THE PROTEIN EFFICIENCY RATIO OF SEVERAL RICE/SOY COMBINATIONS

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INTRODUCTION

Protein-calorie malnutrition is increasingly prevalent in today's world because of rapid population growth without a concomitant increase in the world food supply (1,2,3,4,5,6,7,8). Conventional sources of protein, in particular that of animal origin, are too expensive for the large majority of the populations in developing countries (2,8). Therefore, these populations generally derive up to 80% of their protein from cereal grains, which are both less expensive and more available (1,6,7,8). However, not only is the quantity of protein in cereals low, but the quality of cereal protein is often poor (4,7,9,10,11,12). Efforts to solve the problem of protein-calorie malnutrition should thus be aimed at not only increasing the quantity of protein available, but also the quality of current protein sources.

Although many methods for increasing the world protein supply are under investigation, one of the more feasible solutions is protein complementation. This method is advantageous in that it takes two readily available incomplete protein sources and combines them in a particular ratio to produce a product which has a higher quality protein than either source alone (12,13,14). The combination of rice flour and soybean flour, which results in a higher quality protein was the focus of this study. Rice and soybeans were chosen for several reasons. Rice is used in many countries as the major source of both protein and energy (15,16,17,18,19). However, rice is not considered to be a high quality protein when consumed

without other foods because of the limiting amino acids lysine and threonine (11,17,19,20,21,22,23,24).

Legumes are often used to supplement cereal-based diets of low income families because the amino acid patterns are complementary (1,6,26, 27,28,29,30). Soybeans are higher than rice in total protein (9,28,31) and lysine, although they lack adequate methionine and cystine (8,9,11,20, 21,31,32,33,34,35,36,37). In addition, soybeans have a high fat content, which may help to solve caloric deficits, as well as to spare protein in a marginally adequate diet (9,11). Not only are soybeans available and utilized in rice producing areas, but there are many different soybean products such as soy milk, flour, grits and meal which could be incorporated into a rice diet (34,38). There is currently no data available describing the ratio of soybeans to rice that produces the highest quality protein for human diets.

PROBLEM STATEMENT

With this in mind, the questions to be answered in this study were: Do rice/soybean combinations support better growth in weanling rats than either rice or soybeans alone? What is the particular ratio of rice flour to soybean flour which produces the highest protein efficiency ratio (PER) in weanling rats? Do chemical score evaluations accurately predict PER values in weanling rats?

REVIEW OF LITERATURE

Protein complementation of cereals and legumes has been shown to produce high quality protein products (6,13,39). The adequacy of a protein in a diet depends not only upon its concentration but also upon its quality (2). An understanding of the concept of protein quality is requisite to an evaluation of protein complementation studies. Protein quality has been defined as "an attribute which is dependent upon the amino acid profile of a protein and its amino acid availability relative to the requirements for essential amino acids and non-specific nitrogen by the target species" (40). Most protein sources do not have an essential amino acid pattern that meets amino acid needs when given in minimum physiological amounts (13). The protein source may have specific amino acid deficiencies. On the other hand, most protein sources may also have relative excesses of other specific essential amino acids in relation to the needs of the individual (13). Both deficiencies and excesses of essential amino acids result in a decreased efficiency of utilization of protein sources. High quality protein sources contain an essential amino acid pattern which closely matches the amino acid needs of the individual.

Many *in vitro* indices are available for the determination of protein quality (24,40). The chemical score is a simple index of protein quality which involves a comparison of the amino acid composition of a test food with an amino acid reference pattern (24) or with the amino acid pattern of a high quality protein such as egg (41). The amount of each amino acid

in the test protein is expressed as a percentage of the amount in the reference pattern (24,41,42). The lowest percentage for any of the essential amino acids designates the limiting amino acid and gives an estimate of protein quality.

The chemical scores of various rice flour/soy flour combinations are listed in Appendix A. Amino acid composition data for both rice (43, 44,45) and soybean (43,46) flours was taken from several sources and averaged. The chemical scores were determined using these averages, with egg protein used as the reference standard. Whole egg and suggested reference patterns have chemical scores equal to 100. The chemical score of casein, which is used as a control protein source in protein quality bioassays, has been determined to range from 91.4 to 92.2 (24). However, one study found casein to have a CS of 61.0 (21). The investigators believed that this low value was secondary to the limiting amino acids methionine and threonine. The chemical score of rice has been calculated to range from 52.0 to 68.2, depending on the amino acid reference pattern used (20,24).

Although chemical scores have been shown to be good predictors of protein quality (1,13,21,23,24,39,42,47), it is usually recommended that *in vivo* protein quality tests also be performed. Many problems are associated with using CS as the sole index of protein quality. With CS determinations, no account is taken of the biological availability of the amino acids in a protein source (9,40,42). According to Evans and Witty, "Overall amino acid nitrogen digestibility may differ from total nitrogen digestibility because the non-amino acid nitrogen may be absorbed at a

different rate than that of the amino acid nitrogen" (40). In addition, although a protein may lack a certain essential amino acid, its supplemental effect on a complete diet is not accounted for by chemical scores (40). Further, it has been recently discovered that living organisms have an ability to adapt to low levels of most amino acids (48), most notably lysine (48,49). The rate of catabolism of lysine depends upon its availability. Catabolism of lysine is diminished when lysine intake is low, resulting in its conservation and reutilization (49). Samonds and Hegsted state that "lysine deficient animals will survive for months, whereas threonine deficient animals are moribund within a few weeks" (49). The ability to adapt to low lysine intakes may explain why human populations perform better on high cereal diets than expected from protein quality evaluations (48). Chemical scores for protein may indicate that a protein's quality is lower than it actually is because it does not account for this conservation mechanism in humans.

Other problems with chemical score determinations relate to specific amino acid needs and the accuracy of amino acid composition values for proteins. If egg is used as the reference standard, CS does not account for the amino acid needs of the target species (42). Several amino acid reference patterns are currently available (National Academy of Science (NAS), FAO, whole egg). Chemical scores will vary depending on the reference pattern used (24). The widely different CS values for rice mentioned earlier are the consequence of different amino acid reference patterns. CS also does not consider the difference between maintenance and growth needs for amino acids in a living organism (40,49). Finally,

tabulated amino acid composition values of samples vary widely, and may lead to erroneous conclusions about the sample (24,40). Amino acid composition may also vary with the type and degree of processing the food source is subjected to (1). It is thus evident from the preceding discussion than an in vivo method of protein quality determination is also necessary (1,13,21,39,48).

Methodology for protein quality determination is in a state of flux, and there are many arguments over the most appropriate assay methods and their benefits and limitations (23). Three broad categories of protein bioassays are in use today: growth methods, nitrogen balance methods and indirect methods (10). A listing of the various methods contained within each category appears in Table 1. Each method has specific advantages and disadvantages inherent in its design. Protein efficiency ratio (PER) was chosen for this study because it is presently the officially recognized (50,51,52), most widely utilized and simplest bioassay procedure available for the evaluation of protein quality.

In the PER method, groups of weanling rats are fed diets containing 10% of the test proteins being studied for 28 days. The control group is fed a diet containing 10% casein for the duration of the study. At the end of the assay period, PER is determined by dividing the total weight gain for each diet group by the total protein consumption of each group. The PERs of the test groups are then divided by the PER of the casein group, and multiplied by a standard casein PER of 2.5 (42,48). This "correction factor" is designed to standardize the PER method. Casein has a PER of 2.5, which denotes a good protein quality. An average PER for

egg protein is 3.92 (43), which indicates an excellent protein quality. Although the PER method has become standardized, there are still some problems associated with its use.

TABLE 1

CLASSIFICATION OF PROTEIN BIOASSAYS

Category	Methods
Growth	Protein efficiency ratio (PER) Net protein ratio (NPR) Relative net protein ratio (rNPR) Relative protein value (RPV)
Nitrogen balance	Apparent digestibility (AD) True digestibility (TD) Net protein utilization (NPU) Apparent net protein utilization (aNPU) Biological value (BV)
Indirect	Liver nitrogen

SOURCE: Njaa, L.R.: Biological methods for the evaluation of protein quality. In Nutritional Evaluation of Cereal Mutants. Vienna: Int. Atomic Energy Agency, 1977, p. 60.

The major criticism of the PER method is that results have been shown to vary with the food intake of the animals (10,40,49,53). Food intake varies with the acceptability of the diet. Diets which are poorly accepted by the test animals will have lower PER values, even though their true protein quality may be higher. Net protein ratio (NPR) attempts to correct for variation in food intake by including a term for the use of dietary protein for maintenance (10,49). In the NPR method, an additional group is given a protein free diet. The weight loss of this group is

divided by the protein levels of the test diets and this fraction is then subtracted from PER values. Relative NPR is basically the NPR results standardized by the NPR of a control protein source (50). Both methods are considered to give similar results. Although NPR is considered an improvement over the PER method (40), it has been shown that "the efficiency of protein deposition above maintenance is not linear, and that the adjustment is not as substantial as was originally suggested" (40). Additionally, the NPR method is not widely used; therefore, data for comparison between studies is not available.

Another objection to the PER method is that the PER response varies with the protein level of the diet, i.e., PER is a dose-dependent response (10,40,49,53). Samonds and Hegsted (14,49) believe that the dose-response relationship is curvilinear at low levels of intake and linear at higher levels. The degree of curvature depends upon which amino acid is most limiting in the protein being studied; lysine deficient proteins have greater curvature as the amount of protein is increased because at low levels of intake lysine is conserved (23,49,53). Animals appear to make more efficient use of diets low in protein or low in protein quality (14), thus causing the curvilinear response.

Two slope-ratio assay methods have been designed to account for the dose-response relationship: relative nutritive value (RNV) and relative protein value (RPV). These methods are designed to measure the slope of the dose-response relationship for individual proteins in the region of protein intake where the relationship is linear, "from approximately the maintenance level to the level at which protein is no longer limiting" (49).

The slopes of the test proteins are then compared to the slope of a lactalbumin standard diet (23,49). Although these methods are believed to correct for the problem of varying protein levels, and are considered by some researchers to be the best assays available (48,49), they require an advance knowledge of a protein's quality so that approximate levels of protein can be selected for the study (49). These methods are more precise than necessary for a simple ranking of protein mixtures. Once again, there is little data available on the RPV or RNV values of protein sources.

A third objection to the PER method is that the composition of the weight gain may vary. In other words, weight gain cannot be assumed to represent proportional gain in body protein under all conditions (10,20, 40,48,49,50). Methods which precisely define nitrogen retention such as net protein utilization (NPU) or biological value (BV) are therefore likely to yield more absolute values of protein quality. BV is a measure of nitrogen retained for growth or maintenance and is expressed as nitrogen retained divided by nitrogen absorbed (40,42,48,49). NPU is equivalent to the BV multiplied by the digestibility of the food protein; therefore, it measures both the digestibility and the biological value of the amino acid mixture absorbed from the food source (40,42,48,49).

These nitrogen balance methods have been criticized on the basis that they also assume a linear relationship between the amount of protein consumed and protein quality. Because they are usually measured at suboptimum levels of protein intake, they tend to overestimate the quality of poor quality proteins. The degree of efficiency of dietary utilization at these levels of intake is greater than when the intake is just

sufficient to maintain nitrogen balance or growth (1,23,40,48,49). At such low levels of intake total nitrogen, or some other amino acid besides lysine may cause results not typical of the protein (23). One of the other faults of the nitrogen balance methods is the assumption that the endogenous compartment of nitrogen metabolism is not influenced by the quality or quantity of protein in the diet (42). It is possible that alterations in dietary protein may alter the endogenous nitrogen compartment. These methods tend to be tedious, and it has not been proven that they are better indices of protein quality than other bioassay methods.

A final major objection to the use of growth methods in general is that they involve measurement of a parameter that is not solely dependent upon the quality of the protein in the diet (40,42,48,49). Other factors which may effect protein quality are the fiber content of the diet or the presence of antinutritional compounds (54). These may not only effect growth, but also the utilization of other nutrients. However, attempts can be made to keep the fiber content of the test diets equal, and to inactivate antinutritional compounds.

Most of the other criticisms of the PER method can be allieviated by the use of a standardized procedure, such as that of the Association of Analytical Chemists (AOAC) (55). This method specifies that 21-28 day old weanlings must be used because results can be affected by the stage of growth of the animal (40,51). Because PER has been shown to be influenced by both the sex and the strain of the animals used (47), the AOAC method specifies that male animals of the same species be used. PER results also vary with the duration of the assay period (47,40); the

longer the period of time, the lower the PER values. The standardized method not only specifies a length of 28 days for the assay period, but it also specifies an acclimation period of between three and five days. Diets are standardized for vitamin, mineral, fiber, moisture, protein, fat and carbohydrate contents (20,55). Additionally, all conditions of environment are specified by the AOAC procedure. The final means of decreasing variability of results is the use of ANRC reference casein as the reference protein source (47,50,51,55). ANRC casein is blended and exhibits low variability in amino acid composition (51). Although many criticisms of the PER method exist, most research has demonstrated that it can rank proteins according to their quality (40,49,52).

Although the AOAC method for PER determination specifies a level of 10% protein in the diet (55), many research studies have been performed with lower or higher levels of protein in the diets. One main reason for this deviation is that it is impossible to attain a level of 10% protein when cereals of low protein content (5-8%) such as rice or corn are used as the test protein source (23). Most research on the optimum level of protein in test diets shows that a 10-12% protein level produces a maximum growth response (13,56,57,58). Some estimates of protein needs for maximum growth of rats are as high as 14-18% protein (59,60).

Loosli (61) estimated that for rats under 125 grams, 20% protein was the minimum requirement. However, more recent recommendations of protein levels for PER experiments range from 8-10% protein (49,54). McLaughlan et al. (50), when performing an interlaboratory comparison of

PER, NPR, rNPR, and RNU methods, used a level of 8% protein for the test diets because "high quality proteins such as egg and casein show peak PER values at about 8% protein". PER studies on rice alone, and rice combined with other foods, generally used a range of 5.27-6.0% protein in the test diets (6,7,17,62). Hegsted and Juliano (23) reported that PER responses for rice diets began to decrease after the protein level in the diets reached 10%.

Carcasses and organs of animals involved in PER studies may also provide insights into the quality of protein sources. Juliano (18) reported that carcasses of rats fed rice diets with 5% protein or less had generally lower nitrogen content, but higher crude fat content, than carcasses of rats fed casein diets. Rosenberg and Culik (22) demonstrated that liver protein increased and liver fat decreased as the quality of a rice diet was improved by lysine supplementation. However, Morrison and Campbell (47) concluded that liver weight was not affected by the quality or quantity of protein fed. Finally, Jansen (20) stated that offspring of rats fed white bread fortified with lysine had greater brain weights than those offspring of rats fed unfortified bread. Thus, it is possible that brain, heart and liver weights may be affected by the quality of protein fed to weanling rats.

Protein quality determinations in animals are important in human nutrition because they attempt to predict the following (40):

1) The effectiveness by which a protein supplies amino acids to meet requirements for a stated function
2) The optimum levels of protein needed for a given function
3) Changes in proteins that may occur due to plant breeding or processing procedures

4) How a protein will function to supply the given amino acids in a complete diet.

Despite the inherent problems associated with comparing the results of animal studies to human beings (53), it appears that in most cases results from rat bioassays correlate with human nitrogen balance studies (13,53). Howe et al. (7) state that there appears to be a good correlation between the amino acid requirements for humans and rats, except that the growing rat has higher requirements for total sulfur containing amino acids and lysine. Another small difference is that arginine appears to be essential for rats, but not for humans (62). In contrast to Howe, Bender states that the lysine requirements of the growing rat are approximately equal to the lysine requirements of humans on a "per weight" basis (53).

Other studies report that the amino acid requirements differ for growth and maintenance in both rats and humans (40,49,53). According to Bender (53), "It is not known whether factors that affect the rat assay, such as conservation of amino acids at low levels of intake, are similar in man". Bodwell (52) and Torùn (31) believe that rat assays do not consistently provide accurate estimates of protein nutritive value for humans. They believe that rat assays tend to underestimate the protein quality for humans (31,52), especially in the case of vegetable proteins where methionine is the limiting amino acid (31). Hegsted and Juliano (23) state that proteins low in lysine appear to be much more efficient in maintenance than the promotion of growth. "It is probable that the levels of utilization of protein from milled rice are higher for adult subjects than indicated by the rat assays" (23). In spite of this

conflicting evidence, Bender (53) states that rat assays are acceptable because they do not have to be any more precise than to classify proteins as poor, moderate or good. Use of these protein sources will differ with each human being.

Proteins are needed by man for a variety of purposes: growth, maintenance, pregnancy, lactation and restoration of losses caused by damage or disease. Each purpose requires different proportions of amino acids. A protein of high quality for one purpose may not be good for another (53). Because of this, many researchers feel that the evaluation of proteins should be determined under the conditions of their potential use (14,42). Testing should also consider the adequacy of the total diet in relation to other nutrients, environmental conditions, and age and physiological state of the recipient (24). For these reasons, the protein quality testing of foods in rats by growth methods should be considered as only part of an overall evaluation scheme which also includes nitrogen balance and toxicity effects in animals, and nitrogen balance in human beings (63). Other tests on humans, such as blood and urine constituents (31), should also be considered. Only when thorough testing of food sources in both animals and humans is complete, will their true protein quality be established.

Rice, as a major staple food of the world population, has been studied extensively. Rice contains approximately 7% protein (18); however, it is deficient in lysine and threonine (11,17,19,20,21,22,23,24,25). Although rice is a good source of B-vitamins, its mineral content is low (64). The digestibility of milled raw rice approaches 100% in rats, and decreases upon cooking to approximately 89% (64). Although,

rice may be able to supply protein requirements for maintenance, it is unable to support growth because of the lack of lysine at a time when lysine requirements are increased for tissue synthesis (9). The PER value of rice has been determined to range from 1.32 to 2.3 (average=1.98) (6,13, 17,24,25,62) when fed at a level of approximately 6% protein in the diet. It is known that the PER, and thus the nutritive value of rice in human diets is improved by its consumption with other foods containing a complementary amino acid pattern (17).

Earlier studies on the improvement of the nutritional quality of rice protein were directed towards the addition of lysine and threonine to rice flours (15,22,62,65). In 1951, Pecora and Hundley (15) experimented with supplementation of diets containing 90% rice flour with various amino acids. Supplementation with lysine only did not improve the growth response of the rats; however, the addition of lysine and threonine resulted in a threefold increase in the growth response. Harper et al., in 1955, repeated the previous study, with the addition of a determination of liver fat content (65). The conclusions of this study were that although the addition of lysine and threonine improved the growth response of rats on rice diets, the liver fat content was not significantly decreased until large amounts were added. Supplements of other essential amino acids did not decrease liver fat deposition. Kik, in 1956, confirmed the previous results of improved growth with the addition of lysine and threonine to milled rice diets, and further noted an additional supplementary value of methionine when added to milled rice in the presence of lysine and threonine (62).

In 1957, Rosenberg demonstrated that a rice diet could be improved with lysine and threonine supplementation only (22). Bressani and Valiente (17) confirmed the earlier results of Harper that lysine and threonine supplementation significantly improved growth and decreased liver fat in animals fed rice diets. In the late 60's, when the use of the PER method became more widely utilized, Howe et al. (1967) studied the effects of lysine supplementation on six different varieties of rice (7). The average PER of the rice diets was 1.83 before supplementation and 2.45 after the addition of lysine. Parpia (6) in a similar study found that the addition of lysine increased the PER of rice diets from 1.94 to 2.99. Finally, Jansen (20) studied the amino acid fortification of rice with lysine, methionine and threonine in children, and concluded that these additions did not improve nitrogen retention. Once it had been established that rice diets were improved by the addition of amino acids in rats, investigators began to study the effects of other protein sources on rice protein quality.

Rice has been studied in combination with a variety of legumes and oilseeds (13,17,26,28,29,39), as well as with algae (27), chicken (19), and a variety of other foods (25). The PER's of high protein rice varieties have also been determined (18). In general, rice protein quality is consistently improved by the addition of other foods. Some foods increase the PER values of rice diets much more than others.

Two studies have been performed on the complementation of rice with animal protein sources. Kik (62) investigated the addition of small levels of perch to a rice diet containing 5.27% protein. The PER of rice alone

was found to be 1.72 and perch alone to be 2.68. However, a combination of rice plus 3% perch produced a PER of 2.85, which was better than either source alone. Lee et al. (19) studied the supplementary effect of chicken on a rice diet in young men. Nitrogen retention was not improved by the replacement of a portion of rice with 15% chicken. It was felt that the diets containing 8.0 grams of nitrogen from rice or rice and chicken supplied enough lysine so that a deficiency did not develop. The conclusion was that the protein from rice was reasonably well balanced with respect to essential amino acids when it is the principal source of protein in adult men (19).

Venkataraman studied the supplementary value of algae to rice protein (27). Rice diets were combined with algae in several different ratios. A 1:1 ratio of rice protein to algae protein improved the PER of rice from 2.46 to 2.95. Yadav and Liener studied the effects of dry roasted navy bean flour in combination with several cereal protein sources (28), including rice. A mixture of 60% of protein from rice and 40% from navy beans was better (PER=2.8) than similar combinations of navy beans and oats (PER=2.5), navy beans and barley (PER=2.4) or 100% navy beans (PER=1.5). Bressani and Valiente performed a protein complementation study of rice and black beans (17) and noted that the addition of cooked black beans improved the nutritive value of rice when bean protein replaced rice protein isonitrogenously up to the ratio of 80% rice/20% beans (PER=2.62). Finally, a review article by Swaminathan (25) reports PER studies performed on a variety of other supplementary protein sources. A combination of red gram and amaranth leaf supplying 40% of the protein

in a rice diet improved the PER from 2.09 to 2.18. It can be seen from these studies that supplementation of rice with a variety of other food sources does improve its protein quality.

Soybeans are legumes which have been shown to complement cereal based diets. Full-fat soybean flour contains approximately 35% protein, but it is limiting in the sulfur-containing amino acids methionine and cystine (8,9,11,20,21,31,32,33,34,35,37,63). Soy products are considered to be capable of meeting amino acid and protein requirements of children and adults when they serve as a major source of dietary protein (31). However, soybeans contain a variety of antinutrients such as trypsin inhibitor, phytates and hemagglutinins which decrease their quality (29, 32,34,35,37,54). The trypsin inhibition is thought to retard the demonstration of the quality of the essential amino acid pattern in raw soybeans (37). It is additionally felt that soybeans contain a bitter or "beany" flavor which decreases their acceptance (34,35). Many studies report that the trypsin inhibition is inactivated and that the beany flavor is decreased upon mild heat treatment of the raw soybeans (24,32, 34,35,37,66,67,68).

Rackis and McGhee (66) demonstrated that steam treatment of soybeans for 20 minutes increased the PER from 1.13 to 2.26. Hackler et al. (67) studied the heat treatment of soy milk protein at 121^{o}C. for varying periods of time. Treatment for 5 minutes at 121^{o}C. improved the PER from 0.65 to 2.24. Longer periods of heat treatment resulted in decreased PER values. Kellor (68) states that the PER of fully toasted soybean flour is 2.19, compared to a PER of 1.31 for raw soybean flour. PER values of

heated full-fat soy flour generally range from 2.0 to 2.45 (13,35,38). Thus, soybeans are a good source of dietary protein if properly processed.

Amino acid supplementation of soy has been investigated in a manner similar to the investigation of rice. Methionine fortification of soy has been shown to result in significantly higher PER values (34). However, Torùn et al. report that methionine supplementation of soy in infant feeding studies did not influence nitrogen retention, growth rate or serum albumin levels if adequate levels of protein were provided (31). Soybeans have also been extensively studied and reported as supplements to cereal based diets (11,13,25,36,37,69,70,71,72).

Bressani et al. (11), reported that the addition of 8% soybean flour to a lime treated corn diet increased the PER from 1.0 to 2.25. The addition also increased the protein and energy content of the diet. In another study, Bressani reported that an addition of 8% soybean flour to a corn flour diet increased the PER from 1.3 to 2.6 (72). Bookwalter et al. (69) reported that an addition of 15% soy flour to degermed cornmeal increases PER from 0.2 to 2.0. Marnett et al. (71) determined that the quality of white bread (PER=1.0) could be increased (PER=1.95) by fortification with 12% soy flour. Finally, in a study with sorghum meal, Bookwalter (70) demonstrated that an addition of 15% soy flour to a sorghum meal diet increases the PER from 0.3 to 1.8. It is evident from these studies that soybeans are an effective supplement to cereal based diets. Both Bressani (37) and Swaminathan (25) have written excellent reviews of the use of soybeans in food systems.

Soy has also been studied in complementation studies with rice (26,

29,72) although not as extensively as with other grains. Kardjati (29) studied the protein quality of rice-greengram and rice-soybean diets when the mixtures supplied 10% protein to the diet(s). The actual ratio of rice to soy was not mentioned. The results showed that both mixed diets had higher NPU and PER values than the reported values of rice or legumes when fed as single sources of protein. Bressani studied the value of soybeans as an added supplement to a rice diet (72), and found that when soybeans were added to the diet at a level of 8%, the PER increased from 1.73 to 2.88. Sarwar et al. (26) determined that a ratio of 50% rice protein to 50% soy protein produced a PER of 3.16. The protein quality of rice can therefore be improved by additions of soy.

HYPOTHESIS

The hypotheses to be tested by this research were:

1. The rice flour/soybean flour mixtures will support significantly ($p \leq 0.05$) greater growth in weanling rats, as measured by PER, than either the 100% rice flour diet or the 100% soybean flour diet.

2. The PER of a mixture of 60% of protein from rice flour and 40% protein from soybean flour, at a level of 8% protein in the diet, will be significantly greater ($p \leq 0.05$) than the PER of any of the other test diets, when fed to weanling rats.

3. There will be no significant differences ($p \leq 0.05$) in mean animal organ weights (liver, heart, brain) expressed as percentages of body weight between any of the test diet groups or the control group.

4. PER values will be positively correlated ($p \leq 0.05$) with the calculated chemical scores for all rice flour/soybean flour mixtures.

METHODS AND PROCEDURES

In the initial phase of the experiment, a ten day trial period was performed using nine weanling rats. The casein standard diet, the rice flour diet and the soybean flour diet were tested on these rats for acceptability, average food consumption and spillage problems. The purpose of the trial period was to foresee and correct any possible problems which might occur during the actual PER study.

The specific PER method utilized was that of the Association of Analytical Chemists (AOAC) (55). Eight diet groups, each consisting of 12 male Sprague-Dawley weanling rats were tested. Protein was fed at a level of 8% in all diets, except for the 100% rice flour diet which contained 6.7% protein. A conversion factor of 6.25 was used to convert Kjeldahl nitrogen to crude protein (23). ANRC reference casein was used as the control group protein source (50,51,55). Seven test diets contained the following percentages of protein from rice flour and soybean flour, respectively: 100/0, 80/20, 70/30, 65/35, 60/40, 50/50, 0/100. The labels used for these test diets are included in Table 2.

Riviana Foods (Houston, Texas) provided the rice flour, which is made of ground, long grain white rice. Full-fat soybean flour, which was heated for 30 seconds at 240^{o}F during processing, was procured from Arrowhead Mills (Hereford, Texas). Appendix B contains analyses of the casein, rice flour and soybean flour, as provided by the manufacturers. AIN (American Institute of Nutrition) salt and vitamin mixtures were

obtained from Nutritional Biochemicals, Cleveland, Ohio, and were used according to the specifications of the AOAC (55). Components of the vitamin and salt mixtures are listed in Appendices C and D, respectively. Corn oil, cellulose, water and both cornstarch and sucrose were used in the amounts specified by the AOAC to complete the diets. Appendix E contains a listing of the diet components and the exact amounts which were used to formulate each test diet.

TABLE 2

LABELS USED FOR TEST DIET GROUPS

diet group label	protein sources and percentages
C	100% casein
R	100% rice
S	100% soy
A	80% rice/20% soy
B	70% rice/30% soy
D	65% rice/35% soy
E	60% rice/40% soy
F	50% rice/50% soy

The experimental animals for the PER study were 21 day old weanling, Sprague-Dawley rats, which were obtained from Timco Breeding Laboratories, Houston, Texas. An acclimation period of four days preceded the test, during which a standard diet (Appendix F) was fed to all animals. Test

group weight means differed by no more than one gram on the first day of the assay period. The rats were housed at the VAMC Animal Research facilities in Houston, Texas. Each rat was housed in an individual, screen bottom cage, with food and water provided ad libitum. All conditions of environment and handling methods were kept as uniform as possible. Rat weights were recorded on the first day of the assay period, at the end of each week, and at the end of the study. Food was changed two times each week, and weekly food consumption for each rat was recorded. On the final day, the rats were sacrificed by ether inhalation. The brain, heart and liver was removed from each rat and weighed.

Average weekly and 28 day weight gain and protein intake were calculated and recorded for each group. PER was calculated by the following equation:

$$PER = \frac{\text{avg. weight gain (group) in grams}}{\text{avg. total protein consumption (group) in grams}}$$

Each test group PER was then multiplied by the ratio of (2.5/PER of casein diet) for standardization (48,55).

Food consumption data, PERs and organ weights were statistically analyzed by one way analysis of variance. Changes in food consumption were determined by subtracting the average daily consumption for each group during the fourth week from the average daily consumption for each group from the first week. Change in food consumption was also analyzed by one way analysis of variance. In the event of differences in PERs, food consumption or organ weights ($p \leq 0.05$), a Newman-Keuls pairwise comparison was performed to determine specifically which groups were different. Regression analyses between PER, total protein consumption and organ

weights were performed to determine if any significant ($p \leq 0.05$) relationships existed. In addition, a correlation coefficient was calculated to determine if a significant relationship existed between calculated chemical scores and PERs for each diet group studied. All statistics were run on the Texas Woman's University DEC 20 computer, using the Statistical Package for Social Sciences (SPSS) software package.

RESULTS AND DISCUSSION

Results from this PER study were quite interesting and varied. A table of group means for all variables studied is included in Appendix G. For the purposes of discussion, all references to diet group will be in the form of a ratio, with the first number referring to the percentage of protein from rice flour, and the second number referring to the percentage of protein from soybean flour. The two diets containing 100% of the protein from a single source will be referred to as the 100% rice flour group or the 100% soy flour group.

The standardized protein efficiency ratio was determined to vary significantly between some of the test diet groups. As can be seen from Table 3, the mean PER for all diets tested was 2.16. The PER value for the casein group (3.25) was significantly higher than the PER values for any of the other diet groups. Table 4 contains the results of the analysis of variance, and the Newman-Keuls comparisons of the PER values. Casein is usually given a standard PER value of 2.5 (42,48); however, the PER value of 3.25 from this experiment correlates well with the PER value of 3.4 for casein reported by Bressani et al. (17).

The PER of rice flour (2.07) was not significantly different from the PER values of any of the diet combinations. It was significantly different from the PER values for the casein group, however, and from the PER of the 100% soy diet group ($p \leq 0.05$). The PER value of 2.07 for rice flour is quite similar to the average reported PER value of 1.98

(6,13,17,24,25,62). Although there is a slight upward trend in PER values as the level of protein from soy flour is increased from 20% to 50% (see Figure 1), these results were not significant. The Newman-Keuls pair-wise comparison of PERs showed that there was no significant difference between the 100/0, 70/30, 65/35, 60/40 and 50/50 diet groups. The PER of the 80/20 group was found to be significantly lower ($p \leq 0.05$) than the 65/35, 60/40 and 50/50 diet groups. The substitution of 20% of the protein with soy flour actually decreased the PER of the 100% rice flour diet (Figure 1).

TABLE 3

MEAN PER VALUES BY DIET GROUP

diet group	protein source by %	mean PER value
C	100% from casein	3.25
R	100% from rice	2.07
S	100% from soy	1.08
A	80% from rice + 20% from soy	1.96
B	70% from rice + 30% from soy	2.15
D	65% from rice + 35% from soy	2.18
E	60% from rice + 40% from soy	2.23
F	50% from rice + 50% from soy	2.30
MEAN (97 animals)		2.16

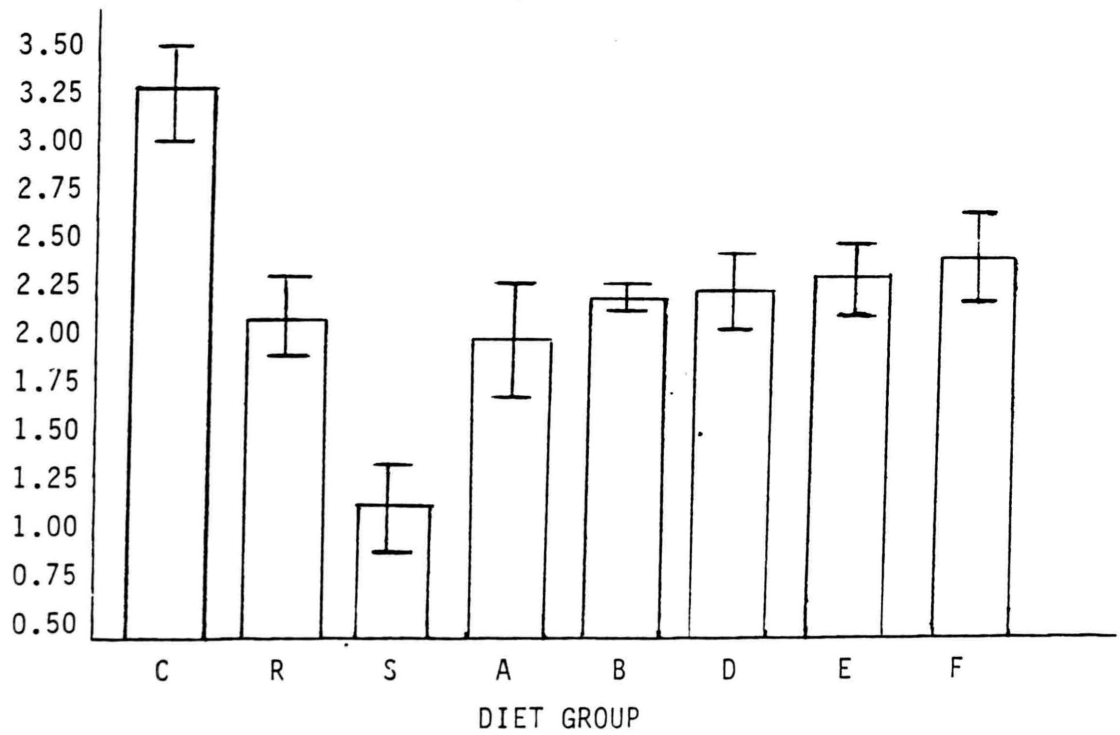
TABLE 4

ANOVA AND NEWMAN-KEULS RESULTS FOR PER VALUES

test	groups	significance
ANOVA	all	$F=98.49$, $p \leq 0.05$
N-K	C>all others	$p \leq 0.05$
N-K	S<all others	$p \leq 0.05$
N-K	B,R and A,D,E,F	N.S.
N-K	A<D,E,F	$p \leq 0.05$

FIGURE 1

TREND IN PER VALUES



Analysis of variance also showed that the 100% soy flour diet had a significantly lower PER ($p \leq 0.05$) than any of the other diet groups. The calculated PER value for soy flour of 1.08 in this experiment is much lower than indicated in the literature. Reported PERs of heated, full-fat soy flour generally range from 2.0 to 2.45 (13,35,38). Raw soybean products before heat treatment have PER values ranging from 0.65 to 1.31 (66,67,68). Hackler et al. determined that heat treatment of soy protein for 5 minutes at 121°C resulted in the greatest improvement in PER (67). The soy flour from Arrowhead Mills used in this study was roasted for 30 seconds at 220°F during processing. It is quite possible that this heat treatment is inadequate for the destruction of anti-nutritive compounds, such as a trypsin inhibitor. Other anti-nutrients and toxins such as phytates and hemagglutinins have also been shown to decrease the protein quality of soybean products (29,32,34,35,37,54) and may have had an effect on the results of this experiment.

Total food consumption (TFC), total protein consumption (TPRO) and total weight gain (TWG) for each group were averaged (Table 5) and analyzed. The mean total food consumption for all 97 animals was 314.9 grams. All of the test diet groups except for the 100% soy flour group and the casein standard group had similar food consumption totals. The casein group consumed significantly more food (TFC=357.1 gm) than the other groups, and the 100% soy flour group consumed significantly less food (TFC=244.8 gm) ($p \leq 0.05$).

These differences have two possible explanations. First, TFC may have been related to the palatibility of the diets. It is generally

felt that soybeans have a bitter or "beany" flavor which decreases their acceptance (34,35). This could explain why the total food consumption of the animals on the 100% soy diet was significantly less. A second factor which might explain differences in total food consumption is the growth rate of the animals in the different diet groups. The 100% soybean flour group had a significantly lower total weight gain ($p \leq 0.05$) than the other diet groups, and the casein standard group had a significantly higher total weight gain ($p \leq 0.05$) than the other groups. Obviously, greater growth rates in the animals would increase food consumption requirements.

TABLE 5

GROUP MEANS FOR TOTAL FOOD CONSUMPTION, TOTAL PROTEIN CONSUMPTION AND TOTAL WEIGHT GAIN

group	mean TFC*	mean TPC**	mean TWG†
casein	357.1	29.2	95.4
100% rice	310.1	20.9	56.6
100% soy	244.8	19.6	27.9
80R/20S	318.3	25.5	65.7
70R/30S	323.9	25.9	72.5
65R/35S	325.7	26.0	74.2
60R/40S	320.5	25.6	74.5
50R/50S	318.3	25.5	76.3
mean for 97 animals	314.9	24.8	68.0

*TFC= mean total food consumption (grams)
**TPC= mean total protein consumption (grams)
†TWG= mean total weight gain (grams)

Total food consumption was included in the data analysis because one of the major criticisms of the use of the PER method for protein quality determinations is that PER is a measure of not only protein quality, but also of total food consumption (10,40,49,53). These studies argue that if total food consumption is decreased, it will result in lower PER values. Thus, a good quality protein with poor acceptability and lower consumption would be assigned a lower PER value than it deserved.

Regression analysis of PER with total food consumption for all 97 animals showed a highly significant ($p \leq 0.05$), positive correlation ($R=.753$). Regression analysis results are included in Table 6. Regression analysis of PER with total food consumption within each group was also performed (Table 7). These results show significant correlations ($p \leq 0.05$) between PER and total food consumption within all of the diet groups except for the 100% soy diet group and two others. The correlation in the 100% soy diet group was only 0.413, as opposed to the overall correlation value of 0.753. This result indicates that the low PER value of the 100% soybean diet was not entirely due to a lower food consumption or poor acceptability, but also due to other factors. In general, however, the analysis of the relationship between total food consumption and PER values does confirm the theory that PER values are correlated with food intake.

TABLE 6

REGRESSION ANALYSIS OF PER WITH
TOTAL FOOD CONSUMPTION AND TOTAL PROTEIN INTAKE*

variables compared	R	F	significance
PER and total food consumption	0.753	123.19	$p \leq 0.05$
PER and total protein consumption	0.737	113.02	$p \leq 0.05$

*NOTE: Comparisons made for total number of subjects (n=97).

TABLE 7

REGRESSION ANALYSIS OF PER
WITH TOTAL FOOD CONSUMPTION WITHIN DIET GROUPS

group	R	F	significance
casein	0.738	11.99	$p \leq 0.05$
100% rice	0.576	4.98	$p \leq 0.05$
100% soy	0.413	2.06	NS
80R/20S	0.721	10.80	$p \leq 0.05$
70R/30S	0.300	0.99	NS
65R/35S	0.597	5.53	$p \leq 0.05$
60R/40S	0.686	8.90	$p \leq 0.05$
50R/50S	0.513	3.92	NS

Total protein consumption was averaged for each group and analyzed using one way analysis of variance and Newman-Keuls comparisons. The results show that the overall mean total protein consumption was 24.78 grams. The casein, 100% rice and 100% soy diet groups differed significantly from the other test diets. The casein group had a significantly higher ($p \leq 0.05$) total protein consumption, and the 100% rice and the 100% soy diet groups had significantly lower ($p \leq 0.05$) total protein consumptions. These results obviously parallel those of total food consumption.

An interesting determination however, is the correlation between PER and total protein consumption. For all 97 animals, PER was positively correlated ($R=0.737$) to total protein consumption ($p \leq 0.05$). This correlation existed for all diet groups except for the 100% soy diet group and two others. For the soy diet group, the correlation ($R=0.413$) was not significant at the 0.05 level. Although the 100% rice diet group and the 100% soy diet group had similar total protein consumption means (Table 5), the PER values varied by 100% (PER 100% rice=2.07, PER 100% soy=1.08). It can be concluded from this that some other factor in the soy rather than the total protein consumed significantly reduced its protein quality.

Changes in food consumption were measured by subtracting the average food consumption during the first week from the average food consumption during the last week. Group means for changes in food consumption are listed in Table 8. Food consumption generally increased throughout the study, with a few significant differences apparent between diet groups. The increase in food consumption for the 100% soy diet group was significantly less than the increases in the casein, 70/30, 65/35, 60/40 and

50/50 diet groups. The increases in food consumption for the casein and the 50/50 diet groups were significantly greater than the increases in the 100% rice, 100% soy and 80/20 diet groups. These results are obviously related to the results for total weight gain. As weight gain occurred within each group, a resulting change in food consumption also occurred.

TABLE 8

CHANGES IN FOOD CONSUMPTION BY GROUP*

group	change in food consumption
casein	+3.10
100% rice	+1.00
100% soy	+0.31
80R/20S	+1.02
70R/30S	+1.92
65R/35S	+2.14
60R/40S	+1.82
50R/50S	+1.46
mean for 97 animals	+1.84

*NOTE: Change in food consumption values represent increases in X grams per day.

Total weight gain patterns between the diet groups were also analyzed (Table 5). The mean total weight gain (TWG) for all 97 animals was 68.0 grams. Most of the test diets produced mean weight gains which were similar to the overall mean. However, analysis of variance showed that

the 100% soy diet group had a significantly lower mean weight gain (mean= 27.9 grams, $p \leq 0.05$), and that the casein standard group had a significantly higher mean weight gain (mean=95.4 grams, $p \leq 0.05$). The 100% rice group was found to have a mean weight gain of 56.6 grams, which was significantly higher than the mean weight gain of the 100% soy diet group, but significantly lower than any of the others ($p \leq 0.05$).

Approximately three weeks into the study, it was noticed that the vitamin mixture used (Appendix C) for the experiment did not contain choline. The suggested rat requirement for choline is approximately 750 milligrams per kilogram of the diet (73). However, this requirement is affected by the levels of vitamin B_{12} and folic acid in the diet, as well as the level of dietary fat. Diets containing over 0.8 percent methionine have been shown to prevent choline deficiency, in the absence of supplementary choline. A choline deficiency in weanling rats is characterized by a critical syndrome which occurs six to eight days after the choline is removed from the diet (73). Fatty infiltration of the liver occurs within 48 hours, and enlargement and degeneration of the kidney develops within 6 to 8 days. The animals will eventually succumb. None of these visible symptoms occurred during the entire assay period. Upon gross anatomical exam, there was no evidence of kidney or liver pathology. Although several of the animals from various groups had hair loss, this was not considered to be a problem related to the dietary intake of the animals. It is felt that the animals probably received adequate choline from the rice flour, soybean flour and the corn starch to meet their requirements.

As reviewed previously, a major criticism of the PER method is that

weight gain cannot be assumed to represent proportional gain in body protein (10,20,40,48,49,50). In order to provide insight into the type of weight gain which occurred in the various diet groups, an analysis of liver, heart and brain weights was performed. Organ weights were also expressed as percentages of total body weight. Table 9 contains group means for organ weights and ratios, and F values for the one way analysis of variance between the groups. Table 10 includes the R values and significance for regression analysis of organ ratios with PER, and total protein consumption with organ ratios.

Liver weight means were significantly different between several of the diet groups. The 100% soy diet group had a significantly lower liver weight mean ($p \leq 0.05$) than any of the other diet groups. The casein standard diet group had a significantly higher ($p \leq 0.05$) liver weight mean than any other diet group. The 60/40 and 50/50 diet groups were found to have significantly higher liver weight means than the 100% rice, 100% soy and 80/20 diet groups. These results are similar to those for total weight gain.

TABLE 9

LIVER, HEART AND BRAIN WEIGHTS AND RATIOS*

group	liver weight	heart weight	brain weight	liver ratio	heart ratio	brain ratio
casein	8.22	0.68	1.51	5.38	0.46	1.00
100% rice	5.67	0.71	1.48	4.98	0.63	1.30
100% soy	4.56	0.58	1.50	5.27	0.69	1.77
80R/20S	5.67	0.75	1.47	4.60	0.61	1.22
70R/30S	6.53	0.58	1.41	5.02	0.45	1.09
65R/35S	6.79	0.67	1.46	5.17	0.51	1.11
60R/40S	7.52	0.58	1.52	5.58	0.44	1.15
50R/50S	7.84	0.55	1.46	5.61	0.42	1.10
mean for 97 animals	6.57	0.64	1.48	5.21	0.52	1.22
F value (ANOVA)	15.44	5.24	1.00	4.11	14.03	28.55
significance	$p \leq 0.05$	$p \leq 0.05$	NS	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

*NOTE: All weights expressed in grams. All ratios represent the weight of the organ divided by total body weight. All values represent group means.

TABLE 10

REGRESSION ANALYSIS OF
ORGAN RATIOS, PER AND TOTAL PROTEIN CONSUMPTION (TPRO)

comparison	R value	F value	significance
PER and Liver ratio	+0.172	2.90	NS
PER and Brain ratio	-0.797	165.61	$p \leq 0.05$
PER and Heart ratio	-0.561	43.64	$p \leq 0.05$
Liver ratio and TPRO*	+0.126	1.54	NS
Heart ratio and TPRO*	-0.584	49.13	$p \leq 0.05$
Brain ratio and TPRO*	-0.805	174.95	$p \leq 0.05$

Analysis of liver ratio data showed that the 80/20 diet group had a mean liver ratio which was significantly lower ($p \leq 0.05$) than the casein, 100% soy, 60/40 and 50/50 diet groups. In other words, the mean liver size for rats in the 80/20 diet group, when expressed as a percentage of total body weight, was much lower. In addition, although the 100% soy diet group had the lowest mean liver weight, when expressed as a percentage of total body weight, this result was not significantly different from most other diet groups. As can be seen from Table 10, a regression analysis of protein efficiency ratio with liver ratio yielded an R value of 0.172, which was not significant at the 0.05 level. Thus, there was no correlation between the relative size of the liver and protein quality as determined by the PER method. Liver ratio was also correlated with

total protein consumption. This regression yielded an R value of 0.126, which was not significant. Therefore, total protein consumption is also not related to the size of the liver, when it is expressed as a percentage of body weight.

The mean heart weight of all 97 subjects was 0.64 grams (Table 9). The only significant differences between the groups, as determined by ANOVA and Newman-Keuls comparisons, occurred between the 80/20 diet group, the 100% rice diet group and several others. The 80/20 diet group had a mean heart weight of 0.75 grams, and the 100% rice diet group had a mean heart weight of 0.71 grams. These values were significantly higher than the mean heart weights for the 70/30, 60/40, 50/50 and 100% soy diet groups ($p \leq 0.05$). It is interesting that the 80/20 and the 100% rice diet groups contained the largest percentages of rice flour. It is possible that some factor in these diets caused an enlargement of the animals' hearts.

Heart weights were also analyzed by expressing them as percentages of total body weight. The mean heart ratio for all diet groups was 0.52%. The 100% rice, 100% soy and 80/20 diet groups, with mean heart ratios of 0.63, 0.69 and 0.61, respectively, had ratios which were significantly higher ($p \leq 0.05$) than all other diet groups. When heart ratio was correlated with protein efficiency ratio, an R value of -0.561 resulted (Table 10), which was significant at the 0.05 level. In other words, the higher the protein efficiency ratio of a diet group, the smaller the heart when expressed as a percentage of body weight. This could be attributed to either the greater body weights associated with good quality proteins, or

some mechanism whereby hearts enlarge with poorer quality proteins. Heart ratio was also negatively correlated with total protein consumption ($R=-0.584$, $p \leq 0.05$).

Brain weights and brain ratios were also analyzed for variance. There was no significant difference in mean brain weights between all diet groups. However, analysis of brain ratios did show significant differences between some of the diet groups. The 100% soy diet group had a significantly higher ($p \leq 0.05$) mean brain ratio than the means of all other diet groups. The casein standard group had a significantly lower mean brain ratio ($p \leq 0.05$). The brain ratio mean for the 100% rice diet group was significantly lower than the mean for the soy group, but significantly higher than all others except for casein ($p \leq 0.05$). The similar brain weights denote that the brain is obviously a precisely regulated organ, despite the quality and quantity of protein consumed by the animal. The differences in brain ratio means could possibly be attributed to the differences in growth between the diet groups. The groups with lower growth rates, such as the 100% soy diet group, thus had higher brain weights when expressed as percentages of total body weight.

As can be seen from Table 10, brain ratio was highly and negatively correlated with protein efficiency ratio ($R=-0.797$, $p \leq 0.05$) for all 97 animals. In other words, with better quality proteins, the brain represents a smaller proportion of total body weight. Brain ratio was also highly and negatively correlated with total protein consumption for all 97 subjects ($R=-0.805$, $p \leq 0.05$). As protein consumption increased, the brain represented a smaller proportion of total body weight. Again, these

results can be attributed to the fact that the brain is preferentially nourished in living organisms. Once the brain has developed, as total protein or protein quality are increased, other parts of the body begin to develop, and the brain represents a proportionately smaller amount of total body weight.

Finally, a regression analysis of protein efficiency ratio with chemical scores for each diet group was performed. PER was highly and positively correlated with chemical score calculations ($R=+0.86$, $p \leq 0.05$). This result supports the general viewpoint expressed in the literature that chemical scores are good predictors of protein quality (1,13,21,23, 24,39,42,47). However, although chemical scores were able to predict the single diet mixture with the best protein quality, the PER results were unable to significantly distinguish between several of the diet mixtures.

SUMMARY AND CONCLUSIONS

The first hypothesis to be tested by this study was that the rice flour/soybean flour mixtures would support greater growth in weanling rats, as measured by PER, than either the 100% rice flour diet or the 100% soybean flour diet. The results did show a significant difference in PER between the diet mixtures and the 100% soybean flour diet. In fact, each test diet had a protein quality which was significantly higher than the soybean flour diet. On the other hand, the PER of the 100% rice flour diet was not significantly different from any of the mixed diets. It was, however, significantly lower than casein and higher than the 100% soy flour diet. The PER of the rice diet was found to be quite similar to values reported in the literature.

An unexpected result was the extremely low PER value for the 100% soybean flour diet. According to the chemical scores and to PER values in the literature, the 100% soybean flour diet should have had a better protein quality than the 100% rice flour diet. However, the PER value for the soy flour diet was 100% lower than the values reported in the literature. It is possible that the soybean flour was not properly heat treated during processing. The substitution of 20% of protein with this brand of soybean flour in a rice flour diet actually decreased the quality of the rice protein, as measured by PER. The PER of the 100% rice flour diet was 2.07, which was decreased to 1.96 upon the addition of 20% soybean flour protein. This result was contrary to the expected improvement

of the rice flour protein by the addition of the soybean flour.

The second hypothesis to be tested by this study was that the PER of a mixture of 60% protein from rice flour and 40% of protein from soybean flour would be significantly greater than the PER values for any of the other test diets. This hypothesis was not supported by the results of this study. The PER of the 60/40 mixture was 2.23, which was not significantly different from any of the diet mixtures except for the 80/20 diet group. The diet groups containing 35%, 40% or 50% of protein from soybean flour had significantly higher PER values than the diet group with 20% of the protein from soybean flour. It is possible that the larger amount of lysine provided by the soybean flour helped to improve the protein quality.

As mentioned earlier, the substitution of 20% of total protein with soybean flour actually decreased the quality of the rice protein. The addition of larger percentages of protein from soybean flour did not significantly improve the quality of the rice flour. However, the addition of any percentage of protein from rice flour significantly improved the quality of the soybean flour. Although there was an upward trend in PER values as larger percentages of soybean flour were added, the results were not significant. It is felt that had the soybean flour had a protein quality similar to that reported in the literature, perhaps the results would have been as predicted. It is possible that the underprocessing of the soybean flour, and/or possibly the presence of toxic factors in the flour significantly altered the results of the study.

The third hypothesis to be tested by the study was that there would

be no significant difference in mean animal organ weights between the test diet groups or the control group. Data for organ weights was arranged into two groups. Group means were calculated and analyzed for both organ weights and organ ratios. Significant differences were found between the groups for liver weights and for heart weights, but not for brain weights. Liver weight and heart weight were affected by the weight gain of the animal, but brain weight remained constant. The analysis of the ratio of organ weights divided by total body weights revealed significant differences between the groups for each organ. Therefore, the hypothesis that there would be no significant differences between organ weights (expressed as % of body weight) was not supported by this study.

Several interesting differences were revealed by the analysis of the organs. The 80/20 diet group had a mean liver ratio which was significantly lower than the other diet groups. In addition, this diet group together with the 100% rice flour diet group had mean heart weights which were significantly higher than the other diet groups. The heart ratios of the 100% soy, 100% rice and 80/20 diet groups were also significantly higher than the other diet groups. From this evidence, it is possible to conclude that some factor associated with the poorer quality protein diets may have led to an enlargement of the heart. Analysis of the brain ratio data showed that as the quality of protein improved, the brain represented a smaller proportion of total body weight. Brain weights alone were not significantly different between the diet groups. It may be concluded that the brain is preferentially nourished during growth, regardless of the protein quality of the diet.

Regression analysis was performed between organ ratios, PER and total protein consumption. These results showed that the liver ratio was not significantly correlated with either total protein consumption or protein quality as determined by the PER method. Brain ratio was highly and negatively correlated to both PER and to total protein consumption. As the protein quality of the diet improved, or as the quantity of protein consumed increased, the brain represented a smaller portion of total body weight. Similar results were found for the correlation between PER and heart ratio and for the correlation between heart ratio and total protein consumption. Both the heart weight and the brain weight, when expressed as percentages of total body weight are related to protein quantity and protein quality. Perhaps these variables should be included in future studies on protein quality in order to provide additional information on the protein quality of the foods being tested.

The final hypothesis to be tested by this study was that the PERs would be positively correlated with the calculated chemical scores of all the diet groups tested. The results of this study supported this hypothesis. Chemical score was highly positively correlated to protein efficiency ratio values ($R=+0.860$, $p \leq 0.05$). Chemical score is therefore a valid predictor of protein quality of test diets, as determined by the PER method.

IMPLICATIONS FOR FUTURE RESEARCH

Several possibilities for future research were generated by this study. One possibility for future research would be a replication of this research with one change. A soybean flour which is known to have been heat-treated for a period of time which allows adequate destruction of anti-nutrients, such as the trypsin inhibitor, would be used instead of the brand used in this study. A similar study could be performed which tested the protein quality of the same brand of soybean flour used in this experiment after subjecting it to various forms of heat treatment. The purpose would be to identify a processing method which would result in the best quality protein possible. The same study reported in this paper could also be performed using a different method of protein quality determination. Finally, future protein quality studies could use organ weights and ratios as additional variables in order to provide additional data from which predictions of protein quality could be made.

APPENDICES

APPENDIX A

CHEMICAL SCORES*

% protein from rice flour % protein from soy flour	100 0	90 10	80 20	70 30	65 35	60 40	50 50	40 60	30 70	20 80	10 90	0 100
Amino Acids												
Isoleucine	84.6	86.1	87.8	89.3	90.1	90.9	92.5	94.1	95.6	97.2	98.8	100.4
Leucine	98.8	98.8	98.8	98.7	98.7	98.7	98.7	98.6	98.6	98.6	98.5	98.5
Lysine	52.0	56.7	61.4	66.2	68.5	70.9	75.6	80.4	85.1	89.8	94.6	99.3
Methionine + Cystine	79.1	77.0	74.2	71.8	70.6	69.4	66.9	64.5	62.0	59.6	57.2	54.7
Phenylalanine + Tyrosine	106.6	105.4	104.3	103.2	102.6	102.1	101.0	99.8	98.7	97.6	96.5	95.4
Threonine	78.3	79.5	80.8	82.0	82.6	83.2	84.5	85.7	86.9	88.2	89.4	90.6
Valine	98.8	97.2	95.7	94.1	93.3	92.5	91.0	89.4	87.9	86.3	84.7	83.2

NOTE: Chemical Score= (amino acid content (mg/gm pro) in mixture/ amino acid content in egg) x 100
Chemical score of egg = 100.
Underlined values indicate limiting amino acid and lowest chemical score for each mixture.

Appendix B

PROXIMATE ANALYSES OF RICE FLOUR, SOY FLOUR AND CASEIN*

	rice flour	soy flour	casein
protein, %	7.5	36.0	95.0
fat, %	0.75	20.0	1.5
fiber, %	0.5	4.0	0.0
ash, %	0.6	4.5	2.05
moisture, %	11.0	8.0	7.0

*NOTE: Information provided by manufacturer.

APPENDIX C

AIN-76 Vitamin Mixture

vitamin	per kg mixture
Thiamin·HCl	600 mg
Riboflavin	600 mg
Pyridoxine HCl	700 mg
Nicotinic acid	3 g
D-Calcium pantothenate	1.6 g
Folic acid	200 mg
D-Biotin	20 mg
Cyanocobalamin	1 mg
Retinyl palmitate or acetate	400,000 IU
dl- -Tocopheryl acetate	5,000 IU
Cholecalciferol	2.5 mg
Menaquinone	5.0 mg
Sucrose, finely powdered	to make 1,000 g

SOURCE: The Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies.

APPENDIX D

AIN-76 Mineral Mixture

mineral	g per kg mixture
Calcium phosphate, dibasic	500.0
Sodium chloride	74.0
Potassium citrate, monohydrate	220.0
Potassium sulfate	52.0
Magnesium oxide	24.0
Manganous carbonate	3.5
Ferric citrate	6.0
Zinc carbonate	1.5
Cupric carbonate	0.3
Potassium iodate	0.01
Sodium selenite	0.01
Chromium potassium sulfate	0.56
Sucrose, finely powdered	to make 1,000.0

SOURCE: The Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies.

APPENDIX E

TEST DIET COMPONENTS*

Component	Diet group							
	casein	100% rice	100% soy	80R/20S	70R/30S	65R/35S	60R/40S	50R/50S
Casein	86.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rice flour	0.0	900.0	0.0	812.7	711.0	660.3	609.5	507.9
Soy flour	0.0	0.0	203.2	40.6	60.9	71.1	81.3	101.6
Corn oil	78.7	40.0	39.4	67.9	66.5	66.0	65.6	65.3
AIN mineral mixture	50.0	45.0	40.8	44.3	44.6	44.7	44.9	45.4
AIN vitamin mixture	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Cellulose	10.0	5.0	1.9	5.1	5.4	5.5	5.7	6.0
Water	44.0	0.0	33.7	0.0	0.0	0.0	0.0	10.5
Sucrose	50.0	0.0	51.0	19.4	51.6	142.4	183.0	253.3
Cornstarch	51.3	0.0	0.0	0.0	50.0	0.0	0.0	0.0
Amadex*	620.0	0.0	620.0	0.0	0.0	0.0	0.0	0.0

NOTE: All components listed in grams per kilogram of diet.
Amadex is a brand of cornstarch.

APPENDIX F

DIET USED DURING THE FOUR DAY ACCLIMATION PERIOD*

Component	Amount in grams
Rice flour	2,000.0
Whole milk powder	1,280.0
Sucrose	720.0
Liver powder	80.0

*NOTE: This diet was used to feed all 97 animals prior to arrangement into diet groups.

APPENDIX G

GROUP MEANS FOR ALL VARIABLES TESTED

Diet group	Variable											
	PER	TFC	TPRO	TWG	chg FC	LWT	HWT	BWT	LR	HR	BR	CS
Casein	3.25	357.07	29.17	95.36	+1.51	8.22	0.68	1.51	5.38	0.46	1.00	91.8
100% rice	2.07	310.05	20.93	56.63	+1.48	5.67	0.71	1.48	4.98	0.63	1.30	52.0
100% soy	1.08	244.78	19.58	27.88	+0.31	4.56	0.58	1.50	5.27	0.69	1.77	54.7
80R/20S	1.96	318.33	25.47	65.74	+1.02	5.67	0.75	1.47	4.6	0.61	1.22	61.4
70R/30S	2.15	323.95	25.91	72.53	+1.92	6.53	0.58	1.41	5.02	0.45	1.09	66.2
65R/35S	2.18	325.68	26.05	74.23	+2.14	6.79	0.67	1.46	5.17	0.51	1.11	68.5
60R/40S	2.23	320.5	25.64	74.47	+1.82	7.52	0.58	1.52	5.58	0.44	1.15	69.4
50R/50S	2.30	318.25	25.47	76.27	+3.30	7.84	0.55	1.46	5.61	0.42	1.10	66.9
Mean 97 animals	2.16	314.88	24.78	67.98	+1.84	6.57	0.64	1.48	5.21	0.52	1.22	——

NOTE: PER=protein efficiency ratio; TFC=total food consumption; TPRO=total protein consumption; TWG=total weight gain; chg FC=change in food consumption; LWT=liver weight; HWT=heart weight; BWT=brain weight; LR=liver ratio; HR=heart ratio; BR=brain ratio; CS=chemical score.

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