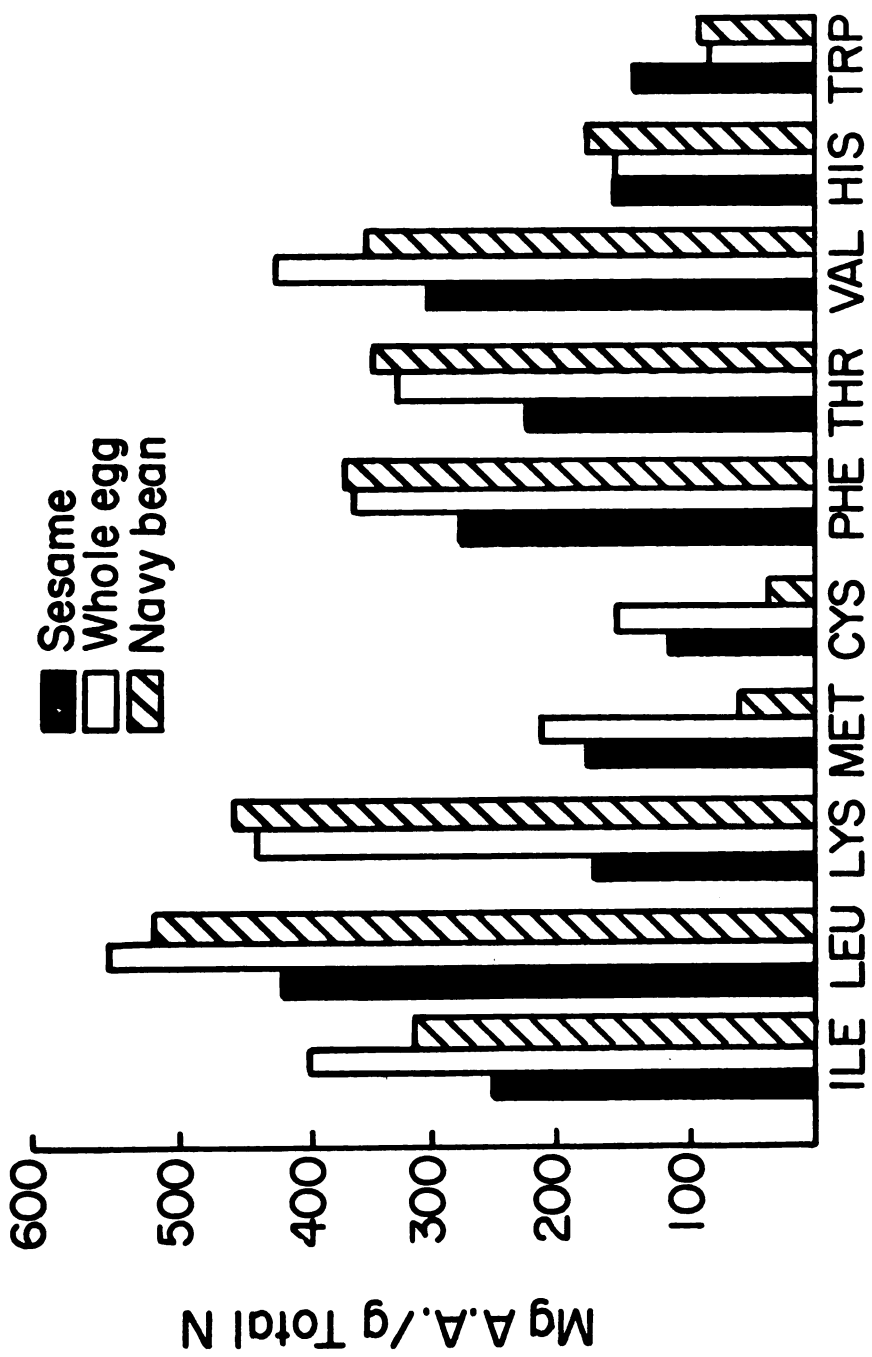

Figure 1. Essential amino acids composition (mg/g total N) of sesame flour, whole egg and Navy beans.

(b) 75% of the protein of the diet was furnished by Navy bean flour and the rest, 25%, came from the sesame flour; (c) sesame flour was the only source of protein; and (d) bean flour provided all the protein in the diet.

The results from this experiment are given in Table 13, and the growth rate and food intake, during the 28 day experimental period, are shown in Figures 2 and 3, respectively.

Group A was fed a 10% casein protein diet and it was used as control to calculate the protein efficiency ratios. The approximate weight gain was 4.35 ± 0.32 g per rat per day, and the feed intake was 13.2 ± 0.75 g per rat per day. The PER for this group was adjusted to 2.5 as recommended by the National Academy of Sciences, National Research Council (1963). All the PER values obtained throughout this work were corrected for casein = 2.50.

Group B was fed a 10% protein diet derived from autoclaved beans. The gain in body weight was 2.33 ± 0.11 g per rat per day. The protein quality of the bean diet was 62% of that for the casein control group. The relatively low efficiency of the bean protein diet in promoting growth is attributed mainly to their low methionine content (Russell et al., 1946; Kakade and Evans, 1965). The protein efficiency ratio (PER) was 1.56 and it is similar to the value obtained by Kakade and Evans (1965).

TABLE 13.--Growth of Rats Fed Standard Diets Containing: Casein; Autoclaved Beans; Sesame Flour; Beans + Sesame Flour (50:50 Protein Ratio) and Bean + Sesame Flour (75:25 Protein Ratio). Total Protein Content of Diets, 10%.

Group	Diet	Gain in Body Weight (g)	Protein Intake (g)	PER[1]	Adjusted PER[1,2]	% PER Casein[3]
A	Casein	1218.0	369.8	3.29 ± .07	2.50 ± .05	100[a]
B	Autoclaved Beans (B)	654.0	319.1	2.05 ± .04	1.56 ± .03	62[b]
C	Sesame Flour (S)	367.0	233.6	1.57 ± .11	1.19 ± .08	48[c]
D	B + S (50:50)	1409.0	464.7	3.03 ± .05	2.30 ± .04	92[d]
E	B + S (75:25)	1329.0	445.8	2.98 ± .03	2.26 ± .02	90[d]

[1] $\bar{X}$ ± SEM.

[2] Corrected for casein = 2.50.

[3] Values followed by the same letter are not statistically different at the 5% probability level.

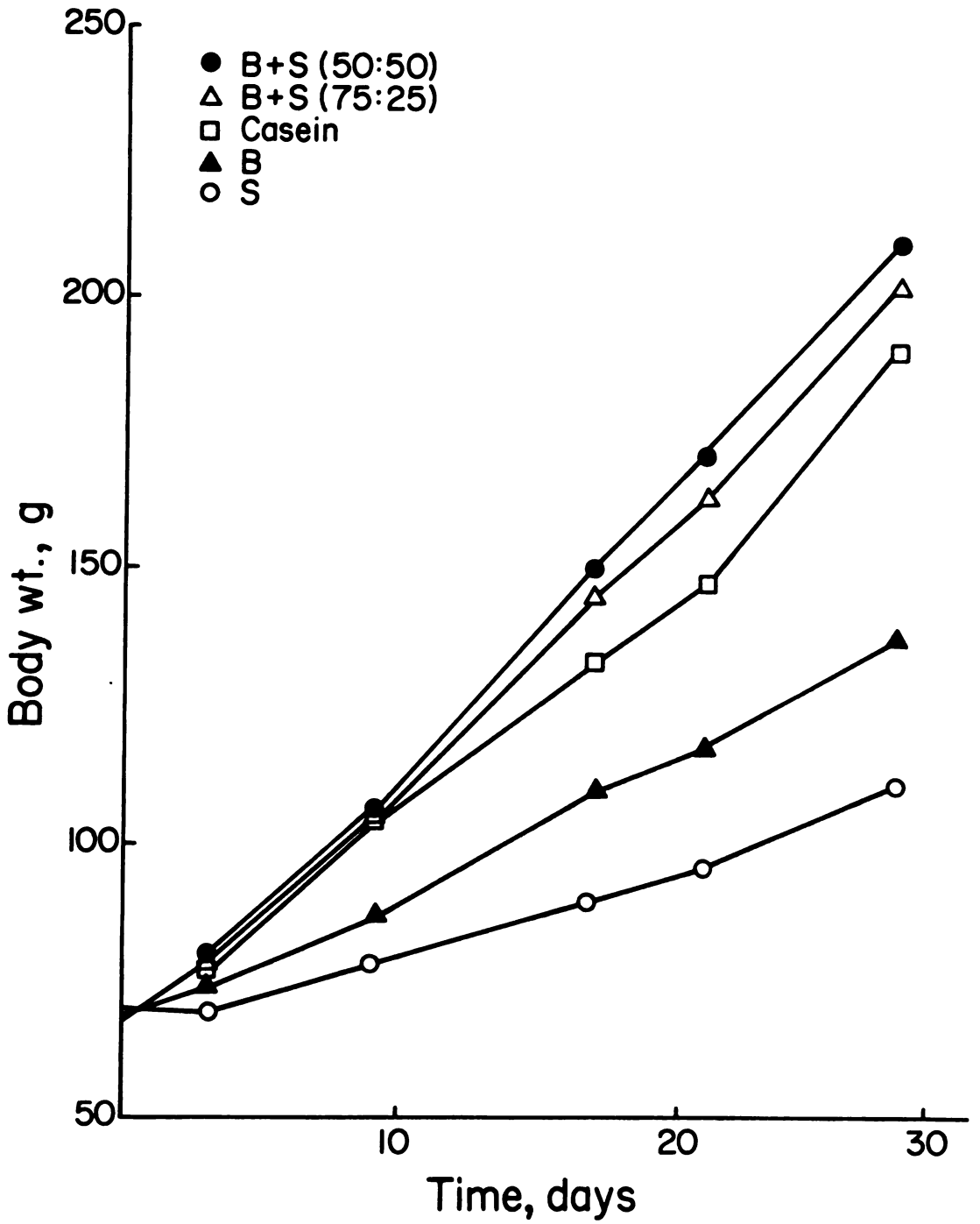

Figure 2. Weight gain of weanling rats (av. of 10) fed standard diets containing: casein; autoclaved beans (B); sesame flour (S); B + S, 50:50 protein ratio; and B + S, 75:25 protein ratio.

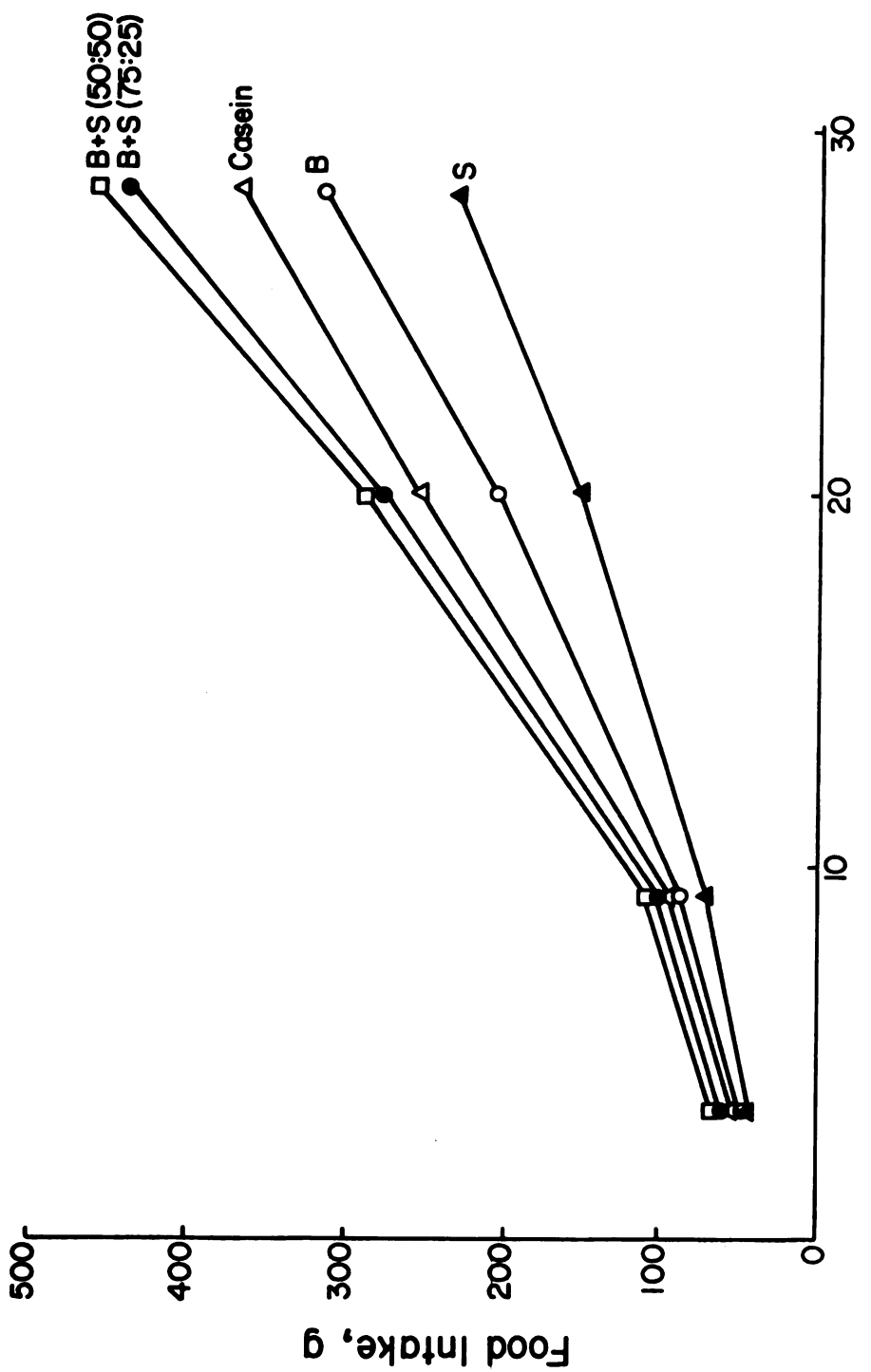

Figure 3. Food intake of weanling rats (av. of 10) fed standard diets containing: casein; autoclaved beans (B); sesame flour (S); B + S, 50:50 protein ratio; and B + S, 75:25 protein ratio.

Group C was fed a diet in which the sole source of protein was from sesame meal. The protein efficiency ratio of this group was 1.19 which represented 48% of that for casein control diet. This is exactly the same as the PER of 1.19 for solvent extracted dehulled sesame reported by Subramamian et al. (1971). The relatively low protein quality of sesame meal is attributed to its deficiency in lysine and somewhat in isoleucine (Lyon, 1972).

In a rat feeding study (Evans and Bandemer, 1967), the effect of fortification on the nutritive value (relative to casein) for sesame meal was demonstrated. Sesame alone had a protein nutritive value of 47%, and when fortified with 0.2% lysine it was raised to 94%.

The average weight gain for Group D (Navy bean: sesame flour, 50:50 protein ratio) was 5.03 ± 0.15 g per rat per day. The rate of growth and food intake were higher than those for the casein diet. The PER was 2.30 and was 92% of that for casein diet.

Rats in Group E (Navy bean:sesame flour, 75:25 protein ratio) gained an average of 4.75 ± 0.16 g per animal per day. The growth rate was higher than the one for casein and the animals consumed more food during the experimental period. The PER was 2.26 or 90% of the PER for casein, which was not statistically ($P < .05$) different from the one obtained for Group D (50:50 mixture).

Alquist and Grav (1944) conducted experiments to study the supplementary effect of sesame and soybean meals and showed that the best gains were made by chicks fed a ration in which the ratio of sesame-soybean protein approached 7:13.

Mankernika et al. (1965) investigated the nutritive value of blends of different plant proteins and reported that protein foods based on 40:30:30 blend of ground nut, soya and sesame flours possessed a fairly high PER value of 2.41.

Evans and Bandemer (1967) studied the supplementary value of sesame protein to that of soybean proteins. They concluded that a diet containing 5% protein supplied by Mexican sesame seed and 5% supplied by Chippewa soybeans promoted better growth than one containing 10% protein furnished by either Mexican sesame or Chippews soybeans. The protein quality of sesame alone was 47% (relative to casein) and the 1:1 mixture of sesame and soybean protein was almost the same as that of casein. The same authors reported that when only one-fourth of the protein was furnished by sesame seed and three-fourths by soybeans, growth was not as good as when soybeans were the sole source of protein.

In the next experiment in this series, we had two objectives. First, we were interested to see if germination of Navy beans, prior to autoclaving, would have any

beneficial effect on the growth of weanling rats, as compared to beans that had been just autoclaved. The second objective was to determine how low the amount of sesame flour in the mixture with Navy beans could be in order to observe an improvement on the nutritive value of the beans.

Results of these tests are presented in Table 14 and Figures 4 and 5. The mixture of Navy bean:sesame flour containing the protein ratios of 87.5:12.5 was also able to promote the growth of rats over that of beans alone.

The average weight gain for Group C (Navy bean: sesame, 87.5:12.5) was 2.52 ± 0.12 g per rat per day. The PER was 1.79 which represented 72% of that for casein and was significantly ($P < .05$) higher than the PER of beans alone. The food intake of this group was almost the same as beans alone, but they gained more weight.

Group D was fed a diet in which the protein source was Navy beans that had been perminated prior to autoclaving. The average weight gain for this group was 1.35 ± 0.14 g per rat per day and the protein efficiency ratio was 1.21. This represents 49% of the casein control group. The total food intake during the 28 days experimental period was low as compared to the casein and autoclaved beans groups (Figure 5). Everson et al. (1943) reported no improvement of the nutritive value of soybeans,

TABLE 14.--Growth of Rats Fed Standard Diets Containing Casein, Autoclaved Beans, a Mixture of Beans: Sesame Flour (87.5:12.5 Protein Ratio) and Germinated - Autoclaved Beans. Total Protein Content, 10%.

Group	Diet	Gain in Body Weight (g)	Protein Intake (g)	PER[1]	Adjusted PER[1,2]	% PER Casein[3]
A	Casein	1054.0	335.7	3.14 ± 0.06	2.50 ± 0.05	100[a]
B	Autoclaved Beans	660.0	324.0	2.04 ± 0.03	1.62 ± 0.02	65[b]
C	B + S (87.5:12.5)	708.0	315.3	2.25 ± 0.04	1.79 ± 0.03	72[c]
D	Germinated - Autoclaved Beans	379.0	249.0	1.52 ± 0.05	1.21 ± 0.04	49[d]

[1] $\bar{X}$ ± SEM

[2] Corrected for casein = 2.50.

[3] Values followed by the same letter are not statistically different at the 5% probability level.

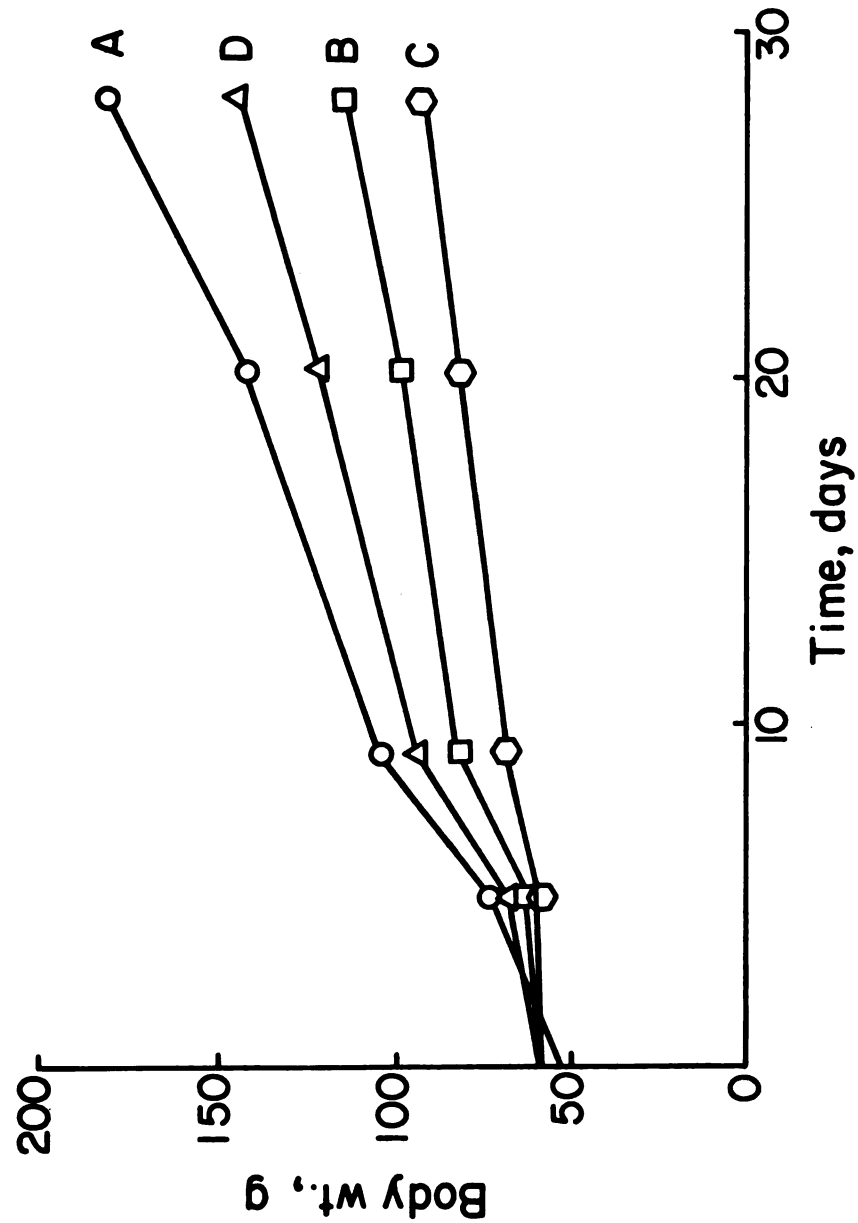

Figure 4. Weight gain of weanling rats (av. of 10) fed standard diets containing: A, casein; B, autoclaved beans; C, germinated and autoclaved beans; and D, mixture of autoclaved beans + sesame flour, 87.5:12.5 protein ratio.

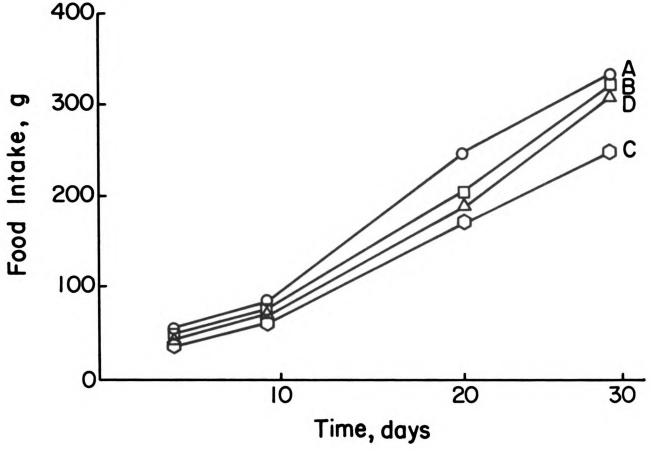

Figure 5. Food intake of weanling rats (av. of 10) fed standard diets containing: A, casein; B, autoclaved beans; C, germinated and autoclaved beans; and D, mixture of autoclaved beans + sesame flour, 87.5:12.5 protein ratio.

as determined by PER, when soybeans were germinated prior to cooking. Chattopadhay and Banerjee (1953) demonstrated that germination improved the nutritional value of certain beans. However, Kakade and Evans (1966), who worked with Navy beans, did not find any beneficial effect of soaking and germination on the nutritive value of the beans. The low growth rate obtained in our study is understandable in view of the low food intake by the animals.

Net Protein Ratios

In order to calculate Net Protein Ratios (NPR), a group of 4 rats was fed a non-protein diet. In a 28 day experimental period, they lost an average 28 ± 1g body weight. It is suggested that this corresponds to the maintenance requirements of rats for protein (Bender and Doell, 1957). The NPR values were determined for casein, Navy bean, sesame and mixtures of beans and sesame flour (87.5:12.5, 75:25 and 50:50 protein ratios) and were in the same order as their PER values. Table 15 indicates the NPR values of the casein and the tested samples. It is again evident that the protein quality of Navy beans is greatly improved by supplementation of sesame flour. In addition, there was no significant difference ($P < .05$) in NPR values between the bean: sesame mixtures with protein ratios of 75:25 and 50:50.

Bender and Doell (1957) determined the NPR, PER and NPU of some animal and plant proteins including

TABLE 15.--Net Protein Ratio (NPR) and % NPR (Casein = 100) of Standard Diets Containing Casein, Autoclaved Beans, Sesame Flour, and Mixtures of Bean:Sesame Flour With Protein Ratios of 87.5:12.5, 75:25 and 50:50. Total Protein Content of all Rat Diets, 10%.

Diet	Protein Intake (g)	Change Body Weight (g)	NPR[1]	% NPR[2]
Casein	369.8	+ 1218.0	3.37 ± 0.11	100[a]
Autoclaved Navy Beans (B)	319.1	+ 654.0	2.14 ± 0.05	64[b]
Sesame Flour (S)	233.6	+ 367.0	1.69 ± 0.07	50[c]
B + S (87.5:12.5)	315.3	+ 708.0	2.33 ± 0.04	69[d]
B + S (75:25)	445.8	+ 1329.0	3.04 ± 0.05	90[e]
B + S (50:50)	464.7	+ 1409.0	3.09 ± 0.03	92[e]
Non-Protein	0.0	- 28.0	----	--

[1] $\bar{X}$ ± SEM

[2] Values followed by the same letter are not statistically different at the 5% probability level.

sesame meal. They reported the NPR of 3.16 and PER of 0.69 for sesame meal. This is not in agreement with our result (NPR = 1.69; PER = 1.19) which might be due to the fact that their experiment lasted only 14 days but ours, 28 days. Furthermore, we found the NPR value of sesame meal to be higher than its PER value. This is because NPR takes into account some allowance for maintenance requirements and proteins low in lysine appear to be much more efficient in maintaining than in promoting growth (Said and Hejsted, 1969 and 1970; Said et al., 1974).

Protein Scores

The essential amino acid composition of Navy bean flour, sesame flour, the three mixtures of Navy bean: sesame flour (87.5:12.5, 75:25 and 50:50 protein ratios) and the new 1973 essential amino acid pattern (FAO/WHO, 1973) were compiled and expressed as milligrams of essential amino acid per gram of total nitrogen (Table 16). In Table 17 the protein scores of the tested samples are shown. This new FAO/WHO (1973) provisional scoring pattern was derived from estimates of amino acid requirements for the normal growth of young children. This pattern has been recently shown by Kaba and Pellet (1975) to be superior to all others tested in correlating with the NPU values obtained with young rats and in predicting the true limiting amino acids.

TABLE 16.--Essential Amino Acid Composition of the Reference Pattern (FAO/WHO, 1973), Navy Bean Flour, Sesame Flour and Mixtures of Navy Bean:Sesame Flours at Different Protein Ratios (Expressed as mg Amino Acid Per Gram Total Nitrogen).

Amino Acids (AA)	FAO/WHO (1973)	Navy Beans[1] (B)	Sesame Flour[1] (S)	B + S (87.5:12.5)	B + S (75:25)	B + S (50:50)
Isoleucine	250	231	188	226	220	210
Leucine	440	419	338	409	399	379
Lysine	340	356	144	330	303	250
Total Sulfur-Containing A.A. Methionine & Cysteine	220	105	294	129	152	200
Total Aromatic A.A. Phenylalanine & Tyrosine	380	525	425	513	500	475
Threonine	250	256	188	248	239	222
Tryptophan	60	75	94	77	80	85
Valine	310	275	244	271	267	260
Total Essential A.A.	2250	2242	1915	2201	2160	2079

[1]Results from this study.

TABLE 17.--Protein Scores of Navy Bean Flour, Sesame Flour and Mixtures of Navy Bean: Sesame Flours at Different Protein Ratios Based on the New Reference Pattern of Essential Amino Acid (FAO/WHO, 1973).

Amino Acids (A.A.)	Navy Beans (B)	Sesame Flour (S)	B:S (87.5:12.5)	B:S (75:25)	B:S (50:50)
Isoleucine	93	88	92	92	91
Leucine	96	90	95	94	93
Lysine	100	50	99	93	78
Total Sulfur-Containing A.A.	48	100	60	72	98
Total Aromatic A.A.	100	100	100	100	100
Threonine	100	88	100	99	96
Tryptophan	100	100	100	100	100
Valine	89	92	89	90	91

The amino acids cysteine and tyrosine were included in the calculation since they have sparing actions on methionine and phenylalnine, respectively.

Based on the protein scores, the Navy bean flour has methionine as the first limiting amino acid, followed by valine and isoleucine. Sesame flour has lysine as its first limiting amino acid, followed by isoleucine and threonine. Table 17 shows that the protein score for Navy bean protein is 48 and it is improved by addition of sesame flour. The protein scores for the bean + sesame with 87.5:12.5 and 75:25 protein ratios are 60 and 70, respectively. These two mixtures still have methionine as their first limiting amino acid, but the degree of deficiency is smaller. However, in the combination of Navy bean:sesame flours with a protein ratio of 50:50, methionine is no longer the limiting amino acid and the score is 78. The sesame flour protein (score:50) was largely improved by the Navy bean flour protein. This is due to the supplementation of lysine, which is the first limiting amino acid in sesame protein, by the Navy bean protein which is rich in this essential amino acid. The protein score, based on lysine, improved from 50 for sesame alone to 78, 93, and 99 for mixtures with protein ratios of 50:50, 75:25 and 87.5:12.5, respectively.

Modified Essential Amino Acid Index (MEAA Index)

This method was originally proposed by Oser (1951) and was modified later by Mitchell (1954). It has an advantage over the protein score method since it rates the proteins based not only on their first limiting amino acids but it takes into account the total individual essential amino acid content of the protein. This approach seems logical since all the essential amino acid must be present at the site of the protein synthesis within a tissue in order to have this process going on efficiently.

However, the computation of the MEAA index for Navy bean flour, sesame flour, and the mixtures of both (Table 18), failed to show the complementary values of the bean and sesame proteins.

Only the sesame flour index showed an increase from 78 to 89 as a result of supplementation with Navy beans. This is because sesame protein has a lower concentration of most of the essential amino acid than Navy bean protein. For the purpose of comparison, the protein scores are also shown in this table.

Evaluation of Protein Quality by Slope Ratio Technique

This technique was developed by Hegsted and his associates (Hegsted and Chang, 1965; Hegsted and Worcester,

TABLE 18.--MEAA Indexes and Protein Scores for Navy Beans, Sesame Flour, and Mixtures of Navy Bean and Sesame Flour at Different Protein Ratios.

	Navy Bean (B)	Sesame Flour (S)	B + S 87.5:12.5	B + S 75:25	B + S 50:50
MEAA Indexes	88	78	89	89	88
Protein Scores	48	50	60	72	78

1966; Hegsted et al., 1968; Hegsted and Neff, 1970). The Relative Nutritive Value (RNV) was defined as the slope of the dose-response curve of the protein under test divided by the slope of the dose-response curve obtained with the standard protein, lactalbumin. A modification of the original RNV has been considered as a potential replacement for the Protein Efficiency Ratio (PER) as the official protein quality determination method in the United States and Canada. The method is considered by its designers to be theoretically and methodologically superior to other protein quality evaluation procedures. The term Relative Protein Value (RPV) was used to describe a modification of the standard RNV procedure where the slope of the regression line linking weight gain with protein consumed was calculated without the non-protein group data. This modification was necessary because of the considerable deviation from linearity that has been found to occur at very low levels of protein intake both

with rats (Hegsted and Neff, 1970) and with human subjects (Young et al., 1975).

This experiment was conducted to evaluate the protein quality of Navy beans, sesame flour and mixtures of bean:sesame flours (with 87.5:12.5, 75:25 and 50:50 protein ratios) using this technique. The gain in body weight was chosen to be the criterion of response since Hegsted et al. (1968) found that the results obtained with weight gain, body water and body nitrogen as measures of response were generally similar. They found that body weight measurements had less variation and the correlation coefficient from 0.89 to 0.91 (average 0.946) between weight gain and body nitrogen was obtained. One reason for the smaller errors when weight gain is utilized is probably because the calculation of weight gain automatically takes into account the variation in size of the animals at the start of the experiment whereas the values used for body water and body nitrogen do not.

The mean weight changes and protein intake for the 24 groups of rats during the 21 days experimental period are shown in Table 19. An increase in food consumption was observed in all cases as the protein content of the diets improved, and the better the protein the more marked the elevation in food intake. The protein intake was well correlated with the average weight gain for all rats. There was, however, some loss

TABLE 19.--Mean (±SEM) Weight Gain and Protein Intakes of Rats Fed Growth-Limiting Amounts of Protein Supplied as Lactalbumin (LA), Navy Beans (B), Sesame Flour (S), and Mixtures of Beans and Sesame Flours with 87.5:12.5, 75:25 and 50:50 Protein Ratios.

Protein In Diet %	LA Diet		Beans Diet		Sesame Diet	
	Weight Gain (g/21 days)	Protein Intake	Weight Gain (g/21 days)	Protein Intake	Weight Gain (g/21 days)	Protein Intake
4	22.3±2.8	6.6±0.4	--	--	--	--
6	50.8±3.8	11.8±0.5	-2.5±1.7	7.1±0.9	2.5±1.6	7.8±0.5
8	81.8±6.1	18.5±0.9	8.0±2.5	10.4±0.7	13.8±1.5	12.7±0.6
10	100.5±3.8	23.9±0.6	20.3±3.4	16.0±0.8	23.3±3.5	16.3±0.7
12	--	--	40.3±1.0	22.3±0.7	32.5±4.4	20.7±1.9
	B+S(87.5:12.5)		B+S(75:25)		B+S(50:50)	
4	--	--	--	--	--	--
6	8.5±2.9	7.9±0.3	21.0±1.6	10.0±0.3	28.8±1.3	10.6±0.3
8	25.5±1.8	13.5±0.3	46.0±5.7	17.1±1.1	58.5±5.7	17.9±1.1
10	54.3±5.5	21.4±1.3	67.5±6.7	23.2±1.0	81.3±8.8	25.5±1.5
12	78.3±8.9	30.1±2.0	20.3±4.5	30.2±0.5	112.8±8.4	35.7±1.9

in body weight for the rats consuming the bean diets at the 6% protein level.

The Relative Protein Value (RPV), was calculated for each test diet as the slope of the regression line of the test diet, and are expressed as a percentage of the slope obtained with lactalbumin in the same experiment. The regression lines were obtained using the weight gain and protein intake of each individual rat for each diet rather than averaging them out as a group. The results are shown in Table 20 and Figure 6.

The Relative Nutritive Value (RNV) calculations were performed in the same way except the regression analysis was done between weight gain and protein intake including the data for the rats fed the nonprotein diet. These animals lost an average of 22.5 g during the experimental period. The correlation coefficients, regression equations, RNV and RPV for the tested samples as well as for the lactalbumin standard, are given in Table 21. The two sets of equations, with and without the zero protein data, were used to calculate the RNV and RPV data, respectively.

The RPV values for Navy bean (B), sesame flour (S), B:S mixtures with 87.5:12.5, 75:25 and 50:50 protein ratios were 57, 52, 67, 71 and 74% (assuming lactalbumin has a RPV of 100), respectively. The RNV values for these diets were 54, 52, 66, 68, and 74% of that for

TABLE 20.--The Relationship of Weight Gain and Protein Intake of Rats Fed Growth-Limiting Amounts of Protein Supplied as Lactalbumin (LA), Navy Beans (B), Sesame Flour (S) and Mixtures of Beans and Sesame Flours with the Following Protein Ratios: 87.5:12:5, 75:25 and 50:50.

Protein Source	Level of Protein (%)	N	R[1]	Y-intercept[2]	Protein Value[3] (Slope)	Relative Protein[4] Value (%)
LA	4,6, 8,10	16	0.99	- 5.79	4.57	100
Beans (B)	6,8,10,12	16	0.94	-19.29	2.62	57
Sesame (S)	6,8,10,12	16	0.98	-16.03	2.37	52
B+S (87.5:12.5)	6,8,10,12	16	0.97	- 8.10	3.07	67
B+S (75:25)	6,8,10,12	16	0.99	-17.87	3.27	71
B+S (50:50)	6,8,10,12	16	0.99	- 5.68	3.39	74

[1]Correlation coefficient.

[2]A group of animals fed a zero-protein diet (N = 4) for 3 weeks lost 22.5 g. The regression lines obtained for the test proteins did not intercept the Y-axis at the point indicated by the zero-control animals.

[3]The slope of the line obtained when gain is regressed on protein intake.

[4]The protein value expressed as a percentage of the protein value obtained for lactalbumin.

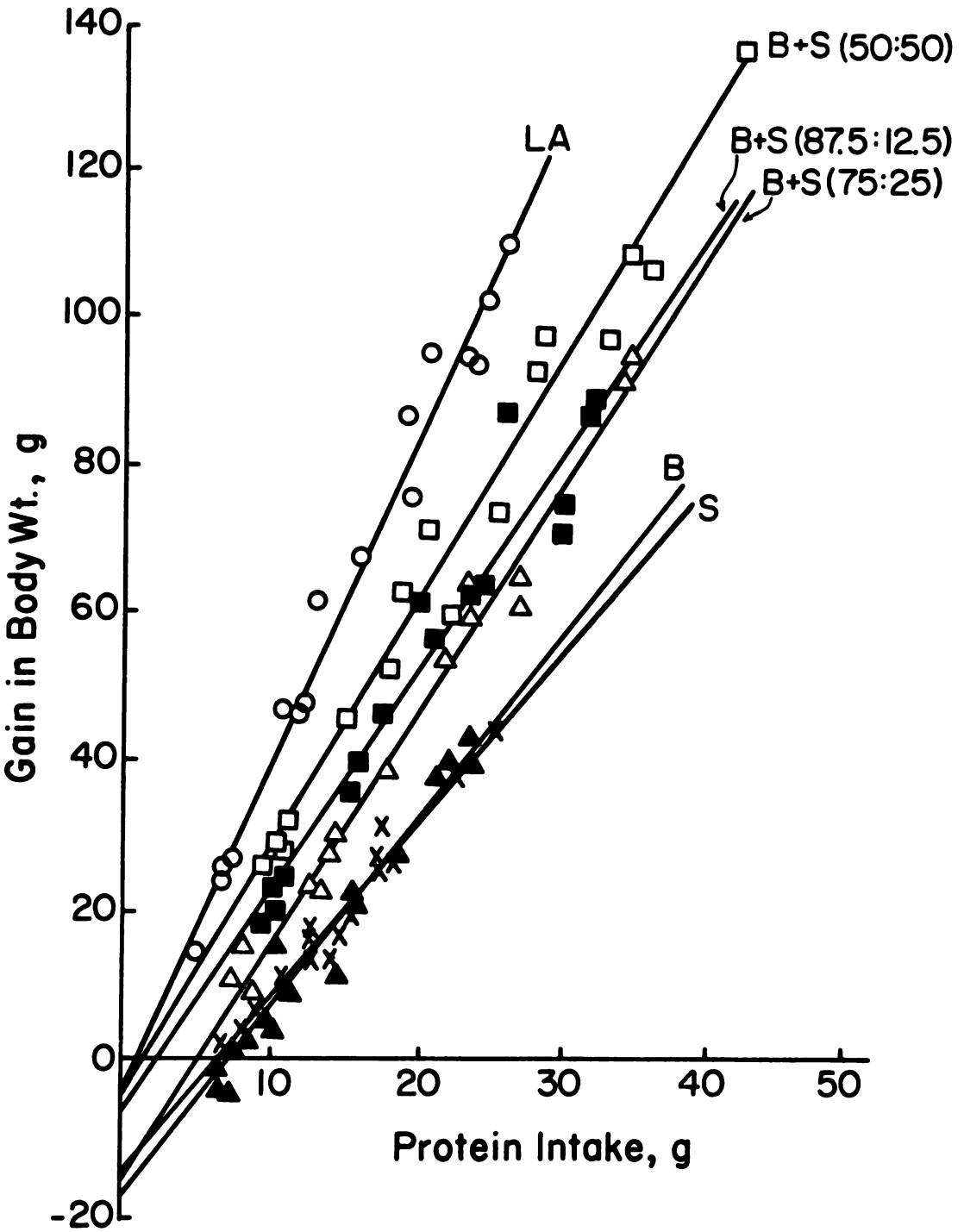

Figure 6. Regression lines relating weight gain and protein intake for rats fed growth-limiting amount of proteins supplied by lactalbumin (LA), autoclaved Navy beans (B), sesame flour (S) and mixtures of beans and sesame flour with protein ratios of 87.5:12.5, 75:25 and 50:50.

TABLE 21.--Regression Equations, Relative Nutritive Values (RNV) and Relative Protein Values (RPV) for Diets Fed to Rats in Growth Limiting Amounts. Protein was Provided by Lactalbumin, Navy Beans, Sesame Flour and Mixtures of Bean and Sesame Flours at Different Protein Ratios.

	Calculated With Zero Protein Data			Calculated Without Zero Protein Data		
Protein Source	Correl. Coef.	Regression Equation	RNV	Correl. Coef.	Regression Equation	RPV
Lactalbumin	0.99	Y=5.14X-15.96	100	0.99	Y=4.57X- 5.79	100
Navy Beans	0.99	Y=2.76X-22.17	54	0.94	Y=2.62X-19.29	57
Sesame Flour	0.99	Y=2.65X-20.50	52	0.98	Y=2.37X-16.03	52
Beans + Sesame (87.5:12.5)	0.99	Y=3.39X-20.55	65	0.97	Y=3.07X- 8.1	67
Beans + Sesame (75:25)	0.98	Y=3.49X-17.71	68	0.99	Y=3.27X-17.87	71
Beans + Sesame (50:50)	0.99	Y=3.78X-16.07	74	0.99	Y=3.39X- 5.68	74

lactalbumin. The RPV determination puts the protein quality of the tested samples in the same order as the RNV estimation. However, the values for RNV are lower than the RPV values since by incorporating the zero protein data the slope of the regression line for lactalbumin is increased more than other diets. This is in agreement with the findings of Chavez and Pellet (1976).

The slope of the lactalbumin line was 4.57 g gain in body weight per gram of protein eaten; and 2.62, 2.37, 3.07, 3.27 and 3.39 g gain per gram of protein eaten for beans (B), sesame (S) and bean:sesame flour with protein ratios of 87.5:12.5, 75:25 and 50:50, respectively (Figure 6). It is also evident that the regression lines, relating the weight gain and protein consumed of the tested proteins did not have a common Y-intercept, nor did they meet at the point indicated by the zero protein group. The intercept of the lactalbumin line falls at -5.79 g, well above the mean of the animals fed the protein-free diet (-22.5 g). This is usually seen for most proteins (Hegsted and Juliano, 1974). Actually this is the main criticism of this technique. However, since it is certain that the regression lines relating dose to response do not go through the same intercept, the slope of the regression line for each protein was

calculated individually and compared to that of the standard lactalbumin protein (Hegsted, 1974).

The slope-ratio technique also shows the great supplementary value of sesame protein for bean proteins. The RPV as well as RNV values of the beans diet improved from 57 and 54% to 74% (mixture 50:50) by addition of sesame flour. A greater improvement was observed for the sesame protein when bean protein was present in the diet (Table 21).

The results of the different testing methods for the protein quality of the beans, sesame and their mixtures with three different protein ratios, are presented in Table 22.

The linear regression equations and correlation coefficients between protein values of the tested diets estimated by the Relative Nutritive Value (RNV), Relative Protein Value (RPV), Protein Efficiency Ratios (PER), Net Protein Ratio (NPR), MEAA indexes and protein scores methods of estimating the protein quality are shown in Table 23.

In general, the correlation between biological assays (RNV, RPV, PER and NPR) were all high with an average correlation coefficient of 0.96. The chemical assays (protein score and MEAA indexes), however, did not correlate with each other and had a low correlation coefficient ($r = 0.50$). Of the two chemical methods,

TABLE 22.--Protein Scores, MEAA Indexes, PER, NPR, RPV and RNV of Navy Beans, Sesame Flour and Mixtures of Navy Bean + Sesame Flour at Different Protein Ratios.

Diet	Methods of Estimation					
	Protein Score[1]	MEAA Index[1]	PER[2]	NPR[3]	RPV[4]	RNV[5]
Autoclaved Navy Beans (B)	48	88	62	64	57	54
Sesame Flour (S)	50	78	48	50	52	52
Mixture A B:S(87.5:12.5)	60	89	72	69	67	66
Mixture B B:S(75:25)	72	89	90	90	71	68
Mixture C B:S(50:50)	78	88	92	92	74	74
Casein	--	--	100	100	--	--
Lactalbumin	--	--	--	--	100	100

[1]Calculated based on the FAO/WHO reference pattern (1973).

[2]Protein efficiency ratios; expressed in relation to casein equal 100.

[3]Net protein ratio; expressed in relation to casein equal 100.

[4]Relative protein value from slope-ratio technique; calculated in relation to lactallumin equal 100.

[5]Relative nutritive value from slope-ratio technique; calculated in relation to lactallumin equal 100.

TABLE 23.--Regression Equations and Correlation Coefficients Between Protein Values of Diets Estimated by Relative Nutritive Value (RNV), Relative Protein Value (RPV), Net Protein Ratio (NPR), Protein Efficiency Ratios (PER), Modified Essential Amino Acid Indexes (MEAA) and Protein Score.

Regression Equation and Correlation Coefficient	Protein Score	MEAA Index	PER	NPR	RPV	RNV
RNV	y=0.687x +20.29 r=0.95	y=1.331x -5 2.37 4=0.66	y=0.473x +28.17 4=0.94	y=0.49x +26.8 r=0.92	y=0.99x -1.14 r=0.99	--
RPV	y=0.667x +23.11 r=0.94	y=1.475x -63.27 r=0.74	y=0.485x +28.94 r=0.97	y=1.814x -43.49 r=0.95	--	y=0.99x -1.14 r=0.99
NPR	y=1.266x -5.0 r=0.94	y=2.706x -160.78 r=0.72	y=0.945x +4.26 r=0.99	--	y=1.814x -43.49 r=0.95	y=0.49x +26.8 r=0.92
PER	y=1.331x -9.24 r=0.94	y=3x -186.48 r=0.75	--	y=0.945x +4.26 r=0.99	y=0.485x +28.96 r=0.97	y=0.473x +28.178 r=0.94
MEAA Index	y=0.178x +75.41 r=0.50	--	y=3x -186.48 r=0.75	y=2.706x -160.78 r=0.72	y=1.475x -63.27 r=0.74	y=1.331x -52.37 r=0.66
Protein Score	--	y=0.178x +75.41 r=0.50	y=1.331x -9.24 r=0.94	y=1.266x -5 r=0.94	y=0.667x +23.11 r=0.94	y=0.687x +20.29 r=0.95

protein score had a good correlation (r = 0.94) with all bioassays tested, but MEAA index method had a relatively poor correlation (r = 0.74) with the biological methods. This was of no surprise, since MEAA indexes failed to show any differences between the protein quality of the diets (Table 22).

It is claimed that the RPV method should be taken as the most accurate estimate available for the protein quality (Hegsted et al., 1968). If this is true, we found the best correlation between RPV and PER (r = 0.97) and between RPV and NPR (r = 0.95). Therefore, NPR was not superior to PER as it was claimed because of the nonprotein control group which makes some allowance for maintenance requirements (Bender and Doell, 1957; Chavez and Pellett, 1976).

Hegsted (1971) claimed that the amino acid score is an inadequate estimate of the nutritive value of proteins. It may not predict protein quality as well as one would wish, but in most instances, prediction from amino acid data (protein score) is reasonably accurate (Chavez and Pellett, 1976).

Hegsted (1971) has claimed further that the slope-ratio technique is superior to other protein quality bioassays. For relatively poor quality protein, this is probably true, beans and sesame alone nevertheless are shown to have a lower protein quality by all the

assays tested and the two mixtures of beans and sesame (with 75:25 and 50:50 protein ratios) were superior by all tests. Furthermore, the slope-ratio technique did not show as high a difference between the higher quality proteins (75:25 and 50:50 mixtures, Table 22) and lower quality proteins (87.5:12.5 mixture, beans or sesame alone) as did the PER and NPR methods; and the estimates of protein quality by standard procedures and by calculation from amino acid data are very close to those obtained with the RPV slope-ratio assay procedure. Chavez and Pellett (1976) came to the same conclusion after estimating the protein quality of different diets by RPV, RNV, PER, NPR, relative NPR and amino acid scores.

Supplementation of Navy Bean Protein with Whole Egg Powder

The purpose of this study was to see if we could get any improvement in the growth of rats by supplementing the beans with small amounts of animal proteins such as egg protein.

Therefore, three different protein ratios of a mixture of beans and whole egg powder were tested and the animals were fed the following diets at the 10% protein level:

a. Casein

b. Autoclaved beans

c. Autoclaved beans and egg powder with bean:egg protein ratio of 90:10

d. Autoclaved beans and egg powder with bean:egg protein ratio of 95:5

e. Autoclaved beans and egg powder with bean:egg protein ratio of 97.5:2.5

The results of this experiment are presented in Table 24 and Figure 7. Protein scores of the tested diets were also calculated which are shown in Table 24.

Group A, casein fed animals, gained 3.69±0.35 g rat per day and the food intake was 11.87±0.87 g per animal per day.

The bean fed animals, Group B, gained 1.50±0.40 g per rat per day, with a food intake of 8.13±0.51 g per animal per day and the PER Value was 1.45.

Supplementation of bean protein with whole egg proteins provided a better growth for the animals than beans alone (Figure 7). The Protein Efficiency Ratios (PER) of a mixture of beans and egg powder with the following protein ratios: 90:10, 95:5 and 97.5:2.5 were 1.71, 1.68 and 1.63, respectively, and protein scores were 56, 52 and 50, respectively. Even though the protein quality of the Navy bean flour improved (as indicated by the PER's and protein scores) by addition of egg protein to the diet, there was no significant difference ($P < .05$) between the PER values obtained for the mixtures of beans and egg with different protein ratios. This could be due to the small contribution of egg protein in preparation of the tested diets.

TABLE 24.--PER and Protein Score of Diets Based on Casein, Autoclaved Beans and Three Mixtures of Beans and Egg Powder, with the Following Protein Ratios: 90:10, 95:5 and 97.5:2.5. Total Protein Content of all Diets was 10%.

Group	Diet	Gain in Body Weight (g)	Protein Intake (g)	PER[1]	Adjusted PER[1,2]	% PER Casein[3]	Protein Score
A	Casein	1035.0	332.5	3.11±0.08	2.50±0.06	100[a]	64
B	Autoclaved Beans (B)	295.0	163.0	1.81±0.04	1.45±0.03	58[b]	48
C	B + Egg (90:10)	497.0	234.1	2.12±0.07	1.71±0.06	68[c]	56
C	B + Egg (95:5)	516.0	246.3	2.09±0.05	1.68±0.04	67[c]	52
E	B + Egg (97.5:2.5)	491.0	242.6	2.02±0.04	1.63±0.03	65[c]	50

[1] $\bar{X}$ ± SEM

[2] Corrected for Casein = 2.50.

[3] Values followed by the same letter are not statistically different ($P < .05$).

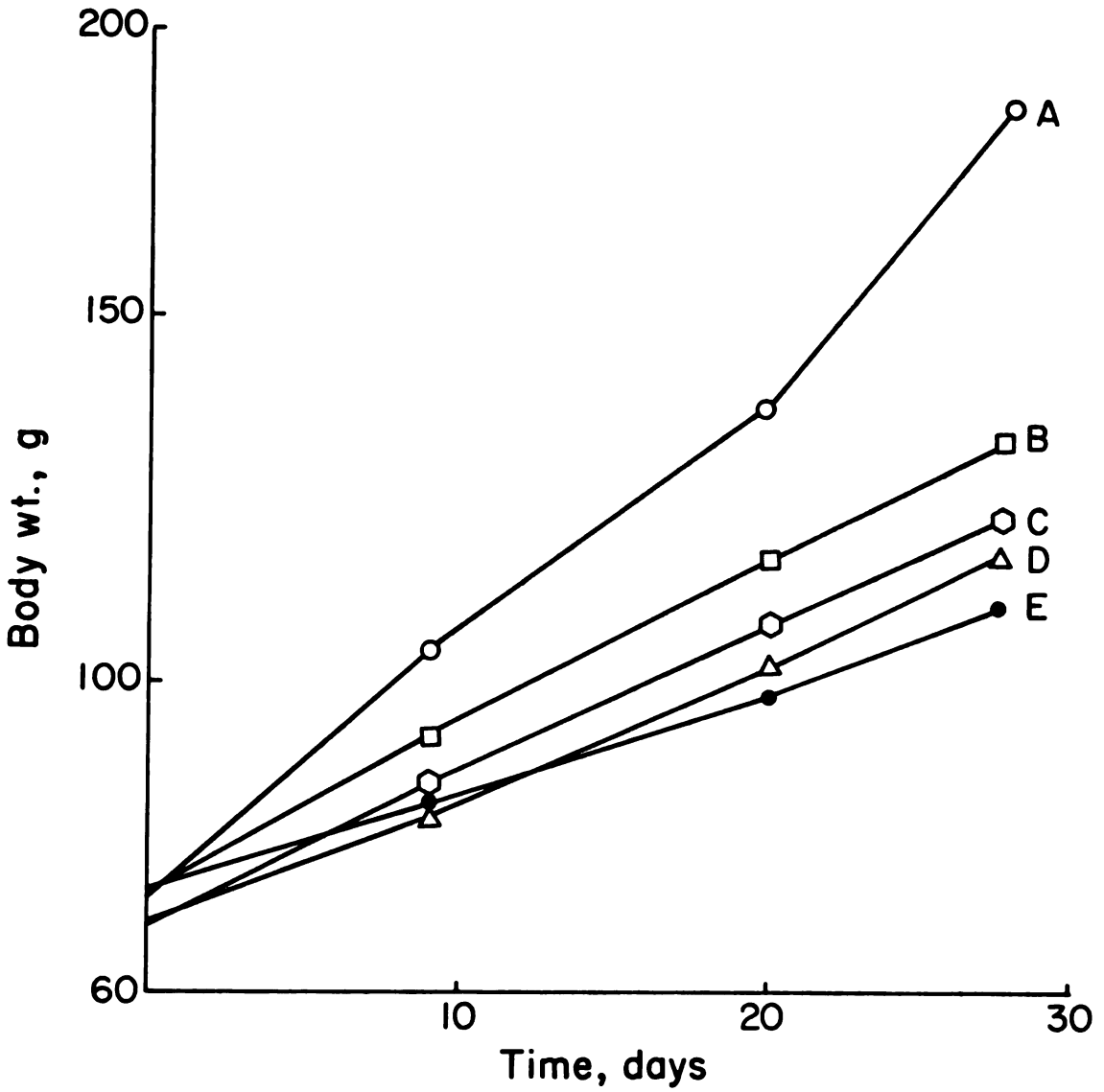

Figure 7. Weight gain of weanling rats (av. of 10) fed standard diets containing: A, casein; B, autoclaved beans + egg, 80:20 protein ratio; C, autoclaved beans + egg, 95:5 protein ratio; D, autoclaved beans + egg, 97.5:2.5 protein ratio; and E, autoclaved beans.

Effect of Processing on the Protein Quality of Navy Beans

The following processing methods were evaluated in this experiment:

a. Canning with no sugar added.

b. Canning with 1.5% sugar in the brine.

c. Home cooking.

d. Autoclaving at 250°F for 10 minutes.

The results of the moisture and protein determination of the samples are shown in Table 25.

TABLE 25.--Moisture and Protein (N x 6.25) Content of Navy Beans Processed by Different Methods, and Dried in Air Forced Drier.

Sample	% Moisture	% Protein (moisture free basis)
Canned Beans	6.2	23.9
Canned Beans (1.5% sugar in the brine)	7.0	23.6
Home Cooked Beans	7.4	23.2
Autoclaved Beans	8.7	23.0

The three criteria used to estimate the quality of the bean protein were: PER, lysine availability and methionine availability.

The first feeding experiment in this series was carried out to test the growth of young rats fed diets based on Navy beans subjected to different types of

processing. The results of this experiment are presented in Table 26, and the growth rate and food intake, during the 28 day experimental period, are shown in Figures 8 and 9.

Group A was fed a 10% protein diet based on casein and it was used as the reference diet to calculate the PER. The weight gain was 5.10±0.55 g per rat per day. The PER value was 3.42 which was corrected to 2.50.

Groups B,C,D and E were fed a 10% protein diet derived from Navy beans which had been subjected to processing. The gain in body weight and food intake of these groups were close as can be seen in Figures 8 and 9. The PER values of the beans subjected to various types of heating, ranged from 1.40 to 1.49 and they were not statistically different ($P < .05$). They represented between 56 to 60% protein quality of the control casein diet.

In order to estimate the availability of lysine in the processed bean proteins, a chemical method using 1-fluoro-2, 4-dinitrobenzene (FDNB) was used (Couch, 1975).

The results of this study are presented in Table 27. Apparently, the availability of lysine in the Navy bean proteins was not impaired by the evaluated processing methods. The availability ranged from 93.3 to 97.5% which indicates that lysine is highly available in the

TABLE 26.--Growth of Rats Fed Standard Diets Containing Casein, Canned Beans (B) Canned Beans with 1.5% Sugar in the Brine (C), Home Cooked Beans (D) and Autoclaved Beans (E). Total Protein Content of all Diets, 10%.

Group	Gain in Body Weight (g)	Protein Intake (g)	PER[1]	Adjusted PER[1,2]	% PER Casein[3]
Casein	1017.0	297.5	3.42±0.05	2.50±0.04	100[a]
B	355.0	180.5	1.97±0.03	1.44±0.02	58[b]
C	348.0	182.9	1.90±0.04	1.40±0.03	56[b]
D	397.0	207.3	1.91±0.04	1.41±0.03	56[b]
E	326.0	159.6	2.04±0.04	1.49±0.03	60[b]

[1] $\bar{X}$ ± SEM

[2] Corrected for casein = 2.50

[3] Values followed by the same letter are not statistically different at the 5% probability level.

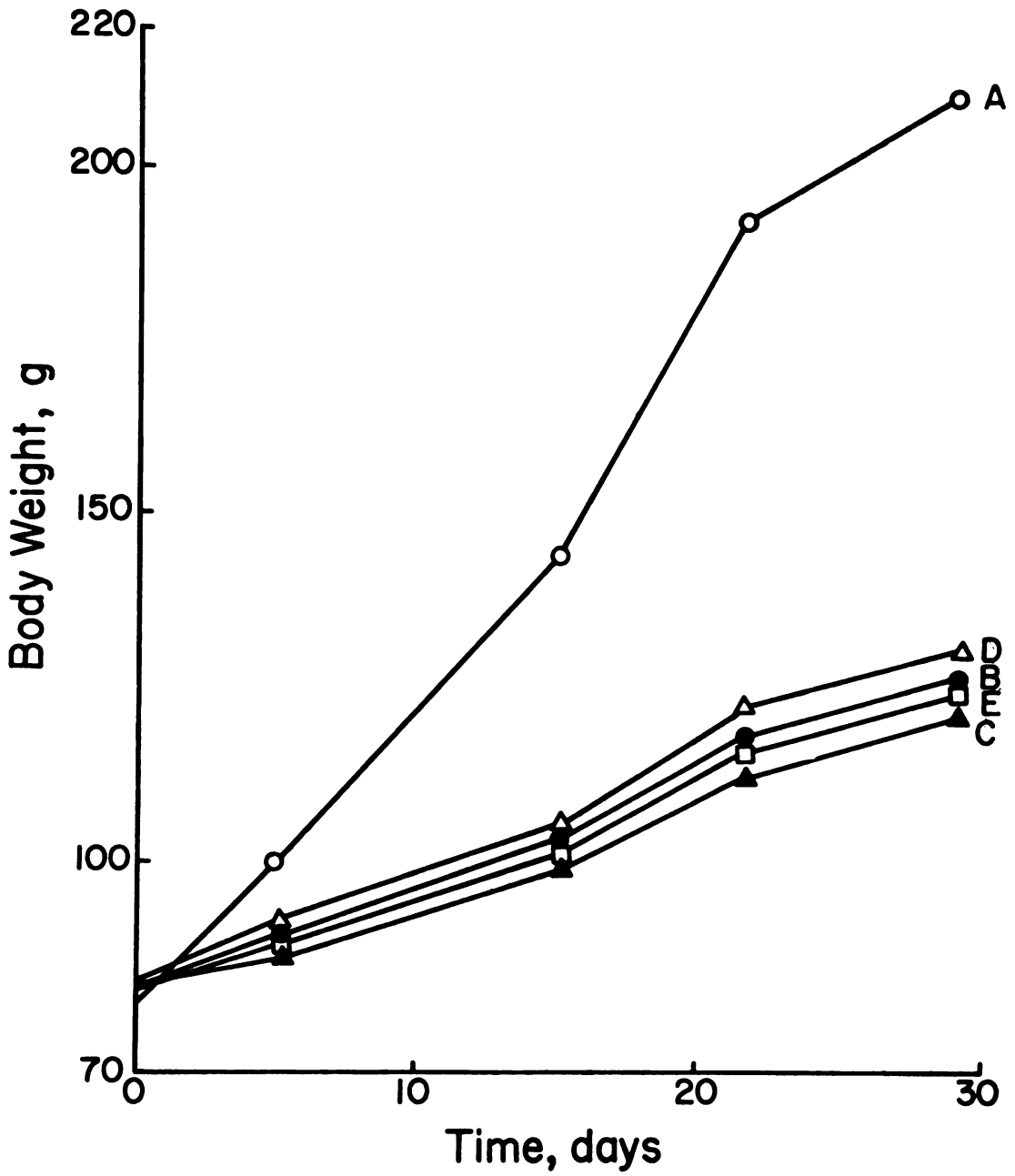

Figure 8. Weight gain of weanling rats (av. of 10) fed standard diets containing: A, casein; B, canned beans (no sugar added); C, canned beans (with 1.5% sugar in the brine); D, home cooked beans; E, autoclaved beans.

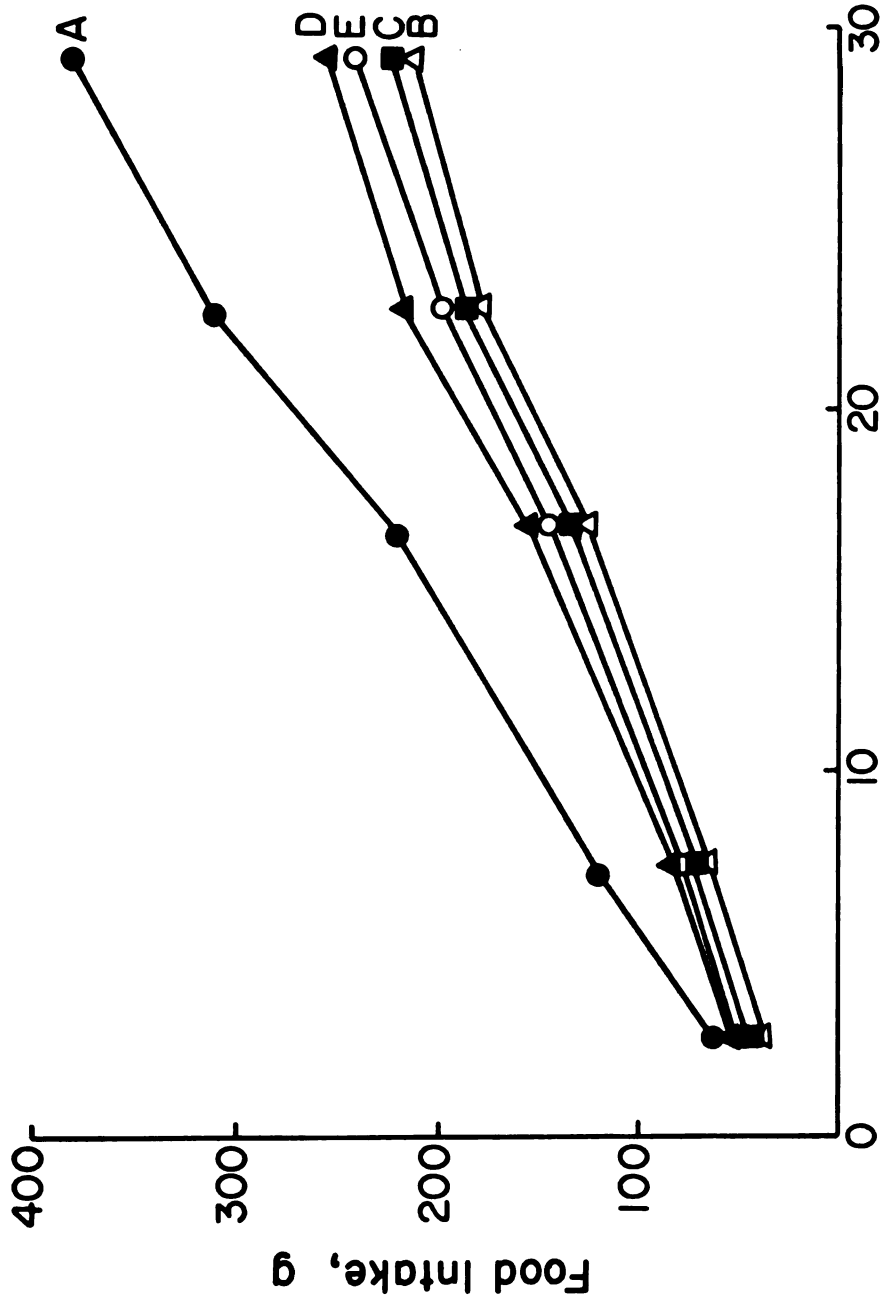

Figure 9. Food intake of weanling rats (av. of 10) fed standard diets containing: A, casein; B, canned beans (no sugar added); C, canned beans (with 1.5% sugar in the brine); D, home cooked beans; E, autoclaved beans.

TABLE 27.--Lysine Availability of Bean Protein Processed as Follows: A, Canned with no Sugar Added; B, Canned with 1.5% Sugar in the Brine; C, Home Cooked; and D, Autoclaved.

Sample	% Available Lysine
A	93.3
B	93.9
C	97.5
D	97.4

Navy bean proteins regardless of the methods of processing used in this study.

The results of the bioassay which aimed at determining the utilization of methionine in processed beans are shown in Table 28 and 29.

Table 28 shows the growth of rats fed standard diets in which the methionine content of the diets was 0.2%. This level does not correspond to maximum growth rate for rats, which is close to 0.6% (NAS, 1972). The 0.2% methionine in the casein diet corresponds to the methionine content of casein itself. The bean diets contained 0.1% methionine originating in the beans and 0.1% L-methionine added as a crystalline compound. The bean methionine availability was calculated as follows:

There are two assumptions made in this calculation: first, that casein methionine is utilized 81% according to

TABLE 28.--Methionine Availability in Processed Beans, Based on Rat Growth Rates, Diets: Canned Beans (B), Canned Beans With 1.5% Sugar in the Brine (C), Home Cooked Beans (D) and Autoclaved Beans (H). L-Methionine was Added to the Diets to Bring the Total Methionine Content to 0.2 and 0.5%.

Diet	Level of Methionine in Diet %	Average Gain in Body Weight	Average Food Intake (g)	Methionine Consumed (g)	G.Wt. Gain / G.Met. Consumed	% Available Methionine[1]
Casein	0.2	88.4	223.2	0.45	198.03	81[2]
B	0.2	79.2	218.0	0.44	181.65	49[a]
C	0.2	83.0	239.5	0.48	173.28	42[a]
D	0.2	78.6	217.3	0.43	180.90	48[a]
H	0.2	80.0	228.2	0.44	183.36	50[a]
Casein-1	0.5	96.2	230.4	1.15	83.51	--
H-1	0.5	70.6	187.2	0.94	75.43	--

[1]Values followed by the same letter are not statistically different at the 5% probability level.

[2]Taken from Schweigert and Guthneck (1954). No value available for 0.5% Methionine in a Casein diet.

TABLE 29.--Efficiency of Methionine Utilization in Bean Diets Fed to Rats (Av. of 5) at Various Levels of Methionine. 0.1% L-Cystine was Added to all Diets (10% Protein).

Diet	G.Met. Consumed (A)	G.Wt. Gain (B)	Efficiency of Methionine Utilization (B/A)
Beans	0.093	27.4	294.62
Beans + 0.1% Met.	0.44	80.0	183.36
Beans + 0.3% Met.	0.389	67.2	172.75
Beans + 0.4% Met.	0.94	70.6	75.43

Schwergert and Guthneck (1954); and second that crystalline L-methionine is utilized 100%.

Casein diet had 0.2% methionine which corresponds to: $0.2\% \times \frac{81}{100} = 0.162\%$ available methionine; this resulted in 198.03 g Wt. gain per rat per g available methionine consumed.

The 0.2% methionine in the autoclaved bean diet (H) resulted in 183.36 g weight gain per rat per g methionine consumed. Some of this growth is due to the pure methionine added to the diet and the remaining growth is due to the bean methionine. The following calculation permits the portion of bean methionine utilized by the growing rats:

0.162% casein available methionine resulted in 198.03 g Wt. gain/g methionine consumed

0.1% added crystalline methionine resulted in X = 122.24 g Wt. gain/g methionine consumed

Therefore: 183.36 - 122.24 = 61.12 g Wt. gain was due to the consumption of the bean methionine, and the availability of the bean methionine is:

$$\frac{61.12\text{g (Wt.gain/rat/14 days caused by consuming bean methionine)}}{122.24\text{g (Wt.gain/rat/14 days caused by consuming pure methionine)}} \times 100$$

$$= 50\%$$

This 50% availability of the autoclaved bean methionine found by growth bioassay corroborates the results of Evans et al. (1974), who investigated the

availability for the rat of methionine and cystine in Navy beans and soybean meal using a microbiological assay (Leuconostoc mesenteroids). Methionine and cystine balance studies were conducted with growing rats and it was reported that about 50% of the methionine and 25% of the cystine in beans were excreted with the undigested protein in the feces. Approximately, 26% of the methionine and 11% of the cystine of soybean meal were excreted in the feces. It was also reported that only small amounts of methionine (1%) or cystine (2-9%) were excreted in the urine.

Our calculation was extended to estimate the methionine availability of beans subjected to other processing treatments (Table 28).

Animals, which were fed canned beans with 1.5% sugar in the brine, consumed more methionine but gained less weight than rats fed canned beans with no sugar added. Therefore, the methionine availability was 42% for the former versus 49% for the latter. The methionine availability of the home cooked beans was 48%.

When the total methionine content of the casein and autoclaved bean diets was raised to 0.5%, the weight gain per gram of methionine consumed decreased drastically. It must be noted that the term "availability" does not describe the phenomenon observed here. The pure methionine added to the diet is readily available to the animal, but

it is simply not utilized for growth. Schweighert and Guthneck (1954) had observed that the growth of protein-depleted rats levelled off when L-methionine was added to casein at levels higher than 200 mg L-methionine per 100 g diet.

Efficiency of methionine utilization for growth would be a better term to describe the relationship between methionine levels in the diet and rat growth. From experiments conducted in this study the following efficiency ratios were obtained (Table 29). The efficiency of methionine utilization is reduced as the methionine level in the diet increases.

It is, therefore, concluded that under the conditions of our experiments, the evaluated processing methods which included: canning with or without added sugar and home cooking did not impair the protein quality of the Navy bean as indicated by the PER, lysine availability and methionine availability values.

Johnson, et al. (1939), in studies of sulfur and nitrogen balances in rats fed diets containing raw, heated and solvent-extracted soybeans, found that equal amounts of sulfur and nitrogen disappeared from the digestive tract regardless of the previous treatment of the meal or beans.

Guttridge (1961) developed a method for the bioassay of methionine using White Leghorn chicks at 11-18

days of age. Using this technique, assays of biological available methionine was made on several soybean samples which had received varying heat treatments. He obtained a high available methionine value, an average of 90% for the tested samples.

Guttridge and Lewis (1964) studied the chick bioassay of methionine and cystine in soybean meals and groundnut meals. They reported an average methionine availability of 85% and 74% for soybean meal and groundnut meal, respectively.

Growth assays with 8-day old crossbred chicks were used to estimate the availability of sulfur amino acids in corn gluten and corn protein by the slope-ratio technique and standard curve method (Sasse and Baker, 1973). The availability estimates for corn gluten meal were 98.9±2.1% and 99.2±1.6% and for corn 96.5±6.5% and 93.9±8.7% using the slope ratio and standard curve methods, respectively.

Miller et al. (1965) measured lysine availability (DNFB method and chick assays) and methionine availability (Streptococcus zymogenes and chick assays) in freeze-dried cod muscle subjected to 16 different conditions of heat treatment, with temperatures from 45 to 116°C and time from 9 to 729 hours; in a few of the treatments the dried muscle was first mixed with 5 or 10% glucose. They reported that mild heat treatment in the presence of 5%

glucose reduced the available lysine by 18% although available methionine was unchanged. From chick and microbial trials it was concluded that heat conditions more severe than 85°C for 27 hours, achieved either by elevation of temperature or by heating for a longer time all brought significant loss of available sulfur amino acids. For the mildest treatment (85°C, 27 hours) the FDNB lysine value was not affected whereas for the others there was a significant fall. The long term treatment (85°C, 729 hours) caused as much apparent damage to methionine as did the short, high temperature treatment (115°C, 27 hours) but considerably less damage to the lysine.

Segal and Motoc (1970) reported that during the heat treatment of green peas important losses of essential amino acids occurred and the amount of loss depended upon the heat treatment, with the largest losses found in arginine, lysine, histidine and methionine. They also found that the presence of glucose in the added brine increased the losses. However, sterilization of peas in cans did not affect the biological value of the proteins significantly because the losses in essential amino acids were low due to short duration of the high temperature process.

Molina et al. (1975) investigated the effect of storage, soaking time (0,8,16 and 24 hours), and cooking

time (10,20, and 30 minutes at 121°C and 15 psi pressure) on the nutritive value of black beans. They reported that cooking time had a statistically significant ($P < 0.05$) effect in lowering the protein quality of the beans processed either immediately after harvesting or after 3 and 6 months of storage. They showed that the total protein content was unaffected either by storage or by any of the processes evaluated; but the methionine and available lysine content of the processed samples tended to increase with storage, independent of the process to which they were subjected.

Braham et al. (1965) found that the optimum cooking temperature for pigeon peas (Cajanus Cajan) was 20 minutes at 121°C and 16 pound pressure and a longer heating time lowered the nutritive value of the beans.

With respect to cooking periods, Bressani et al. (1963) have recommended autoclaving at 16 psi and 121°C for 10 to 30 minutes for black beans (Phaseolus vulgaris). Heating for longer periods resulted in a decrease in the nutritive value of the protein, due to changes in the essential amino acid content of the bean, specially in the content of available lysine, which decreased proportionally to the increases in cooking time.

Miller (1973) studied the effect of processing on the nutrient retention and the PER value of pinto bean products. Three processing methods were investigated:

(a) instant drum-dried bean powder processed in water (the regular method and by prior HCl treatment); (b) cooking for two hours at 210°F, and (c) retorting for 45-90 min. at 250°F (soak-blanched canned beans). They found no difference in the PER values of these bean products.

Zinc Supplementation of Navy Beans

The statements have been made "that a relationship exists between zinc and the utilization of phytate containing plant seed proteins and that when properly supplemented with zinc these proteins were equal in quality to animal proteins" (Oberleas and Prassad, 1969); and "the reluctance on the part of many nutritionists to supplement adequate zinc in experimental diets has resulted in the belief that plant seed proteins are inferior--when in fact they may be quite comparable to animal protein" (Oberleas, 1973).

There are many factors that can influence the availability of zinc in the diet. The presence of chelates in the diet influences the availability. The phytate present in animal diets reportedly interferes with the intestinal absorption of zinc (Oberleas, et al., 1962). Zinc phytate complexes can be formed in the gastrointestinal tract making zinc unavailable.

The purpose of this study was to test the effect of zinc supplementation of Navy beans on the growth of weanling rats. The animals were fed the following diets at a 10% protein level.

a. casein
b. autoclaved beans
c. autoclaved beans + 3.8 ppm zinc
d. autoclaved beans + 6.2 ppm zinc
e. autoclaved beans + 16.2 ppm zinc
f. autoclaved beans + 21.2 ppm zinc
g. autoclaved beans + 0.5% D, L-Methionine
h. autoclaved beans + 0.5% D, L-Methionine + 10.9 ppm zinc

The analysis of Navy bean flour revealed a zinc content of 29 ppm. The casein used in this study contained 34 ppm zinc, the corn starch 7 ppm, and no zinc was detected in the corn oil.

The results of a four-week ad libitum feeding are shown in Table 30 and Figure 10.

The highest average gain in body weight was achieved by Group G and H, in which beans were supplemented with methionine and methionine plus zinc, respectively. The lowest weight gain was for Group B, in which no zinc (as a pure salt) was added to the bean diet. The Protein Efficiency Ratio (PER) of the bean diet fortified with methionine was 2.80 which was statistically higher than the one for the casein diet (PER = 2.50). Further

TABLE 30.--Effect of Methionine and Zinc Supplementation of Autoclaved Navy Beans on the Growth of Rats.

Group	Protein Source	Zinc Content ppm	Weight Gain (g)	Protein Intake (g)	PER[3]	Adjusted PER[3,4]	% PER Casein[5]
A	Casein[1]	15.1	114.3	33.7	3.39±0.11	2.50±0.08	100[a]
B	Aut. Bean[2]	16.6	48.3	26.7	1.81±0.09	1.33±0.07	53[b]
C	Aut. Bean[1]	20.4	49.5	25.8	1.92±0.07	1.42±0.05	57[c]
D	Aut. Bean[1]	22.8	53.4	28.3	1.89±0.06	1.40±0.04	56[c]
E	Aut. Bean[1]	32.8	60.3	31.5	1.91±0.08	1.41±0.06	56[c]
F	Aut. Bean[1]	37.8	51.2	26.9	1.90±0.07	1.40±0.05	56[c]
G	Aut. Bean[2] +0.5% Met.	16.6	152.0	40.1	3.79±0.05	2.80±0.04	112[d]
H	Aut. Bean[1] +0.5% Met. +10.9 ppm Zn	27.5	153.9	40.0	3.85±0.04	2.84±0.03	114[d]

[1]Zinc was added as $ZnSo_4 \cdot 7 H_2O$.

[2]Zinc was not added as a pure salt.

[3]$\bar{X}$ ± SEM

[4]Corrected for casein = 2.50.

[5]Values followed by the same letter are not statistically different at the 5% probability level.

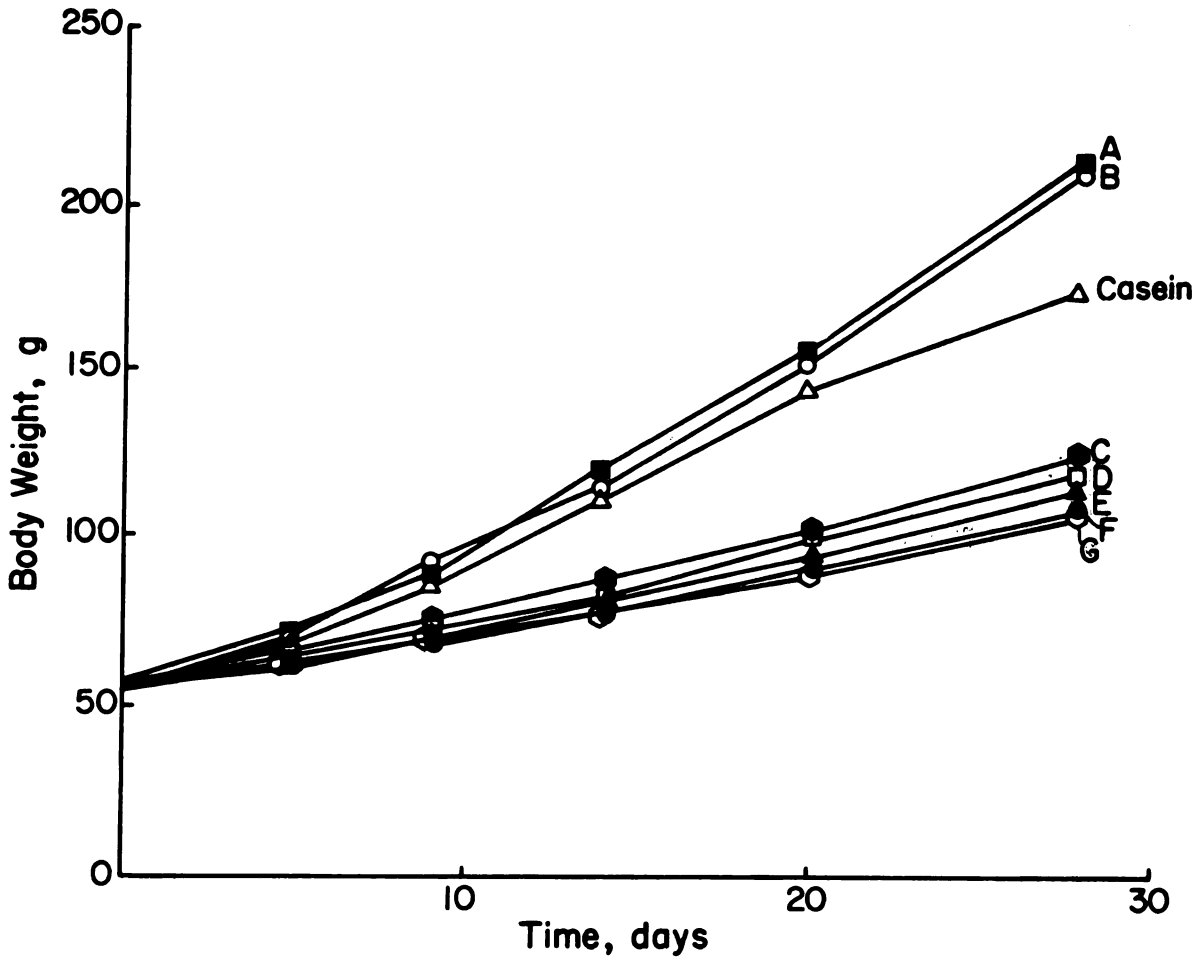

Figure 10. Growth curves of rats (av. of 10) fed bean diets supplemented with Zn and D,L-methionine: A, beans + 0.5% D,L-methionine; B, beans + 0.5% methionine + 10.9 ppm Zn; C, beans + 16.2 ppm Zn; D, beans + 6.2 ppm Zn; E, beans + 21.2 ppm Zn; F, beans + 3.8 ppm Zn; G, beans; and casein.

supplementation of this diet with zinc increased the PER value only one percent (PER = 2.84) which was not statistically significant at the 5% probability level.

The beans alone provided about 14 ppm zinc to the rat diet. An increase of 6% in PER was observed with an addition of about 6 ppm zinc to the diet. However, no indication of improvement, as determined by gain in body weight and PERs, was observed by addition of graded levels of zinc up to a total concentration of about 38 ppm. This can be seen in Figure 11 which shows the percent PER of bean proteins, relative to casein equal 100, as a result of increasing zinc content of the diets. It is apparent that maximum rat growth was obtained on a diet in which Navy beans were the only source of protein with a zinc concentration of 19.8 ppm.

However, when the bean diet was supplemented with methionine the growth rate increase was 110% in terms of PER. Furthermore, the very modest additional growth obtained by supplementing the bean diet with zinc disappeared when the bean protein quality was upgraded by addition of methionine. Therefore, the contention that addition of zinc can upgrade the quality of a plant-seed protein to that of animal protein (Oberleas and Prasad, 1969) is invalid, at least in the case of Navy bean protein and when protein efficiency ratio is used as an index of protein quality. On the other hand, this

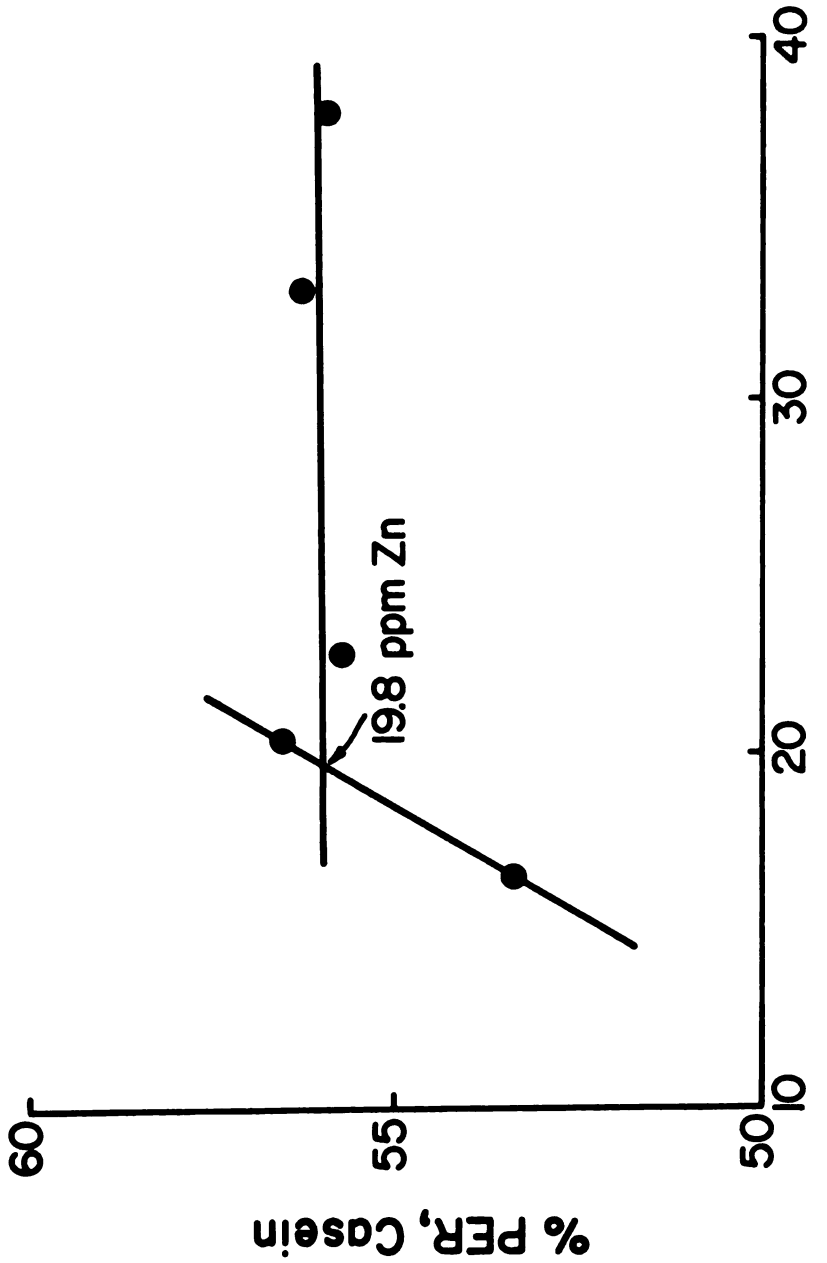

Figure 11. Protein efficiency ratios (PER) of bean diets supplemented with various quantities of Zn. The PER of a complete casein diet was set at 100.

experiment clearly demonstrates the great supplementation value of methionine for Navy bean protein which is severely deficient in this amino acid.

Oberleas and Prasad (1969), on the basis of an experiment in which pure phytic acid was added to a soy protein diet, had concluded that "a relationship exists between zinc and the utilization of phytate containing plant seed proteins and that when properly supplemented with zinc these proteins were equal in quality to animal proteins."

The aim of this study was to investigate the effect of zinc supplementation to the diets in which the phytic acid present was the one naturally occurring in the Navy bean flour. Our results agree with those of Forbes and Yoke (1960) who showed that 17.8 ppm zinc resulted in maximum growth rate when soy protein was the sole source of protein in rat diets. In fact, if the glucose used in the soy protein experiment contained zinc at a level close to that of the corn starch used in our experiment, the optimum zinc value of Forbes and Yoke would have been even closer to ours (19.8 ppm Zn).

Antunes (1975) showed that the amount of zinc absorbed by the rats was essentially the same for the Navy bean diets with or without addition of 55 ppm zinc.

The bean flour contained 1.3% phytic acid (Lolas and Markakis, 1975). All diets in which autoclaved bean

flour was the sole source of protein contained 0.6% of naturally occurring phytic acid. Assuming that the growing rat requirement for zinc to be 12 ppm in a 90% dry diet (NAS, 1972), the bean diet necessitated the presence of an additional 8 ppm zinc. If all of this additional zinc was chelated by phytic acid of the beans, and made unavailable to the rat, only 0.002% of the phytic acid was used in the binding of zinc.

SUMMARY AND CONCLUSIONS

The amino acid composition of Navy bean (Phasealus vulgaris L.) flour of the Sanilac variety and sesame seed (Sesamum indicum L.) defatted flour was determined by the Beckman Model 120°C amino acid analyzer.

The supplementation effect of Navy bean protein with sesame protein was studied in a series of experiments. Mixtures of Navy bean flour with defatted sesame flour were prepared.

All bean flours used in these experiments were autoclaved for ten minutes at 250°F (121°C). Diets were prepared containing the following protein ratios: all bean protein; all sesame protein; 87.5 bean protein to 12.5 sesame protein; 75 bean protein to 25 sesame protein; and 50 bean protein to 50 sesame protein. Protein quality of the diets were evaluated by the Protein Efficiency Ratio (PER), Net Protein Ratio (NPR), and slope-ratio methods. The FAO/WHO group (1965) procedure was used to calculate protein scores based on the FAO/WHO reference pattern (1973). The Modified Essential Amino Acid (MEAA) indices (Mitchell, 1954) of the tested diets was also computed using the same reference pattern (FAO/WHO, 1973).

The PER for the bean diet compared to 100 for that of casein, was 62, and 48 for the sesame meal. For the 87.5:12.5, 75:25, and 50:50 mixtures of beans:sesame flour, the PER's were 72, 90, and 92%, respectively. The NPR values for beans (B), sesame (S), B:S (87.5:12.5), B:S (75:25), and B:S (50:50) were 64, 50, 69, 90 and 92%, respectively, compared to 100 for casein. The Relative Protein Value (RPV) and Relative Nutritive Value (RNV) from slope-ratio techniques were claculated when the corresponding lactalbumin values were set at 100. The RPV for the tested diets were, 57, 52, 67, 71 and 74% respectively. The RNV were, 54, 52, 66, 68 and 74%, respectively.

The protein scores (PS) of Navy beans, sesame meal and mixtures of Navy beans:sesame flours with protein ratios of 87.5:12.5, 75:25 and 50:50, were 48, 50, 60, 72 and 78, respectively. The MEAA indices were 88, 78, 89, 89 and 88, respectively.

The protein values of the tested diets from rat bioassays and chemical methods were compared. The correlation coefficients between the evaluated biological methods were high with an average of 0.96. The correlation coefficient between the chemical methods (PS and MEAA indices) was low ($r = 0.50$), and of the two methods tested, only protein score gave a good correlation with the biological assays ($r = 0.94$).

The supplementary effect of whole egg on the Navy bean protein was also studied, and the protein quality of the beans improved by the presence of egg protein in the diet. The mixtures of beans and egg powder with three different protein ratios were prepared. The PER values relative to casein 100, of the Beans (B), B:Egg (97.5:2.5), B:Egg (95:5) and B:Egg (90:10) were 58, 65, 67 and 68 respectively.

In the second series of the experiments, the protein quality of the Navy beans subjected to different processing methods was estimated. The processing methods evaluated were: Canning with or without sugar, home cooking and autoclaving. The PER, methionine availability (growth assay with wealing rats) and lysine availability (reaction with 1-fluro-2, 4-dinitrophenol benzene) were used to estimate the protein quality of the processed beans. The PER values (compared to casein = 100) for the canned beans, canned beans with 1.5% sugar in brine, home cooked beans and autoclaved beans were, 58, 56, 56 and 60% respectively; the methionine availability (compared to crystalline methionine equal to 100) were, 49, 42, 48 and 50%, respectively; the lysine availabilities were, 93.3, 93.9, 97.5, and 97.4% respectively. It was concluded that the evaluated processing methods did not impair the protein quality of the Navy beans as indicated by the PER, methionine availability and lysine availability values.

A study was conducted to determine if zinc supplementation can upgrade the quality of the bean protein. Increasing quantities of zinc were added to 10% protein diets in which the sole source of protein was autoclaved Navy beans. The concentration of zinc in the diets varied from 16.6 to 37.8 PPM. These diets were compared to a standard casein diet in order to determine PER values. A very small increase in growth (6% in PER) was observed when the natural Zn content in the bean diet (16.6 ppm) was raised to 20 ppm zinc by adding $ZnSO_4 \cdot 7H_2O$. Further supplementation of the bean diets with zinc did not promote the growth. Supplementation of the same diet with 0.5% D, L-methionine resulted in a much greater increase in growth rate (110% in PER). Furthermore, the very modest additional growth obtained by supplementing the bean diet with zinc disappeared when the bean protein quality was upgraded by adding methionine.

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