PROTEIN QUALITY EVALUATION OF CORN TORTILLAS, WHEAT FLOUR TORTILLAS, PINTO BEANS, SOYBEANS AND COMBINATIONS OF THESE

by

Mauro Eduardo Fernando Valencia

A Thesis Submitted to the Faculty of the

COMMITTEE ON AGRICULTURAL BIOCHEMISTRY AND NUTRITION (GRADUATE)

In Partial Fulfillment of the Requirements For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 7 5

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: _____

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

_____ _____

MITCHELL G. VAVICH Date

Professor of Agricultural Biochemistry

ACKNOWLEDGMENTS

The author is deeply indebted to Dr. Mitchell G. Vavich, Dr. Bobby L. Reid, and Dr. Charles W. Weber for their cordial guidance and assistance in the planning and execution of this study. The author would like to express his deep gratitude to Mrs. Phyllis Maiorino, Mr. Angelo J. Longo, and Dr. Ye-Shan Peng for valuable technical assistance.

The author wishes to thank the professors, staff, and secretaries of the Department of Agricultural Biochemistry and Department of Poultry Science for their cooperation. Acknowledgment is also made to the National Council of Science and Technology (CONACYT) in Mexico City and The University of Sonora in Hermosillo, Sonora, Mexico, for sponsoring and supporting the author.

TABLE OF CONTENTS

TABLE OF CONTENTS--Continued

LIST OF TABLES

ABSTRACT

The purpose of this study was to investigate the protein quality of corn tortillas, wheat flour tortillas, pinto beans, corn-soy tortillas (90:10), corn-soy tortillas (80:20), corn tortillas-pinto beans (50:50), and wheat flour tortillas-pinto beans (50:50) mixtures as measured by the protein efficiency ratio (PER) and net protein ratio (NPR) methods. Protein score, apparent amino acid digestibility, and digestible energy values were determined as well.

Protein score values were calculated with and without consideration of amino acid digestibility. Protein score values showed better correlations with PER and NPR when apparent amino acid digestibility was considered.

Aside from the control diet (soybean meal + 0.05% DL methionine) the corn-soy combinations had higher PER's and NPR's than any of the other diets tested. Protein quality of wheat flour tortillas improved significantly when consumed in combination with pinto beans. No such effect was observed with corn tortillas consumed with pinto beans. PER values correlated significantly with NPR's.

The corn-soy tortilla combinations offer excellent possibilities for improving protein quality of Mexican

diets. This is important if we consider the economic restrictions of low income families in Mexico.

INTRODUCTION

The majority of people in Mexico depend on grains and a few legumes for their source of protein. Animal protein is expensive and limited to families of medium- and high-income status; therefore, protein sources such as milk, meat, and eggs are unaffordable to low-income families. These deficiencies, unavoidably, cause a serious imbalance in diet.

It was considered important to study the protein quality of the most common grains and legumes used for human consumption in Mexico, such as corn, wheat, and pinto beans; and to investigate whether various combinations of these result in improved protein quality in the diets of Mexicans.

Soybeans, which are not as commonly consumed by humans as pinto beans, were included in this study because of special nutritional and processing characteristics. The idea of using soybeans and corn as a combination was derived from the basis of the amino acid composition of the two. Corn is known to be deficient mainly in lysine, but not in methionine; soybeans are low in methionine, but are good sources of lysine. Another advantage is that soybeans can be processed exactly as corn to make a product called "tortillas." Since corn tortillas are widely consumed in

Mexico, it was felt that improvement of protein quality in this particular food would be of great dietary significance.

The combinations of corn and soybeans in this study were done in two proportions: the first combined 90% corn and 10% soybeans and the second 80% corn and 20% soybeans. The reasons for using these combinations are: (1) a higher percentage of soybeans is not economically feasible, and (2) the mill used for the process becomes too oily, and the cost of equipment maintenance rises. It must be considered that higher soybean percentages could produce a change in flavor resulting in a problem of acceptability.

The pinto bean is a very popular food item and is usually consumed with corn tortillas. However, in the northern states of Mexico, such as Sonora, wheat flour tortillas, or sometimes bread, are consumed with pinto beans. For this reason, two additional combinations were studied: corn and pinto beans in a proportion of 50:50, and wheat flour (as tortillas) and pinto beans in the same 50:50 ratio. The combinations were chosen because of personal observations and the opinions of Mexican housewives regarding common eating habits.

The combination of different grains with legumes is perhaps the most practical approach to improve the protein quality of the diets of many population groups throughout the world.

There is still much research to be done in this field considering there are sources such as milo, cottonseed meal, safflower meal, and others that have not been fully explored with regard to human nutrition.

Technical problems involved in research concerning human usage of milo, cottonseed meal, safflower meal, and so on require further investigation, but still offer great possibilities.

For the purpose of protein quality evaluation, albino mice were used to determine protein efficiency ratio (PER) and net protein ratio (NPR). Protein score, apparent amino acid digestibility, and digestible energy were also determined in this study.

LITERATURE REVIEW

Amino Acid Requirements

The unique functions in the animal body served by amino acids resulting from protein digestion are all anabolic in character. They relate to the replacement of essential tissue constituents that have been degraded in catabolic reactions, or the formation of new tissue constituents in growth. In the rapidly growing animal, the latter functions dominate the body's requirements for amino acids. In the mature animal the replacement functions may dominate the amino acid requirements, but the growth functions still persist, since some tissues continue to grow throughout life.

The amino acids needed for growth are used mainly for the synthesis of the protein molecules entering into the structure of protoplasm. This function requires the simultaneous presence in the tissues of all the amino acids that the body cannot manufacture. The absence of any one will block the synthetic process. The amino acids needed for maintenance are used mainly for the formation of creatine, carnosine, glutathione, ergothionine, thyroxine, adrenaline, and other nitrogenous tissue constituents or tissue products that are catabolized. For these replacement functions the

assortment of amino acids needed is simple and will vary from one type of synthetic reaction to the other. The absence from the diet of any one essential amino acid will block one or more of these anabolic reactions but not all. Hence a dietary protein like gelatin or zein, deficient in one or more of the essential amino acids, may be partially utilized in maintenance as McCollum and Steenbock (1912) showed many years ago in metabolism experiments on growing pigs. Gelatin has a biological value of zero for growth, since no growth will occur when gelatin is the sole dietary source of amino acids. However, for maintenance gelatin has a biological value of about 25 (Arnold and Shad, 1952; Block and Mitchell, 1946; Rhode, Perkins, and Vars, 1949) which indicates that many of the replacement reactions of maintenance do not require the amino acid entirely lacking from the gelatin molecule, i.e., tryptophan. Maintenance will also involve some protein synthesis for the growth of epidermal structures, for the replacement of red blood cells destroyed during metabolism, and probably for many other purposes, although the net aggregate in terms of nitrogen may not be large in proportion to the total replacement aggregate.

During the last 20 years many attempts have been made to determine the relative proportions in which the essential amino acids are required by the growing rat. While there have been very few attempts to determine the amino acid requirements of mice, there is no evidence that

they differ from those of the rat, and a few measurements of protein quality that have been made using mice are similar to those obtained with rats. Accordingly, a single "target pattern" has been proposed for both species by Coates et al. (1969) that will be discussed further on.

When diets are compounded using those sources of protein which are commonly available, calculations of the proportions of amino acids in the resulting mixture will show, in the majority of cases, that the sulfur-containing amino acids, methionine and cystine, are those which most seriously fall short of the "target pattern."

Reports in the literature indicate a sparing effect of some non-essential amino acids. Cystine has been found to replace about three-fourths of the total requirements for the sulfur-containing amino acids in adult rats (Womach, Harlin, and Lin, 1953). Rose and Womach (1946) and Armstrong (1955) reported that an appropriate amount of tyrosine reduced the minimum requirement of phenylalanine. Rama Rao, Metta, and Johnson (1964) also showed that the need for phenylalanine when tyrosine was in excess was reduced from 0.7% to 0.4% and the need for methionine in the presence of excess cystine was reduced from 0.5% to 0.16%.

Protein Quality Evaluation

Determination of the nutritional value of proteins is inherently nonspecific. In the case of vitamins or essential minerals one deals from the assay standpoint with the evaluation of a single essential nutrient at a time. In the assay of protein value, conversely, one must be concerned with the qualitative and quantitative adequacy of at least nine amino acids. There are also problems of amino acid balance and utilization to be considered. Thus, although blood carries amino acids to all tissues, only one of the proteins of the blood is nutritively balanced. The keratins of skin, feathers, and hair, on the other hand, which have fair balance of amino acids, are largely undigestible.

It is inevitable that different assay methods differ in classifying proteins as to nutritive value. The different assay methods have differing parameters of measurement and quite different end points. Despite the vagaries inherent in this type assay, protein evaluation methods have classified most food proteins in the same general order of adequacy. The balanced proteins of meat, milk, and eggs have been used as standards of excellence. By the same token, the major grain proteins, low in one or more essential amino acids are generally improved in feeding value by appropriate amino acid fortification. Balance of amino acids is usually achieved in man by a sufficiently varied

food intake. Balance can also be purposefully achieved, as in the case of manufactured feeds for poultry and swine, by a minor fortification of a single protein source, such as soy bean meal with methionine.

We now know the approximate amino acid needs of several species. Knowing the essential amino acid composition of a protein one can predict its feeding value with fair accuracy. One cannot predict, however, failures in digestibility or effects of processing, some of which are not revealed by composition analyses. Protein methods, which reflect true feeding value, continue therefore to provide the ultimate in biological evaluation. Indirect methods of protein evaluation confirm and complement the direct feeding methods.

Chemical Score and Protein Score

Block and Mitchell (1946) developed a method that relates the amino acid composition of proteins and their nutritive value. For this, they used the amino acid composition of egg as a standard of reference. The chemical score was calculated by the formula:

$$\text{chemical score} = \frac{\text{per cent of amino acid in test protein}}{\text{per cent of amino acid in egg protein}} \times 100$$

The amino acid with the least score was accepted as the limiting amino acid.

The chemical score has been criticized because of (1) the assumption that egg protein is ideal for the experimental animal does not always hold true and (2) the accuracy of amino acid estimation is not precise.

A modification of the chemical score method is reported by FAO/WHO (1965) as protein score, where the difference from the chemical score determination comes from computing the sulfur amino acids methionine and cystine together and the aromatic amino acids tyrosine and phenylalanine as a whole. This modification takes into consideration the sparing effects of cystine (Womach et al., 1953) and tyrosine (Rose and Womach, 1946; Armstrong, 1955).

Growth Method and Protein Efficiency Ratio (PER)

The way in which different proteins support growth in young rats has been the most general and widely used criterion of protein value. In 1919 Osborne and his coworkers introduced the concept of "protein efficiency ratio" as a refinement of the simple growth method. The weight gain per gram protein intake was measured for several proteins, and it was found that varying levels of protein in the diet gave different protein efficiency ratios. A rather definite level was found for each individual protein which produced the greatest gain per gram protein ingested. These levels for casein and a lactoalbumin were 12% and 7.9%, respectively. In general, it has been found that the

better the protein, the lower the level in the diet required to produce the highest protein efficiency ratio. This is a clear reflection of the importance of the proper nutritive balance of all the amino acids to produce optimum metabolic efficiency. In practice, however, it became fairly customary to determine protein efficiency ratios at a level of 10% of dietary protein.

The PER determination has been criticized on the basis that it can vary according to the level and type of protein in the diet. Sure (1955), for example, pointed out that the PER of wheat tended to improve with increasing protein level in the diet, rolled oats yielded essentially the same PER at 7 to 12% protein levels, and egg and milk proteins gave decreasing values with increasing protein level. By this time attention was given to the need for finding the dietary level of a given protein that would yield maximum efficiency as visualized originally by Osborne, Mendel, and Ferry (1919). PER varies with the length of the experimental period. A three-week growth experiment, for example, yields a higher PER than a four- or five-week experiment. The determination makes no allowance for maintenance. The method assumes that the gain in body weight is an indicator of protein synthesized by the body; and the PER varies with food intake.

In spite of these factors protein efficiency ratio is still a method of choice for evaluating protein quality

according to many researchers in the field of biological evaluation.

Biological Value (BV)

The second of the older methods of protein assay is biological value. The concept of "biological value" was first introduced by Thomas (1909) in terms of per cent of digestible nitrogen from a test food which was retained by the adult human. Mitchell (1923) on the other hand applied the method to the growing rat, thus including the requirements for both growth and maintenance. Mitchell et al. (1945) brought the method to an eventual high stage of precision as applied to the growing rat.

Biological value is expressed as nitrogen retained divided by nitrogen absorbed (Mitchell, 1923). It is determined by nitrogen balance and is applicable to humans as well as laboratory animals. In strict quantitative sense, BV may be expressed more explicitly as:

$$BV = \frac{I - (F - F_o) - (U - U_o)}{I - (F - F_o)}$$

where I is nitrogen intake, U is urinary nitrogen, F is fecal nitrogen. U_o and F_o are urinary and fecal nitrogen excreted when subjects are maintained on a nitrogen-free or nearly nitrogen-free diet. Biological value calculated from this equation accounts for metabolic and endogenous nitrogen

losses. If the correction is not made, that is if U_o and F_o are not in the equation, BV obtained is designated "apparent biological value." The practice of ignoring metabolic nitrogen is not uncommon, so many values for BV represent apparent biological value of proteins.

Nitrogen Balance Index

Nitrogen balance index is essentially the same as biological value (Allison, 1955). It may be determined from the slope of the line when nitrogen balance is plotted against absorbed nitrogen. More simply, nitrogen balance index may be calculated from the following equation:

$$\text{Nitrogen Balance Index} = \frac{B - B_o}{A}$$

where B is nitrogen balance, B_o is nitrogen balance when nitrogen intake is zero, and A is absorbed nitrogen. Since B_o represents metabolic nitrogen, the nitrogen balance index is a measure of dietary nitrogen retained. This method is not used as often as are some other measures of protein quality.

Net Protein Utilization (NPU)

Quantitatively NPU is represented by a simple formula:

$$\text{NPU} = \frac{\text{N retained}}{\text{N intake}}$$

The NPU thus is equivalent to biological value x digestibility and is a measure both of the digestibility of food protein and the biological value of the amino acid mixture absorbed from food.

Miller and Bender (1955) described the method. The NPU like the calculation for nitrogen balance accounts for the amount of nitrogen needed for maintenance. A group of subjects on a non-protein diet provides the "endogenous or maintenance nitrogen" correction. The body nitrogen deposited as new tissue by the test group is thus estimated with referece to the total nitrogen consumed.

NPU represents the proportion of food nitrogen retained, whereas both BV and nitrogen balance index represent the proportion of absorbed nitrogen retained. NPU is therefore related directly to dietary intake of nitrogen. Nitrogen retention may be measured by nitrogen balance studies in the human and by nitrogen balance or direct analysis of the animal body.

If carcass analysis is used as a measure of nitrogen retention it is necessary to have two groups of experimental animals equivalent in weight and age. One group is analyzed at the beginning of the study and the second group is placed on the experimental diet and analyzed at the end of the study period. Nitrogen retention is determined as average body nitrogen of animals at the end of the experimental period minus body nitrogen of the comparable group

of animals sacrificed at the beginning of the experimental period. When nitrogen balance is used as a criterion of retention the following formula may be used:

$$NPU = \frac{I - (F-F_o) - (U-U_o)}{I}$$

Abbreviations here are the same as those already given for biological value. If correction for endogenous losses in urine and feces is not made, NPU value is designated "apparent NPU."

Net Protein Ratio

Taking into consideration the drawbacks of the protein efficiency ratio that (1) no allowance is made for maintenance requirements of the test animal, (2) that the result varies with food intake, and (3) the assumption that the gain in body weight is indicative of the protein tissue laid down; a modification is described whereby a control group of animals fed on a protein-free diet is included in the experiment, and the difference between the weights of this group and the test group is used in the calculation instead of merely weight gain. This procedure allows for maintenance requirements and also permits the evaluation of poor proteins which do not promote growth and for which, consequently, PER cannot be measured.

Bender and Doell (1957) reported this new aspect of the biological evaluation of proteins. They used two groups

of four animals, balanced as regards litter-mates and combined body weight in each test. One group was fed on the test protein, the other on a non-protein diet. At the end of the 7 or 10 day feeding period the animals were weighed and the protein intake measured.

The net protein ratio can be expressed as follows:

$$NPR = \frac{\text{gain in weight of test group} + \text{loss in weight of non-protein group}}{\text{protein intake}}$$

To illustrate the remark that was made earlier about poor proteins which do not promote growth and for which PER cannot be measured, the following example will be presented. Effect of food intake on PER and NPR measured on rats (theoretical calculations). Assumptions: 2 gm protein required for maintenance; 1 gm protein produces 5 gm increase in body weight.

Protein eaten	Change in body weight (gm)	PER	NPR
0	-10	--	--
1	- 5	(cannot be determined)	5/1 = 5
2	0	0/2 = 0	10/2 = 5
3	5	5/3 = 1.7	15/3 = 5
4	10	10/4 = 2.5	20/4 = 5
5	15	15/5 = 3.0	25/5 = 5

The correctness of this theoretical treatment was borne out by results obtained with dried skim milk tested on six occasions and with bread fortified with lysine tested on

five occasions (Bender and Doell, 1957), when ad libitum feeding resulted in varying food intakes. The PER's varied with food consumption, but the NPR's and NPU's were relatively constant.

Bender and Doell (1957) measured NPU's on thirty-five proteins and mixtures of proteins, each value being replicated several times. From the same data the PER's and NPR's were calculated. Replicated PER's showed wide variations (mean coefficient of variation 41.0) but NPR's were relatively constant (mean coefficient of variation 7.4). NPR was highly correlated ($p < 0.01$) with NPU (computed from the mean values): $y = 3.3 + 15.5X$ ($y = NPU_o$, $X = NPR$); $r = 0.986$; $p < 0.01$.

Application of the Chromic Oxide Indicator Method to Balance Studies

The determination of nutrient balance is a basic technique which has been applied extensively in nutritional studies of digestibility, utilization, and retention. The procedure most frequently followed is to collect all the excrement produced during the experimental period and, by comparison with the total food intake, to determine the "disappearance" of the nutrient under study. This procedure is laborious and time consuming.

The basic data needed to establish a balance comprise the amounts of excrement derived from the measured food intake over a period of time sufficiently long to give

a representative sample characteristic of the experimental conditions employed. The procedure can be facilitated by any method which will aid in establishing the amount of excrement equivalent to a given amount of food. In one such method, a known amount of a completely indigestible index material is incorporated in the diet; the amount of food from which a sample of feces was derived can be calculated from the respective concentrations of index material in food and feces. Chromic oxide (Edin, 1918; Edin, Kihlen, and Nordfeldt, 1944; Schürch, Lloyd, and Crampton, 1950; Whitson et al., 1943) and lignin (Kane, Jacobson, and Moore, 1950) have been employed as index substances.

Less work in this field has been done with poultry than with other species. Determination of true digestibility with birds is complicated by the fact that urinary and fecal excrements are voided together.

The studies which have been made are typified by those of Whitson et al. (1943) and Olson (1950). The former workers used barium sulfate as the index material in determining fat utilization by growing chickens. Olson (1950) applied the "Edin Indicator Method" to digestibility studies with mature hens, geese, and turkeys on restricted dietary regimens. In both cases the balance experiments were carried out on birds kept in individual cages.

The insoluble nature of chromic oxide and the practicability of its use as an index substance have been well established in the studies already mentioned.

Dansky and Hill (1952) applied the chromic oxide method to balance studies with groups of growing chickens under ad libitum feeding conditions, with particular reference to the following: (1) length of collection period necessary to assure a representative sample of excrement, (2) reproducibility of results in successive collection periods, (3) recovery of ingested chromic oxide, and (4) comparison of balance data by the index and total collection methods.

In summary the following equation is used for nutrient digestibility (Edwards and Gillis, 1959):

% of digestible nutrient =

$$100 - \left[\frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in feces}} \times \frac{\%\text{ nutrient in feces}}{\%\text{ nutrient in feed}}\right] \times 100$$

Apparent Amino Acid Digestibility

The problem of describing both the quantity and availability of each essential amino acid in foodstuffs is presently limited by methodology. Many methods have been developed to investigate the quality of various proteins, as well as to determine the availability of amino acids contained within them. However, none of these methods have been successfully used on sufficient samples and/or

different types of proteins to investigate the availability of the total amino acids spectrum simultaneously. Consequently, reliable data describing the specific availability of essential amino acids in proteins is limiting and, for some amino acids, is not available.

Payne et al. (1971) and Reid and Weber (1973) have reported apparent digestibility of amino acids measured using the ratio of the individual amino acids in feed and feces to a chromic oxide marker, using the following formula, according to Edwards and Gillis (1959) modification:

Apparent amino acid digestibility as % =

$$100 - \left[\frac{\text{feed } Cr_2O_3}{\text{feces } Cr_2O_3} \times \frac{\text{fecal amino acid}}{\text{feed amino acid}}\right] \times 100.$$

Apparent nitrogen digestibility can be calculated in the same manner.

METHODS

Preparation of Tortillas

The tortillas were prepared according to commercial procedures. Preparation of the corn masa: The whole corn grain was placed in hot water (90-92°C) with 0.8-1.0% lime for approximately 20-45 minutes. After this period, the corn was separated from the chaff by gravity and held for 12 hours at a lower temperature of about 80°C. The corn was then washed several times to remove the lime. The lime-treated corn was milled in a special rock mill. The product obtained is called masa, from which the tortillas were shaped.

Corn-soy tortillas, 90:10 and 80:20, were processed in the same way as corn alone.

Pinto beans were cooked in water for 3 hours in a steam jacketed kettle at 99°C.

Wheat flour tortillas were purchased in a local store. The wheat masa was prepared with wheat, water, and shortening. These items were then dried in an oven at approximately 45°C and ground in a Willey mill to 10 mesh.

The standard reference protein in these studies was soybean meal with 0.05% dietary supplemental methionine. The protein sources were incorporated into diets at 7.5% crude protein level, as follows: (1) control (soybean meal

+ 0.05% methionine), (2) corn, (3) corn-soy 90:10, (4) corn-soy 80:20, (5) pinto beans, (6) wheat flour, (7) pinto beans-corn 50:50, and (8) pinto beans-wheat flour 50:50.

Nitrogen and Crude Protein Determinations

The nitrogen determinations were done by the standard macro Kjeldahl method and crude protein was calculated as N x 6.25. The protein sources were analyzed for crude protein prior to the preparation of the diets and the diets were prepared to contain 7.5% crude protein. After preparation of the diets, Kjeldahl determinations were made to determine the exact content of crude protein in each diet. The Kjeldahl procedure was also used to analyze the N content of the collected feces at the end of the feeding trial.

Amino Acid Analysis

Samples for amino acid analysis were hydrolyzed by Reid's (1975) method, from the Poultry Science Department, The University of Arizona, as follows: Hydrolyses of samples were performed using 25 ml of 6 N HCl and approximately 0.1 gm of sample, in flat bottom round 250 ml flasks covered with 50 ml beakers and hydrolyzed for 16 hours in an autoclave at 18 lb/in^2 and 120-125°C. Duplicates of each sample treatment were used. To one of these samples sodium thioglycolate was added to protect methionine. The other sample was hydrolyzed without the sodium thioglycolate.

The presence of sodium thioglycolate protects methionine but destroys cystine, hence hydrolysis with and without sodium thioglycolate provides a measure of both methionine and cystine. The HCl was removed by evaporation in a Rotovac. The hydrolyzate was extracted with 10 ml citrate buffer solution, pH 2.2, from which 0.75 ml were used for the analysis in a Beckman Model 121 automatic amino acid analyzer.

Composition of Diets

The composition of the basal diet is given in Tables 1 and 2. The test diets were formulated to contain 7.5% crude protein, the basal diet comprised 24.8% of the experimental diets and cerelose was used to make a total of 100% after addition of the protein test material.

Soybean meal was used as a control protein source and was supplemented with 0.05% dietary DL-methionine. The mixing amounts of the complete diets are presented in Table 3.

Protein Efficiency Ratio (PER)

Male and female weanling mice of the Charles River CD-1 strain were housed in stainless steel wire mesh cages. Aluminum bottom containers with a stainless steel wire screen and stainless steel top were used as feeders to prevent spillage. Eight treatment groups including the control were arranged by weight (8-10.5 g) with five male

Table 1. Basal diet premix and vitamin mix purified.

Ingredient	%	Mixing amount
Basal diet premix		
Corn Oil	5.0	700
Dicalcium Phosphate	3.0	420
Salt	0.2	28
Trace Min. Mix	0.2	28
Vit. Mix, Purified	4.0	560
Solka Floc	3.0	420
Choline Cl (50%)	0.2	28
Cerelose (Glucose)	9.0	1260
Cr_2O_3	0.2	28
	24.8	3472

Vitamin mix, purified

	% of mix	1307.52 gm. of mix	% of Diet @ 4%
Vitamin A (30,000 IU/gm.)	.833	10.892	10,000 IU/kg.
Vitamin D_3 (15,000 ICU/gm.)	.160	2.092	960 ICU/kg.
Myvamix E (20,000 IU/#)	.499	6.525	8.8 IU/kg.
Santoquin	.125	1.634	.005
Thiamin HCl	.020	.262	.00088
Riboflavin	.030	.392	.0012
D-calcium Pantothenate	.038	.497	.00152
Pyridoxine HCl	.010	.131	.0004
P-amino Benzoic Acid	.050	.654	.0020
Inositol	2.500	32.688	.1000
Niacin	.220	2.877	.0088
Choline Chloride	14.6674	191.779	.2206
Vitamin B_{12}	.075	.981	.0038
Biotin	.0005	.006	.00002
Folic Acid	.0050	.065	.00020
Menadione	.0165	.216	.00066
Cerelose	80.7506	1055.830	
Total	100.000	1307.52	

Table 2. Trace mineral mix -- Amount supplied in finished diet when used at 0.2% of feed.

Element	mg/lb	PPM (mg/kg)
Iron	18.242	40.0
Zinc	54.580	120.0
Molybdenum	0.936	2.0
Manganese	54.580	120.0
Calcium	152.936	336.0
Copper	3.600	8.0
Iodine	1.362	3.0
Cobalt	1.362	3.0

Table 3. Diets (gms).

Ingredients	Diets							
	1 (Control) Soybean Meal	2 Corn	3 Corn-Soy 90:10	4 Corn-Soy 80:20	5 Pinto Beans	6 Wheat Flour	7 Beans-Corn 50:50	8 Beans-Wheat 50:50
Basal Premix	620	620	620	620	620	620	620	620
Soybean Meal + 0.05% DL-Met.	390.62 + 1.25	--	--	--	--	--	--	--
Corn	--	1677	--	--	--	--	--	--
Corn-Soy 90:10	--	--	1346	--	--	--	--	--
Corn-Soy 80:20	--	--	--	1131.5	--	--	--	--
Pinto Beans	--	--	--	--	813	--	--	--
Wheat Flour	--	--	--	--	--	1620	--	--
Beans-Corn 50:50	--	--	--	--	--	--	1100.75	--
Beans-Wheat 50:50	--	--	--	--	--	--	--	1077.5
Cerelose	1488.13	203	534	748.5	1067	260	779.25	802.5
Total Weight	2500	2500	2500	2500	2500	2500	2500	2500

and five female mice per treatment group. Each treatment group was housed in 5 cages with one mouse of each sex in a single cage. The treatment groups were exposed to 12 hours of light and 12 hours of darkness in a room maintained at 24 ± 2°C. The mice were fed the experimental diets ad libitum for a period of three weeks. Deionized water was given ad libitum. A weighed fresh portion of diet was supplied twice a week and the remaining diet was dried and weighed so that the amount of food consumed could be calculated. Feces were collected twice a week and stored under refrigeration for analysis at a later date. The mice were weighed weekly.

PER was calculated by the following equation:

$$PER = \frac{\text{weight gain (g)}}{\text{protein consumed (g)}}$$

Duncan's (1955) multiple range test and standard error analysis were employed to evaluate statistical significance.

Net Protein Ratio (NPR)

Similar grouping and feeding procedures were employed for the NPR determination. In addition to the control group and the seven test diet treatments a comparable group of mice was placed on a nitrogen-free diet to determine the weight loss during the three week experimental period.

NPR values were calculated according to Bender and Doell (1957) as follows:

$$NPR = \frac{\text{gain in weight of test group} + \text{loss of weight of nitrogen-free diet group}}{\text{protein intake}}$$

The same statistical procedures were employed.

Apparent Digestibility of Amino Acids and Digestible Energy

The apparent digestibility determinations of amino acids and digestible energy were calculated by the Cr_2O_3 balance method as described by Edwards and Gillis (1959), modified.

Triplicate samples of feed and feces were weighed into 125 ml Erlenmeyer flasks. Ten ml of concentrated HNO_3 were added to each sample and the mixture was allowed to stand overnight. The samples were heated on a hot plate until 2-3 ml of liquid remained. Fifteen ml of 60% $HClO_4$ were added for complete oxidation of the inorganic materials. The heat was increased and the digestion continued until the solution turned from green to yellow or orange. The digested samples were removed from the hot plate and were allowed to cool for a few minutes. A few ml of deionized H_2O were added until the orange color became yellow. The samples were transferred quantitatively to 100 ml volumetric flasks using deionized H_2O. When the samples were completely cooled, they were diluted to mark, shaken,

and allowed to stand overnight. With a minimum of disturbance to the sediment in the bottom of the flasks, 5 to 10 ml of each sample were poured into Coleman tubes and read in a Coleman Junior II Spectrophotometer at 444 mμ using deionized H_2O as a reference. Absorbance of each sample was recorded. The working standard was prepared essentially in the same manner taking different dilutions to make a standard curve and to determine a K value for the calculation of samples:

$$\% \ Cr_2O_3 = \frac{O.D. \times K \ (standard)}{sample \ wt \ (g) \times 100}$$

The apparent digestibility values for amino acids and digestible energy were calculated according to Edwards and Gillis (1959) as follows:

$$\% \text{ of nutrient retained} =$$

$$\left(\frac{\% \ Cr_2O_3 \text{ in feed}}{\% \ Cr_2O_3 \text{ in feces}} \times \frac{\% \text{ of nutrient in feces}}{\% \text{ of nutrient in feed}} \right) \times 100.$$

Gross energy of the diet and feces were determined using a Parr oxygen bomb colorimeter and the chromic oxide balance method was used to determine digestible energy.

Protein Score

Protein score values (FAO, 1965) were calculated using the "target pattern" for mice and rats (Coates et al., 1969) and the analyzed amino acid values of the different

diets. The following equation was used for the calculations:

$$\text{protein score} = \frac{\text{X amino acid in diet}}{\text{X amino acid in "target pattern"}} \times 100$$

The protein score value is the lowest amino acid, also called the most limiting.

Protein score was estimated also with a modification, taking into consideration the determined apparent amino acid digestibilities, as follows:

$$\text{protein score} = \frac{\text{(X amino acid) digestibility}}{\text{X amino acid in "target pattern"}} \times 100$$

The most limiting amino acid was taken as the protein score value.

RESULTS AND DISCUSSION

The results of the amino acid analysis of the diets fed are presented in Table 4. Values are expressed as per cent of the protein (g amino acid per 16 g of nitrogen). The amino acid values for the single protein sources agree with amino acid values reported in the literature, considering the range of values that exists because of differences in grain varieties and in their protein content.

Table 5 shows the amino acid composition of the fecal samples of the test treatments and are expressed as g amino acid per 16 g of nitrogen.

Table 6 shows essential amino acid requirements of rats and mice as reported by various investigators and the proposed amino acid "target pattern" for both species (Coates et al., 1969).

Apparent Amino Acid Digestibility

Apparent amino acid digestibility values for each diet were calculated using the amino acid compositions of diet and feces and the per cent Cr_2O_3 in diet and feces, according to the following equation:

$$\text{apparent amino acid digestibility} = 100 - \left[\frac{\%Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}} \times \frac{\%\ \text{amino acid in feces}}{\%\ \text{amino acid in feed}} \right] \times 100.$$

Table 4. Amino acid content of diets containing various sources of protein, g/16 g nitrogen.

Amino Acids	1 Soybean Meal + Met	2 Corn	3 Corn-Soy 90:10	4 Corn-Soy 80:20	5 Pinto Beans	6 Wheat Flour	7 Beans-Corn 50:50	8 Beans-Wheat 50:50
Lysine	5.939	2.649	3.591	3.890	6.713	2.003	5.593	6.477
Histidine	2.506	3.147	3.010	2.621	2.751	1.959	3.036	3.041
Ammonia	2.898	3.054	2.713	2.284	1.822	3.712	2.095	2.688
Arginine	7.009	5.044	5.768	5.772	6.147	3.438	5.931	6.231
Aspartic Acid	10.807	7.459	8.973	9.061	11.794	4.270	10.441	11.240
Threonine	3.518	3.949	4.029	3.734	4.386	2.691	4.295	4.531
Serine	4.172	4.962	4.966	4.472	5.243	3.982	5.248	5.811
Glutamic Acid	17.014	23.899	22.742	19.800	14.556	50.718	17.294	23.982
Proline	4.748	10.679	9.185	7.296	3.660	10.170	5.572	6.192
Glycine	3.940	3.961	4.138	3.889	3.934	3.340	4.037	4.396
Alanine	3.883	8.761	7.531	5.987	3.903	2.644	5.324	4.288
Cystine	1.462	1.501	1.017	1.784	0.580	1.078	0.428	0.530
Valine	4.801	5.859	5.763	5.222	5.513	4.051	5.577	5.982
Methionine	1.469	2.504	2.122	1.741	1.000	1.519	1.394	1.138
Isoleucine	4.261	4.192	4.405	4.176	4.672	3.589	4.508	5.041
Leucine	7.046	16.352	13.895	10.936	8.055	6.507	10.532	8.910
Tyrosine	3.086	4.688	4.343	3.668	3.191	2.814	3.673	3.641
Phenylalanine	4.467	5.344	5.412	4.741	5.293	4.377	5.341	5.867

Table 5. Amino acids in feces of mice fed diets containing various sources of protein, g/16 g nitrogen.

Amino Acids	1 Soybean Meal + Met	2 Corn	3 Corn-Soy 90:10	4 Corn-Soy 80:20	5 Pinto Beans	6 Wheat Flour	7 Beans-Corn 50:50	8 Beans-Wheat 50:50
Lysine	5.136	5.323	5.031	5.254	5.547	5.606	5.258	5.662
Histidine	1.808	1.893	1.949	1.924	2.175	1.836	2.230	2.181
Ammonia	4.061	3.142	3.351	3.391	3.260	3.399	3.161	3.255
Arginine	4.252	4.070	3.756	3.695	4.252	4.050	4.142	4.318
Aspargine	9.883	9.215	9.344	10.154	10.138	9.461	9.529	10.031
Threonine	4.089	4.691	4.406	4.420	4.913	4.716	4.734	4.759
Serine	3.832	3.595	3.781	4.242	4.192	3.756	4.025	4.256
Glutamic Acid	11.806	10.903	10.726	11.875	10.344	11.816	10.163	11.111
Proline	3.278	3.862	3.874	4.411	3.912	3.388	4.436	3.930
Glycine	4.176	4.842	4.594	4.798	4.695	4.572	4.671	4.936
Alanine	4.549	5.226	4.488	4.400	5.144	4.988	4.858	5.186
Cystine	0.452	0.672	0.550	1.298	1.107	0.684	0.993	5.243
Valine	4.720	4.943	4.470	4.524	5.410	5.030	5.195	5.334
Methionine	1.433	1.788	1.596	1.649	1.685	2.288	1.617	2.120
Isoleucine	4.042	3.897	3.469	3.558	4.407	3.953	4.080	4.335
Leucine	6.536	5.978	5.318	5.416	6.426	5.679	6.181	6.482
Tyrosine	2.709	3.087	2.784	2.844	3.184	3.013	3.080	3.276
Phenylalanine	3.840	3.325	2.995	3.067	3.849	3.194	3.578	3.880

Table 6. Requirements for essential amino acids expressed as % of protein.

	Rat									Mouse		Human milk	Whole egg	Target pattern
	1	2	3	4	5	6	7	8	9	10	11	12	12	
Arginine	--	5.0	9.0	--	4.6	--	--	--	0.23	--	1.9	4.0	6.6	5.0
Histidine	4.0	2.5	2.9	1.8	2.0	2.0	--	2.5	0.28	--	3.4	2.2	2.4	2.5
Lysine	10.1	9.0	11.8	5.2	3.7	6.5	--	7.5	0.54	--	9.0	6.4	6.4	6.0
Tyrosine	--	3.0	--	--	4.7	--	--	--	--	--	4.8	5.7	4.2	4.0
Tryptophan	1.0	1.1	1.0	1.0	1.5	1.3	--	1.3	--	--	1.9	1.7	1.6	1.5
Phenylalanine	4.6	4.2	7.7	4.9	6.3	--	--	7.5	0.85	--	14.0	4.8	5.8	5.0
Methionine + Cystine	7.5	5.0	5.4	4.7	4.4	--	4.6	5.0	0.16	3.0	8.0	4.4	5.5	4.5
Threonine	5.0	5.0	3.5	4.1	2.6	4.2	--	4.2	0.50	--	6.8	4.6	5.1	4.0
Leucine	8.0	7.0	7.3	7.8	9.5	8.2	--	6.7	0.69	--	12.5	9.2	8.8	8.0
Isoleucine	5.0	5.5	4.8	4.3	5.1	5.0	--	4.2	0.57	--	8.7	6.6	6.6	5.0
Valine	7.0	5.5	5.4	5.0	6.2	--	--	5.8	0.62	--	9.7	6.7	7.3	5.5

1. Rose, Oesterling, and Womach (1948).
2. Rama Rao, Metta, and Johnson (1959).
3. Rogers and Harper (1964).
4. Bender (1961).
5. Fisher (1954).
6. McLaughlan and Illman (1967).
7. Harstook and Mitchell (1956).
8. NRC (1962).
9. Peng (1975) as % of diet.
10. Leveille, Sauberlich, and Shockley (1961).
11. Bauer and Berg (1943).
12. FAO (1965).

These data are shown in Table 7 and are expressed as per cent. These data indicate that the limiting amino acids, namely lysine in corn and wheat, cystine and methionine in pinto beans, cystine in the pinto beans-corn combination, and methionine in the pinto beans-wheat combination were less digestible than the rest of the amino acids, contrary to what would be expected. It would seem logical that the limiting amino acids would be absorbed more readily or at least as readily as the non-limiting amino acids.

This can be explained by the contribution of endogenous amino acids from pancreatic and intestinal mucosa enzymes or bacteria present in the digestive tract. Snook (1964a, 1964b) estimated the amount of endogenous nitrogen secreted using chromic oxide to nitrogen ratios in the intestinal contents and feces of rats. Based on their calculations the amount of endogenous nitrogen entering the intestine during the 12 hour digestion period was 70 mg in rats fed a protein-free diet and 125 mg in rats fed a diet containing 15% whole egg protein. The latter value would be about equal to the nitrogen intake of rats fed a 15% protein diet. Snook, in a continuation of those studies, fractionated the various pancreatic proteolytic enzymes collected at various points along the small intestine. He found that peptides and amino acids reduced the rate of degradation of the enzymes and that a slower degradation occurred when protein was included in the diet.

Table 7. Apparent amino acid digestibilities of diets containing various sources of proteins (%).

Amino Acids	1 Soybean Meal + 0.05 Met	2 Corn	3 Corn-Soy 90:10	4 Corn-Soy 80:20	5 Pinto Beans	6 Wheat Flour	7 Beans-Corn 50:50	8 Beans-Wheat 50:50
Lysine	91.03	70.88	80.62	80.41	84.64	75.67	83.07	86.67
Histidine	92.52	91.28	91.04	89.37	85.31	91.88	86.77	89.07
Arginine	93.71	88.30	90.99	90.65	87.15	89.76	87.42	89.43
Aspartic Acid	91.38	82.09	85.60	83.78	84.03	80.74	83.57	86.39
Threonine	87.94	82.78	84.87	82.87	74.90	84.77	79.95	83.99
Serine	90.48	88.89	89.47	86.27	85.15	91.80	86.19	88.83
Glutamic Acid	92.81	93.39	93.47	91.31	86.80	97.98	89.42	92.93
Proline	92.84	94.76	94.17	91.25	80.14	97.11	85.66	90.32
Glycine	90.01	82.28	84.65	82.14	77.83	88.10	79.16	82.88
Alanine	87.85	91.35	91.76	89.36	96.26	83.60	83.57	81.56
Cystine	97.08	93.51	92.52	89.46	64.53	94.49	58.22	79.50
Valine	89.80	87.77	89.27	87.46	81.76	89.21	82.22	86.40
Methionine	89.88	89.65	89.60	86.29	68.69	86.91	79.11	71.59
Isoleucine	91.06	86.53	89.11	87.66	82.47	90.42	83.70	86.89
Leucine	91.25	94.70	94.71	92.83	85.18	92.42	89.43	88.91
Tyrosine	90.90	90.46	91.13	88.78	81.46	90.69	84.90	86.28
Phenylalanine	91.08	90.98	92.35	90.64	86.49	93.65	87.94	89.92

On the other hand, Beilorai et al. (1973) evaluated the absorption and digestion of protein in chicks fed heated soybean meal, using yttrium-91 as a reference substance. In heated soybean meal-fed chicks a cumulative digestion of 90% was attained; 70% in the duodenum and an additional 20% in the remaining segments. In the duodenum of the raw soybean meal-fed chicks extensive nitrogen secretion, proportional to the raw soybean meal levels in the diets occurred and the secreted nitrogen was reabsorbed in the upper jejunum. The net digestion values in the duodenum were also proportional to the raw soybean meal levels in the diets. When the duodenal nitrogen secretion was taken into account it was concluded that, as in chicks fed heated soybean meal, most of the food protein was digested in the duodenum of raw soybean meal-fed chicks. The 20% difference in the cumulative net digestion between the heated soybean meal and the raw soybean meal groups was probably the result of the inhibition of proteolysis in the segments beyond the duodenum.

The corn-soy combinations and the control showed that all the amino acids had apparent digestibilities above 80%. Table 7 shows the results obtained in our experiment and will be discussed further in terms of other evaluations and comparisons.

Protein Score

Protein score values were calculated on the basis of the amino acid "target pattern" for mice and rats as shown in Table 8. The protein score value was taken as the most limiting amino acid for each diet. Wheat flour showed the lowest protein score among treatments with a value of 33.38; pinto beans, 35.11; bean-wheat combination, 37.07; bean-corn combination, 40.48; corn, 44.15; corn-soy combination (90:10), 59.85; the control soybean meal + 0.05% DL-methionine, 65.13; and the highest was the corn-soy combination (80:20), 78.33.

Also, protein score values were calculated taking into consideration the amino acid apparent digestibility for comparison with the amino acid "target pattern" for mice and rats. In this case pinto beans had the lowest protein score value among treatments with a score of 23.57; wheat flour, 25.06; beans-wheat combination, 27.45; beans-corn combination, 30.03; corn, 31.29; corn-soy (90:10), 48.25; corn-soy (80:20), 52.13; and the highest was the control soybean meal + 0.05% methionine with a value of 60.87 (Table 9).

Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR)

Protein efficiency ratio (PER) was used to evaluate protein quality. The PER values are expressed as grams gain in body weight per gram of protein consumed during a

Table 8. Protein score values of diets containing various sources of protein.

Amino Acids	1 Soybean Meal + 0.05 Met	2 Corn	3 Corn-Soy 90:10	4 Corn-Soy 80:20	5 Pinto Beans	6 Wheat Flour	7 Beans-Corn 50:50	8 Beans-Wheat 50:50
Lysine	98.98	44.15	59.85	86.44	100.00	33.38	93.22	100.00
Histidine	100.00	100.00	100.00	100.00	100.00	78.36	100.00	100.00
Arginine	100.00	100.00	100.00	100.00	100.00	68.76	100.00	100.00
Threonine	87.95	98.72	100.00	93.35	100.00	67.28	100.00	100.00
Valine	87.29	100.00	100.00	94.95	100.00	73.65	100.00	100.00
Methionine + Cystine	65.13	89.00	69.76	78.33	35.11	57.71	40.48	37.07
Isoleucine	85.22	83.84	88.10	88.10	93.44	71.78	90.16	100.00
Leucine	88.08	100.00	199.00	199.00	199.00	81.34	100.00	100.00
Phenylalanine + Tyrosine	83.92	100.00	100.00	93.66	94.27	79.90	100.00	100.00
Protein Score	65.13	44.15	59.85	78.33	35.11	33.38	40.48	37.07

Table 9. Protein score of experimental diets corrected for digestibility.

Amino Acids	1 Soybean Meal + 0.05 Met	2 Corn	3 Corn-Soy 90:10	4 Corn-Soy 80:20	5 Pinto Beans	6 Wheat Flour	7 Beans-Corn 50:50	8 Beans-Wheat 50:50
Lysine	90.10	31.29	48.25	52.13	94.70	25.26	77.44	93.56
Histidine	92.74	100.00	100.00	93.70	93.88	72.00	100.00	100.00
Arginine	100.00	89.07	100.00	100.00	100.00	61.72	100.00	100.00
Threonine	77.34	72.80	85.38	77.35	82.13	57.02	85.84	95.14
Valine	78.38	93.50	93.54	83.04	81.95	65.70	83.37	93.97
Methionine + Cystine	60.87	73.45	63.14	68.83	23.57	51.95	30.03	27.45
Isoleucine	77.60	72.55	78.51	73.21	77.06	64.90	75.46	87.60
Leucine	80.37	100.00	100.00	100.00	85.77	75.17	100.00	99.03
Phenylalanine + Tyrosine	76.39	100.00	99.49	83.92	79.74	73.90	86.83	93.45
Protein Score	60.87	31.29	48.25	52.13	23.57	25.26	30.03	27.45

three week period of ad libitum feeding (Table 10). Statistical analysis using Duncan's (1955) multiple range test showed a significant difference between the control and the test treatments and among some of the test treatments themselves (Table 10).

Pinto beans and wheat flour showed no significant difference but were different from the rest of the diets. Corn, beans-corn, and beans-wheat were not significantly different in PER value but were lower than the rest of the diets. Corn-soy (90:10) and corn-soy (80:20) showed no significant difference but differed significantly from the other diets. The control soybean meal + 0.05 DL-methionine was significantly higher in PER than all the other diets.

Net protein ratio (NPR) values are shown in Table 10. Statistical analysis was done using Duncan's multiple range test. Again significant difference was found between the control and the rest of the diets and among the diets themselves. Pinto beans and wheat flour did not differ significantly from each other; but were different from the other diets. Corn was not different from pinto beans, but had a higher NPR than wheat flour and NPR was lower than the rest of the diets. The bean-corn and bean-wheat combinations were not different from each other and had higher NPR values than corn, wheat, and pinto beans; but were lower than corn-soy (90:10), corn-soy (80:20), and the control. Corn-soy (90:10) was lower than corn-soy (80:20)

Table 10. PER and NPR values of the experimental diets.

Protein source in the diet	PER	NPR
Soybean meal + 0.05 Methionine (control)	$2.77^{d1} \pm 0.096^2$	$2.10^{f1} \pm 0.054^2$
Corn-Soy 80:20	$2.25^{c} \pm 0.078$	$1.65^{e} \pm 0.072$
Corn-Soy 90:10	$2.06^{c} \pm 0.084$	$1.39^{d} \pm 0.083$
Beans-Wheat 50:50	$1.72^{b} \pm 0.068$	$1.10^{c} \pm 0.058$
Beans-Corn 50:50	$1.69^{b} \pm 0.067$	$1.09^{c} \pm 0.078$
Corn	$1.59^{b} \pm 0.053$	$0.69^{b} \pm 0.048$
Wheat Flour	$1.33^{a} \pm 0.068$	$0.39^{a} \pm 0.107$
Pinto Beans	$1.25^{a} \pm 0.040$	$0.46^{ab} \pm 0.075$

1. Means having different letter superscripts are significantly different at the 0.05 level of probability.

2. Standard error of mean.

and the control, but higher than the rest of the diets. Corn-soy (80:20) was lower than the control, but had the highest NPR value of all the diets.

As mentioned in the literature review Bender and Doell (1957) found that NPR's and NPU were highly correlated, $r = 0.986$, $p < 0.01$. In our experiment NPU values were not determined. However, a regression analysis was done for NPR's and PER's and were found to be highly correlated, $r = 0.979$, $p < 0.01$. So in spite of the drawback argued against PER's, it is as good as the NPR method for evaluating protein quality.

Furthermore, the correlations were calculated for PER and protein score, NPR and protein score with and with out digestibility being considered. PER and protein score were correlated significantly ($r = 0.846$, $p < 0.01$) as were NPR and protein score ($r = 0.836$, $p < 0.01$).

However, when the apparent amino acid digestibility was considered for the calculation of protein score and correlated with PER and NPR, the results showed a better relationship in both cases. PER and protein score showed a correlation coefficient of 0.963 ($p < 0.01$) while NPR and protein score showed a correlation coefficient of 0.927 ($p < 0.01$).

These results apparently show the importance of digestibility consideration since protein score alone predicts the value of a protein but does not consider

digestibility. Our results show that when apparent amino acid digestibility was considered, the values showed a closer relationship to those obtained by other protein quality evaluation methods such as PER and NPR.

Energy Determinations

Gross energy determinations were carried out on the diets and feces using a Parr bomb colorimeter. The results are shown in Table 11. From these values digestible energy was calculated using the Cr_2O_3 balance method and expressed as per cent digestible energy.

No apparent relationship was found with biological protein evaluations and digestible energy except that pinto beans showed the lowest digestible energy value and had the lowest PER. Aside from this, the variation in the energy levels of the diets did not seem to influence the PER and NPR values.

The standard deviations for digestibility and gross energy values, expressed as Kcals/g, were 0.054 and 0.034, respectively.

Table 11. Gross and digestible energy values of experimental diets fed to mice.

Protein source in diets	Feed GE (Kcal/g)	Feces GE (Kcal/g)	% DE (%)	DE Kcal/g (Kcal/g)
Soybean Meal + 0.05% Methionine (control)	3.71	3.37	90.68	3.40
Corn	4.26	3.59	86.12	3.67
Corn-Soy 90:10	4.21	3.56	88.31	3.72
Corn-Soy 80:20	4.11	3.66	87.12	3.58
Pinto Beans	3.86	3.70	82.21	3.17
Wheat Flour	4.23	3.47	92.87	3.93
Beans-Corn 50:50	3.97	3.78	82.87	3.29
Beans-Wheat 50:50	4.03	3.68	86.07	3.47

SUMMARY AND CONCLUSIONS

Aside from the control diet (soybean meal + 0.05% DL-methionine) the corn-soy combinations had higher PER's and NPR's than any of the other diets tested. Protein quality of wheat flour tortillas improved significantly when consumed in combination with pinto beans. No such effect was observed with corn tortillas consumed with pinto beans.

PER correlated significantly with NPR, therefore PER was as good a method as NPR to measure protein quality.

Protein score correlated better with PER and NPR when apparent amino acid digestibility was considered. These results emphasize the importance of digestibility.

Considering the PER and NPR values and the protein score determinations of the corn tortillas-pinto beans (50:50) combination and the wheat flour tortillas-pinto beans (50:50) mixture it would appear that only small amounts of animal protein would be needed in the diet to provide optimum protein quality. This is important if we consider the economic restriction of low income families in Mexico. However, the corn-soy tortilla combinations offer better possibilities for improving protein quality of Mexican diets than any of the other experimental diets used in this study.

REFERENCES

Allison, J. B. (1955). Biological Evaluation of Protein. Physiol. Rev., 35:664.

Armstrong, M. D. (1955). The Phenylalanine and Tyrosine Requirements of the Rat. J. Biol. Chem., 213:409.

Arnold, A., and Shad, J. S. (1952). Evaluation of Nitrogen Source Materials by an Intraperitoneal Rat Test. J. Nutr., 48:377.

Bauer, C. D., and Berg, C. P. (1943). The Amino Acids Required for Growth in Mice and the Availability of the Optical Isomers. J. Nutr., 26:51.

Bender, A. E. (1961). Determination of the Nutritive Value of Proteins by Chemical Analysis. In Progress in Meeting Protein Needs of Infants and Preschool Children. Publs. Natn. Res. Coun., Wash., 843:407.

Bender, A. E., and Doell, B. H. (1957). Biological Evaluation of Proteins: A New Aspect. Brit. J. Nutr., 11:440.

Bielorai, R., Tamir, M., Alumont, E., Bar, A., and Hurwitz, A. (1973). Digestion and Absorption of Protein Along the Intestinal Tract of Chicks fed Raw and Heated Soybean Meal. J. Nutr., 103:1291.

Block, R. J., and Mitchell, H. H. (1946). The Correlation of the Amino Acid Composition of Proteins with their Nutritive Value. Nutr. Abst. Rev., 16:249.

Coates, M. E., O'Donoghue, P. N., Payne, P. R., and Ward, R. J. (1969). Laboratory Animal Handbooks 2. Dietary Standards for Laboratory Rats and Mice. Nutritional and Microbiological Recommendations. London: Laboratory Animals LTD.

Dansky, L. M., and Hill, F. W. (1952). Application of the Chromic Oxide Indicator Method to Balance Studies with Growing Chickens. J. Nutr., 47:449.

Duncan, D. B. (1955). Multiple Range and Multiple F Tests. Biometrics, 11:1.

Edin, H. (1918). Orienterande Försök Över Ansvandbarheten av en på "Ledkroppsprincipen" Grundad Metod att Bestämma en Foderblandings smaltbarhet. Centralanstalten för Försöksväsendent på Jordbruksområdet. Stockholm Medd. Nr., 165:1.

Edin, H. G., Kihlen, G., and Nordfeldt, S. (1944). A Summarized Description of "Edin's Indicator Method" for the Determination of the Digestibility of Feeds and Feed Mixtures. Ann. Agr. Coll. Sweden, 12:166.

Edwards, H. M., and Gillis, M. B. (1959). A Chromic Oxide Balance Method for Determining Phosphate. Poultry Sci., 38:569.

FAO (1965). Protein Requirements. F.A.O. Nutr. Mtg. Rep. Ser. 37.

FAO/WHO (1965). Protein Requirements: Report of a Joint FAO/WHO Expert Group. WHO Technical Rep. Ser. 301.

Fisher, R. B. (1954). Protein Metabolism. London: Methuen.

Harstook, W. E., and Mitchell, H. H. (1956). The Effect of Age on the Protein and Methionine Requirements of the Rat. J. Nutr., 60:173.

Kane, E. A., Jacobson, W. C., and Moore, L. A. (1950). A Comparison of Techniques Used in Digestibility Studies with Dairy Cattle. J. Nutr., 41:583.

Leveille, G. A., Sauberlich, H. E., and Shockley, J. W. (1961). Sulfur Amino Acid Requirements for Growth of Mice Fed Two Levels of Nitrogen. J. Nutr., 75: 455.

McCollum, E. V., and Steenbock, H. (1912). Studies of the Nutrition of the Pig. Wisconsin University Agr. Expt. Sta. Res. Bull., 21:53.

McLaughlan, J. M., and Illman, W. I. (1967). Use of Free Plasma Amino Acid Levels for Estimating Amino Acid Requirements of the Growing Rat. J. Nutr., 93:21.

Miller, D. S., and Bender, A. E. (1955). The Determinations of Net Utilization of Protein by a Shortened Method. Brit. J. Nutr., 9:382.

Mitchell, H. H. (1923). A Method of Determining the Biological Value of a Protein. J. Biol. Chem., 58: 873.

Mitchell, H. H., Hamilton, T. S., Beadles, J. R., and Simpson, F. (1945). The Importance of Commercial Processing for the Protein Value of Food Products. J. Nutr., 29:13.

NRC (1962). Nutritional Requirements of Domestic Animals. Number 10. Nutritional Requirements of Laboratory Animals. Publs. Natn. Res. Coun., Wash., 990.

Olson, N. (1950). Smaltbarhetsförsök med Fjärdefa. Kungl Lantbrukshögskolan och Statens Lantbruksförsök Statens Husdjursförsök. Stockholm Medd Nr., 43:1.

Osborne, T. B., Mendel, L. B., and Ferry, E. L. (1919). A Method of Expressing Numerically the Growth-Promoting Value of Proteins. J. Biol. Chem., 37: 223.

Payne, W. L., Kifer, R. R., Snyder, D. G., and Combs, G. F. (1971). Investigation of Apparent Amino Acid Digestibility of Fish Meal Protein Using Cecectomized Adult Male Chickens. Poultry Sci., 50:143.

Peng, Y. S. (1975). Department of Agricultural Biochemistry, University of Arizona. Personal communication.

Rama Rao, P. B., Metta, V. C., and Johnson, B. C. (1959). The Amino Acid Composition and the Nutritive Value of Proteins. I. Essential Amino Acid Requirements of the Growing Rat. J. Nutr., 69:387.

Rama Rao, P. B., Metta, V. C., and Johnson, B. C. (1964). The Amino Acid Composition and Nutritive Value of Proteins. IV. Phenylalanine, Tyrosine, Methionine and Cystine Requirements of the Growing Rat. J. Nutr., 73:38.

Reid, B. L. (1975). Department of Poultry Science, University of Arizona. Personal Communication.

Reid, B. L., and Weber, C. W. (1973). Dietary Protein and Sulfur Amino Acid Levels for Laying Hens During Heat Stress. Poultry Sci., 52:1335.

Rhode, C. M., Perkins, W. M., and Vars, H. M. (1949). Nitrogen Balances of Dogs Continuously Infused with 50 Per Cent Glucose and Protein Preparations. Am. J. Physiol., 159:415.

Rogers, Q. R., and Harper, A. E. (1964). Amino Acid Diets for Rats. Fedn. Proc. Fedn. Am. Socs. Exp. Biol., 23:186.

Rose, W. C., Oesterling, M. J., and Womach, M. (1948). Comparative Growth on Diets Containing Ten and Nineteen Amino Acids with Further Observations Upon the Role of Glutamic and Aspartic Acids. J. Biol. Chem., 176:753.

Rose, W. C., and Womach, M. J. (1946). The Partial Replacement of Dietary Phenylalanine by Tyrosine for Purposes of Growth. J. Biol. Chem., 166:429.

Schürch, A. F., Lloyd, L. E., and Crampton, E. W. (1950). The Use of Chromic Oxide as an Index for Determining the Digestibility of a Diet. J. Nutr., 41:629.

Snook, J. T. (1964a). The Role of the Gastrointestinal Tract in Protein Metabolism. Blackwell Scientific Publications, LTD., Oxford.

Snook, J. T. (1964b). Response of Digestive Enzymes to Dietary Protein. J. Nutr., 82:409.

Sure, B. (1955). Relative Nutritive Values of Proteins in Foods and Supplementary Values of Amino Acids in Pearled Barley and Peanut Flour. J. Agr. Food Chem., 3:789.

Thomas, K. (1909). Arch. Anat. Physiol. Abt. 219.

Whitson, D., Carrick, C. W., Roberts, R. E., and Hauge, S. M. (1943). Utilization of Fat by Chickens--A Method for Determining the Absorption of Nutrients. Poultry Sci., 22:137.

Womach, M., Harlin, A., and Lin, Peihsing (1953). The Influence of Non-Essential Amino Acids on the Requirements of the Adult Rat for Isoleucine, Methionine and Threonine. J. Nutr., 49:513.