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EVALUATION OF THE GROWTH-SLOPE ASSAY FOR THE DETERMINATION OF PROTEIN QUALITY IN CEREALS

by

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ABSTRACT

Development of triticale and high lysine mutants in maize and other cereal grains has focussed attention on methods for assessing protein quality. The purpose of the present study was to evaluate the suitability of the growth-slope assay and more specifically the relative protein value assay for determination of protein quality in cereal grains. Relative protein value (RPV) is the slope of the regression line for weight gain on nitrogen intake for a test protein expressed as a percent of the slope of the regression line for a reference protein. Several lines of wheat, rice, and triticale, together with wheat flour and wheat gluten, were assayed in a series of experiments with growing rats. The reference protein (casein) and each of the protein sources (except Intan rice) were substituted for the cornstarch in a protein-free basal diet at levels to provide 0.37, 0.80, and 1.28% dietary nitrogen. Intan rice which contained 0.92 % nitrogen, replaced 47, 62, and 100% of the starch. Groups of 12 weanling male Wistar rats, 4 per dietary protein level, were assigned to casein and each of the cereals being tested. A group of 4 rats also was assigned to the protein-free basal diet in each assay. The 4 rats were housed individually, and weight and feed consumption were measured during a 14-day period. The data was subjected to multiple regression analysis to evaluate the effect of initial weight as well as nitrogen intake

on growth response. Initial weight affected the growth response per unit of nitrogen consumed. The goodness of fit of the regression line of weight gain on nitrogen intake was improved when initial weight of the rats was taken into consideration. Both multiple and simple regressions were essentially linear for all cereals, and the inclusion of the data for the protein-free treatment group in the calculation had only minimal effects on the slope and intercept of the regression lines, and correlation between nitrogen intake and weight change. Some problems were encountered in the assay. In the assessment of protein quality of rice, the RPV of Intan rice was 108 compared to casein set at 100. In addition, duplicate RPV's were not obtained for wheat flour in 2 separate experiments, and the variability among the four casein groups raises the question of its suitability as a reference protein. Nevertheless, the RPV assay appeared to distinguish among the various cereals and cereal products on the basis of protein quality.

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INTRODUCTION

Concern over malnutrition in the less developed countries, in particular protein-calorie malnutrition, together with the fact that cereals frequently provide a substantial proportion of the dietary protein in these countries, has brought considerable emphasis on the development of cereal varieties with high protein contents of improved nutritional quality. The development of triticale and high lysine mutants in cereal grains such as maize and barley, has focused attention on the biological methods for assessing protein quality. Miladi and Hegsted (1972) have suggested that none of the methods currently available for the evaluation of protein quality are entirely satisfactory.

The relative growth-slope assay was first proposed by Hegsted and Chang (1965) as a more satisfactory method of protein evaluation than PER and other commonly used rat growth assays. The International Union of Nutritional Scientists and the Protein-Calorie Advisory Group (PAG) of the United Nations, currently consider the slope ratio assay the preferred rat assay for protein evaluation. There has been debate over two factors in the growth-slope assay. Concern has been expressed over the range of protein intakes for which there is a linear relationship between growth and nitrogen intake. The other question that has been raised is whether the protein-free treatment group should be included in calculating the growth

response in the evaluation of cereals. The PAG of the U.N. has recommended that cereals be evaluated using diets providing 2, 5, and 8% protein. However, they have not made a recommendation concerning the inclusion of the protein-free group in the regression calculation.

The purpose of the present study was to evaluate the growth-slope assay for the determination of protein quality in cereals.

REVIEW OF LITERATURE

CEREAL PROTEIN SUPPLY

Cereals contribute more in the human diet to both calories and protein than any other single group of staples. The FAO Provisional World Plan for Agricultural Development, published in 1969, stated that cereals, particularly wheat, rice, maize, sorghum, millet and barley, provided more than 50% of calories and protein for the people of Africa south of the Sahara, more than 60% for the peoples of Asia, and more than 65% for the people of the near East.

Theoretically there is no protein shortage in the world (Porter, Rolls, 1973). Shellenberger (1973) states that there appears to have been a tendency in reports to overstate the deficiency of protein food supplies in developing countries. Based on FAO estimates and projections for the years immediately ahead, there is an adequate supply of protein to meet human needs. This does not mean that inadequate distribution, lack of purchasing power, and cultural practices will not result in protein malnutrition among children, especially in lower income families. Evidence indicates, for example, that protein-calorie malnutrition does exist in the less-developed countries (L.D.C.) and that it is most damaging to the preschool child (Hornstein, 1973). Statistically, two-thirds to four-fifths of all deaths that occur in the L.D.C.'s are among this group and some 30% of the children fail to reach the age

of five. The Pan-American Health Organization showed protein-calorie malnutrition to be either the primary cause or a major factor in these deaths.

Although cereals provide a substantial portion of the dietary protein in the L.D.C.'s, most of the common varieties of cereal grains are deficient in certain essential amino acids. The most common limitation is lysine (Mertz, 1974). Since grains are relatively low in cost and widely available as a source of protein, emphasis has been on the development of varieties with high protein content of improved nutritional quality. In 1964 Mertz et al. reported the effect of the opaque-2 gene on the quality of maize endosperm protein. The opaque endosperm contained 3.39 gm. lysine/100 gm. protein, whereas the normal endosperm contained 2.0 gm. lysine/100 gm. protein. Harpstead (1971) and a team of investigators in Columbia carried out nutritional evaluations of high lysine corn using pigs, in 1967. They demonstrated improved growth using high lysine corn as compared to regular corn. At the same time Pradillo et al. began a series of trials using the new corn in the treatment of children suffering from malnourishment. Their demonstration of the use of the new corn in "curing" children from malnourishment excited considerable interest in its value as a food. F. C. Byrnes in 1969 was also able to cure kwashiorkor in children, with a diet containing 8% protein, using opaque-2 endosperm cornmeal as the only source of protein (Mertz, 1974).

Since then an intense search for high protein quality

cereal mutants has been the focus in many plant breeding programs all over the world. In addition to the mutant genes contributing to high protein quality in maize, Mertz (1974) reported the discovery by Ingverson and co-workers in Denmark, and Axtel and co-workers in the United States, of high lysine mutants of barley and sorghum respectively. This raises the hope that all cereals of economic importance may eventually be improved.

Triticale, the first man-made cereal, has the same potential for high protein content with improved amino acid balance as has high-lysine corn. It is produced by cross-breeding of wheat and rye, and will grow on arid sandy soils, under climatic conditions which wheat will not tolerate. Under certain ecological conditions, its yield outperforms that of wheat and rye. In addition, the slightly higher lysine content has conferred a certain nutritional advantage over wheat.

TRITICALE

The development of triticale has raised the optimism of plant breeders, to the potential of improving the protein quality of cereal grains. Because triticale is relatively new it will be considered in more detail. The protein content of common wheat varieties varies from 6-23%, with an average of 12.9% on a dry weight basis (Hulse and Spurgeon, 1974). The protein content of rye is slightly lower, ranging from 6.5 to 15.0%. However, the biological value of protein in rye is higher than that of wheat primarily because of its higher lysine content. In some instances triticale appears to combine

the high total protein content of wheat and the high lysine content of rye.

The International Maize and Wheat Improvement Centre (CIMMYT) reports that several lines of triticale have a lysine content equal to that of high-lysine corn and a total protein content much higher than that of high-lysine corn. Preliminary tests of the 1972-73 crops indicated that some lines had lysine contents as high as 4.35% of the total protein, compared to the average lysine content of 3.14% for all triticale lines and 2.6% for wheat. In addition, the average protein content was slightly higher than for most of the common wheat varieties (Hulse and Spurgeon, 1974). Villegas et al. (1970) reported that spring wheat and durum wheat protein were approximately equal in lysine content on an equal nitrogen basis; triticale and rye protein were 20 and 30% higher, respectively.

Ashmed and McDonald (1974) calculated the chemical score for triticale grain and mill fractions, using egg protein as the reference. They suggested lysine as the first limiting amino acid and methionine second limiting. Kies and Fox (1970) found lysine to be the first limiting amino acid in both wheat and triticale for the adult human. Nitrogen retention improved for their subjects when lysine was added as a supplement either singly or in combination with tryptophan and methionine.

Knipfel (1969) evaluated the protein quality of each of the cereals, triticale, wheat, and rye, using the growing rat. The PER of triticale was equal to the PER of rye, whereas that

of wheat was significantly lower. When the cereals were fed at the 5% protein level and supplemented with 5% casein, the PER values for both supplemented triticale and supplemented rye were equal to the PER for casein fed alone, whereas the PER for the wheat-casein combination was lower. Examination of the amino acid levels in the test diets and in the blood plasma of rats fed these diets, indicated that lysine was less limiting in rye than in triticale and wheat. These observations raise the question of amino acid availability in cereals, since even though lysine appeared to be less limiting in rye than in triticale, growth was similar for the two diets.

EVALUATION OF CEREAL PROTEIN

The efforts of plant breeders to improve the quality of cereal protein has focussed attention on the factors that determine protein quality and the methods used to evaluate it. Protein quality may be defined as the capacity of a protein or mixture of proteins to satisfy the nitrogen and essential amino acid requirements of an organism. The quality of the protein is determined not only by the amino acids present, but also by the nutritional availability of these amino acids. Protein evaluation in the usual sense implies the determination of the nutritive value by direct biological methods or by indirect methods such as amino acid analysis. In the broader sense protein evaluation also includes appraisal of organoleptic qualities as well as appraisal of the possible toxic factors contained in certain protein foods (Porter and Rolls, 1973). All biological methods essentially are based on two

parameters, growth and nitrogen balance. There is considerable variation, however, in the growth and nitrogen balance assays, and controversy exists over the adequacy of the various methods. As many as 1500 investigations dealing with the nutritive value of protein have been reported (Hegsted and Chang, 1965). In spite of all this work, Hulse reported that the Protein-Calorie Advisory Group (PAG) of the United Nations, pointed out as late as 1971 the need for nutritional food quality guidelines including methods for protein evaluation. Miladi and Hegsted (1972) also have suggested that none of the methods currently available for the evaluation of protein quality are entirely satisfactory.

Ideally methods for evaluating protein quality should be quick, relatively inexpensive, and reproducible. If the food under study is for human use, the requirement of the test animal should bear a close relationship to that of the human. Although the growth rates for weanling rats and human infants differ widely, Bernhart (1970) found that they required similar amounts of nitrogen per gram of weight gain. In addition, they have similar average requirements for essential amino acids (except for S-amino acids) per gram of growth.

METHODS OF EVALUATING CEREAL PROTEIN

I. Amino Acid Score (Chemical Score)

The quality of protein in a food is dependent largely on the level of the most limiting amino acid present. The National Academy of Sciences (1963) has defined the limiting amino acid as: "the essential amino acid of a protein which shows the

greatest percentage deficit in comparison with a standard". The chemical score, or amino acid score as it is currently called, predicts protein quality from the amino acid profile of the test protein. The score is calculated by comparing the amount of each essential amino acid in the test protein to the amount of the amino acid found in the reference or standard protein using the formula:

$$\frac{\text{content of amino acid in test protein}}{\text{content of same amino acid in reference protein}} \times 100$$

The lowest score obtained for any of the essential amino acids, is the amino acid score for the test protein. Mitchell and Block (1946) first proposed the chemical score and suggested egg protein as the reference protein because of its known high biological value. Since then, various reference proteins have been suggested. In 1957 the FAO Committee on Protein Requirements proposed the use of a provisional reference pattern based on human amino acid requirements. It became evident that the FAO reference pattern contained excess tryptophan and sulphur containing amino acids and in 1965 the joint FAO/WHO Expert Group on Protein recommended that egg or human milk be adopted as the reference protein. This decision was again reversed in 1973 when the joint FAO/WHO Expert Committee proposed the amino acid scoring pattern based on the amino acid requirements of young children.

The validity of ideal amino acid patterns may be challenged, since many questions have been raised concerning protein metabolism, for which adequate answers are not available. Most animals have varying abilities to conserve specific amino

acids and the mechanisms controlling this process are unknown. Data indicate that amino acid requirements probably vary depending on the protein status of the subjects. In addition, there may be substantial differences in the pattern of amino acids required for growth and maintenance (Hegsted, 1971).

Amino acid scores do not consider the digestibility of the protein and the availability of the amino acids. Nevertheless, amino acid scores may be very useful as a preliminary screening method for determining the potential biological value of a protein and for focussing on the limiting amino acid. Rama Rao et al. (1964) used the pattern of essential amino acid requirements for the growing rat as the reference standard for estimating the nutritive value of a variety of proteins. They calculated the requirement index (geometrical mean of the essential amino acids, each expressed as a percentage of its standard value), for eleven proteins and found that the values obtained correlated highly with the published biological values for the protein. Hegsted (1971) suggested that amino acid scores may be the most logical method for evaluating protein quality. The development of automated amino acid analyzers has made the method more feasible.

II. Amino Acid Availability

The nutritive value of a protein is dependent not only on its content of essential amino acids but also the biological availability of these amino acids (Kuiken and Lyman, 1948). As discussed previously, both triticale and rye produced equivalent growth, but the triticale diet contained less lysine, suggest-

ing that the lysine in triticale was more available (Knipfel, 1969). Similarly, the lysine in opaque-2 corn was found to be more available than the lysine in normal corn (Klein et al, 1972).

Amino acids are unavailable if they are in regions of a protein protected chemically or physically from the action of proteolytic enzymes or if they are linked to other chemical moieties through bonds not readily hydrolyzed by digestive enzymes. Cross-linking is probably the most important chemical mechanism restricting biological utilization. Because of its epsilon-amino group, lysine is particularly susceptible to side reactions and cross-linking, thereby rendering it unavailable. Finley and Friedman (1973) have classified four ways in which lysine becomes nutritionally unavailable:

1. Lysine may be buried in a protein matrix in a particular sequence or conformation which is slow to hydrolyze or is not hydrolyzed at all by animal proteases. Such lysine may appear as chemically available by hydrolysis methods and yet be totally unavailable nutritionally.
2. Lysine can be cross-linked to an aspartyl or glutamyl residue on another protein or in the same protein molecule. The aspartyl or glutamyl-lysine complex remains after proteolysis and appears nutritionally unavailable.
3. Substantial losses of lysine may result when the protein is exposed to moderate heat under alkaline

conditions. Dehydroalanine formed under these conditions reacts with the epsilon-amino group to form lysinoalanine which is unavailable.

4. A more common reason for loss of lysine is the reaction of reducing and non-reducing sugars with lysine, rendering it unavailable.

Amino acid availability may be determined using both in vitro and in vivo methods. The in-vitro methods are based on: a) comparisons of the rates at which amino nitrogen or free amino acids are released from different proteins when they are incubated in vitro with proteolytic enzymes; or b) measurements of the percentage of free epsilon-amino groups of lysine in different proteins by the fluorodinitrobenzene (FDNB) procedure. The in vivo methods are based on fecal analysis, growth and nitrogen balance. The values for availability obtained by these methods differ considerably. The fecal analysis method measures the amount of unabsorbed amino acid and therefore is a measure of digestion and absorption, whereas growth and nitrogen balance assess not only digestibility but also the efficiency of utilization by the body of absorbed amino acids.

In general, fecal analysis methods give higher values than those obtained by the growth method. Kuiken and Lyman (1948) reported 92.8% availability for lysine in wheat based on fecal excretion in the rat. Similar values of 92.4% availability for lysine in wheat using young rats and 90.3% availability using old rats were reported by Giovanetti et al. (1970), based on fecal analysis. Gupta et al. (1958) however,

using the growth of young rats as an index of availability, reported 70% availability for lysine in wheat flour. Guthneck (1953) reported lysine in wheat germ to be 65% available based on gains in body weight of protein depleted adult rats. Calhoun et al. (1960), using weanling rats, observed 99% availability of lysine in a gluten basal diet based on fecal analysis, but 80% availability using growth assays. Such a wide discrepancy could indicate that factors other than digestion or absorption limits the availability of lysine from gluten. De Muelenaere et al. (1967), using the two methods, reported conflicting results. They observed that the availability of lysine in corn gluten, rice and rice protein, appeared to be lower with the fecal analysis method than with growth assays. The lower values for lysine by the fecal method could be explained either by growth stimulation due to factors other than lysine, or by factors which influence the fecal analysis method. The excretion of amino acids in the feces may be influenced by intestinal microbiological activity. If lysine is synthesized or destroyed by the intestinal microflora, it would result in respectively lower or higher values for availability. However, Kuiken (1952) was not able to detect any effect of intestinal microflora, but admitted that more work was needed in this before conclusions could be drawn.

Klein et al. (1972) found that the lysine in normal corn was 67 to 73% available, compared to 50% found by Gupta et al. (1958). Klein et al. used four levels of corn in their diets and suggested that Gupta et al., by using only one level of

corn, had too few observations on the growth curve to estimate lysine availability with the accuracy that four levels provided. This suggests that the slope-ratio technique, which is discussed later, may be suitable for testing amino acid availability.

III. Nitrogen Balance

Most classical methods for evaluating protein quality are based on the nitrogen balance technique. It is essentially the determination of nitrogen retained while the test protein is fed. The measure of nitrogen retained may be expressed in terms of the amount of nitrogen absorbed, as in biological value (B.V.), or it may be expressed in terms of the amount of dietary nitrogen ingested, as in net protein utilization (N.P.U.).

a) Biological Value - The B.V. of a protein is the percentage of absorbed nitrogen retained in the body, and is obtained by measuring fecal and urinary nitrogen when the test protein is fed and correcting for the amounts excreted when a nitrogen-free diet is fed. The assumption is made that the endogenous (fecal) and metabolic (urinary) nitrogen are constant and can be subtracted from the test values (Hegsted, 1971). There is limited information to suggest that this may not always be true. Brush et al. (1947) found a B.V. of over 100% for methionine alone by adding it to a nitrogen-free diet and thus decreasing nitrogen excretion. Biological value must be measured at or below the maintenance requirement and does not necessarily reflect the quality of the protein when it is

fed in sufficient amounts to allow for growth.

b) Net Protein Utilization - Like B.V., N.P.U. estimates nitrogen retention, but unlike B.V., it considers the digestibility of the protein. N.P.U. is defined as the percentage of the dietary nitrogen retained (Hegsted, 1971). The difference between the body nitrogen content of animals fed a nitrogen free diet and those fed a test protein, is divided by the amount of nitrogen consumed from the test protein. In animals of the same age, there is a high correlation between nitrogen and body water content (Hegsted and Neff, 1970). Thus the substitution of body water measurement for body nitrogen measurement has been suggested as a simpler method of estimating nitrogen retention.

Both B.V. and N.P.U. methods have been criticized for overestimating nutritional quality. Proteins completely lacking in an essential amino acid would have a chemical score of zero, yet, as pointed out by Hegsted (1971), Mitchell found gelatin to have a B.V. of 20%. Since animals will not survive on gelatin alone, this must be an over-estimation of the real nutritive value. Said and Hegsted (1970) concluded that a lysine-free diet will yield an N.P.U. of approximately 40, and is not characteristic of the protein quality. The theory that amino acid score, B.V. and N.P.U. measure the same thing, is based on the assumption that protein synthesis should be limited to an equal degree by a comparable degree of deficiency of any essential amino acid, and that protein synthesis should cease if the diet is devoid of any essential amino acid. Thus a diet

with an amino acid score of zero would be expected to be equivalent to a protein-free diet. This theory is not entirely compatible with experimental observations, since diets devoid of various amino acids do not produce comparable losses in body protein, and only in some instances are the losses comparable to those obtained with a nitrogen-free diet (Hegsted, 1971).

The measurement of B.V., N.P.U., and net protein ratio (N.P.R.) are all based upon the measurement of the differences in nitrogen retention between a nitrogen-free diet and a test diet. In essence they constitute a slope-ratio assay based upon 2 points only, a blank and an experimental point. It is assumed in all of these tests that nitrogen retention is linearly related to the amount of test protein fed. If this is not true, the tests are to some degree invalid (Said and Hegsted, 1969). The same assumption is made in the slope-ratio growth assay, to be discussed in a subsequent section, but it offers a means of estimating the degree to which the growth curve departs from linearity.

IV. Protein Efficiency Ratio (PER)

PER is defined as the ratio of body weight gain to the weight of protein consumed. Like any growth assay it assumes that weight gain reflects protein synthesis and although widely accepted, the PER method has been criticized for several aspects.

1. There is no proportional relationship among PER values. A protein with PER of two does not necessarily have twice the nutritive value of a protein

with a PER of one.

2. Results may be influenced by food intake.
3. Maximum PER values are not obtained at the same dietary protein level for different proteins.
4. No allowance is made for the maintenance of body tissue.

Hegsted (1974) concluded from a collaborative study among 8 laboratories that PER underestimated the nutritive value of low quality protein. In addition, PER determination showed the greatest variability among the 8 laboratories of the methods being tested.

V. Net Protein Ratio (NPR)

One of the major criticisms of PER methodology is the lack of any consideration for maintenance. The NPR assay attempts to overcome this criticism and is similar to PER except that the mean weight loss of a group of rats fed a protein-free diet is added to the mean weight gain of the test group. Thus allowance is made for body tissue replacement and maintenance.

VI. Relative Protein Value

The slope-ratio growth assay was proposed by Hegsted and Chang (1965) as a satisfactory method of protein evaluation. This method has recently been considered by the International Union of Nutritional Scientists because of the short-comings of the PER and NPR assays. These latter methods tend to rank proteins according to nutritive value, but frequently give

little information on the relative nutritive value of different proteins. The growth-slope assay is based on the response in weight gain to different protein intakes. Since it is assumed that the response is linear and thus directly related to the nutritional requirements of the organism, the assay overcomes many of the criticisms directed at PER and NPR assays.

The assay involves the calculation of a regression line $y = a + bx$ (where y is weight gain and x is protein intake), for body weight change of animals fed various levels of the test protein. The slope of the line (b) represents the nutritive value (NV) of the test protein. In a recent collaborative study, nutritive values varied among laboratories for a series of test proteins. It was suggested that these results were related to the variability of the animals used and perhaps the environmental conditions under which the tests were conducted (Samonds and Hegsted, 1974). When a reference protein was included, such as casein or lactalbumin, and the slope of the test protein expressed relative to that of the reference protein (RNV), the variation in some cases was reduced or eliminated. Inclusion of a reference protein was more successful in reducing laboratory or laboratory-protein variation when calculating RNV than was a similar correction for PER.

The International Union of Nutritional Scientists and The Protein-Calorie Advisory Group (PAG) of the United Nations have proposed the slope-ratio assay or relative protein value (RPV) as the preferred growth assay. The term RPV was used by the PAG rather than RNV. The basis of their proposal stems

from the results of a collaborative study in which 8 laboratories compared RPV with 3 other methods; NV, PER and NPR. The study indicated that all of the methods distinguished among proteins which differ appreciably in quality. The assay methods did not, however, rank the proteins in the same order except that all rated lactalbumin as the highest quality protein. The NV and RPV methods ranked defatted meat and casein next to lactalbumin, soy flour and soy isolate somewhat lower, but these assays did not rank all four proteins in the same order. The ranking pattern with the PER and NPR methods were even less consistent, suggesting that NV and RPV were preferred methods.

The slope-ratio assay is dependent on body weight change being linear over the range of intakes studied for the test protein. That this relationship holds has been demonstrated over a fairly wide range of protein intakes and with proteins of different quality (Samonds and Hegsted, 1974). This relationship may not extend to zero protein intake. There has been debate over two factors in the growth-slope assay. First, the range of protein intakes which will produce a linear relationship between growth and intake; and second, whether or not the zero protein level should be included in the calculation of the regression line. Studies show the importance of having a sufficient range of protein levels, since the particular levels selected may influence the slope of the line. Hegsted and Chang (1965) found regression lines to be essentially linear over a considerable range of protein intake, and antici-

pated little difficulty in selecting appropriate levels for assay. It must be recognized, however, that relatively high protein values were used. The lowest protein levels studied were 2.2, 3.4, 8.5, and 11.4% respectively for lactalbumin, casein, soy protein, and wheat gluten. They found that the y-intercepts of the regression lines for these protein sources tended to be slightly higher than that actually found with rats fed a protein-free diet, suggesting that there may be departure from linearity at low levels of protein intakes. Studies indicated that amino acids are conserved to varying degrees when they are in short supply (Young and Taylor, 1973). Diets free of an essential amino acid usually do not duplicate the results found with protein-free diets and this is particularly true for proteins deficient in lysine (Hegsted and Neff, 1970). This may explain why the dose response lines are not necessarily linear at very low levels of protein intake, and why intercepts of the regression line often fall above the point defined by feeding a protein-free diet (Samonds and Hegsted, 1974).

Bressani et al (1974) reported that the dietary protein intakes in the lower range (1, 2 and 3%), tended to overestimate the protein quality of cereals while dietary intakes in the higher range (3, 4 and 5%) in general underestimated the protein quality of cereals. The omission of the zero protein level generally resulted in an overestimation of the protein quality of cereals unless the calculation was based on a large number of protein levels. Nevertheless, inclusion of the values for animals fed the zero protein level seemed to be important when

the estimates of the intercepts for the test protein were not statistically different from those established experimentally regardless of the number of proteins used in the calculation. Hegsted and Juliano (1974) have offered an apparent explanation for the improvement of regression analysis when the zero-protein values with certain animals are included. They state that: "Since rice is believed to be limiting in lysine and at least sometimes limiting in threonine and perhaps other amino acids, the nature of the dose-response lines which may be expected is not clearly known. It should also be noted that when deviation from a common intercept is relatively small, forcing the lines through a common point serves to stabilize the regression line. Since the slope will be relatively inaccurate if the range of protein tested is limited, this may actually yield a better estimate of the true RNV even though it can not be strictly justified." On the other hand, Hegsted (1974) reported that the slope of the regression lines excluding the values for rats fed the zero protein diet appeared to be the most legitimate measure of protein quality on the basis of the collaborative study. He did not, however, present data on which he based his conclusions. McLaughlin and Campbell (1974) concluded that the slope ratio assay could be improved by omitting the group of rats fed the zero protein diet. They reviewed data available in the literature.

It is generally agreed that the narrower the range of protein intakes tested, the less accurate the estimate of protein quality. Thus when evaluating the protein quality of

cereals, the problem is compounded since both quantity and quality are low. Hegsted and Juliano (1974) encountered difficulties in assays of the nutritive quality of rice. Contrary to the expectations that regression analysis of proteins limiting in lysine would yield intercepts above the intercepts obtained with lactalbumin and a protein-free diet, lower intercepts were noted for Intan, IR22 and IR8 rice. On the other hand, the IR480-59 rice diet yielded a high value for the intercept, and predicted an actual weight gain at zero protein intake. Also, the RPV for Intan and IR8 rice were 92 and 98 respectively, which was considerably above the expected value. The RPV for IR480-59 rice was not improved when the sample was supplemented with lysine and threonine, even though these amino acids are limiting in rice. They found that by selection and/or manipulation of the data, they were able to produce results more consistent with expected values. Although there are obvious limits to the amount of manipulation which may be done, they justify it on the basis that methodology of protein evaluation is in a state of flux and that some insights into the problems encountered may be gained by careful inspection instead of blind acceptance of the data.

SUGGESTED METHODOLOGY

The methodology for the determination of protein quality is in a state of flux. There is controversy related to the most appropriate assay method. Considerable research is still required before the assay procedures for the biological evaluation of protein quality can be standardized. Nevertheless,

the PAG of the United Nations presently regards the RPV assay as the preferred rat assay. They recommend that proteins be evaluated at the 2, 5, and 8% protein level, and suggest that casein (ANRC high nitrogen casein) be used as the reference protein. They have not made a recommendation concerning the inclusion or exclusion of the zero protein level, which suggests that additional research is needed.

METHODS AND MATERIALS

A number of cereals (Table 1) were evaluated for protein quality using the growth-slope assay as recommended by the Protein Advisory Group of the United Nations System in PAG Guideline 16: Protein Methods for Cereal Breeders related to human nutritional requirements. In a preliminary study, wheat gluten supplemented with lysine was fed with the object of determining if weight gain was linear with protein intake in the growth slope assay. Since there was an apparent difference in nutritive value among triticale samples grown in different localities, digestibility studies were carried out on these samples.

ANIMALS

Weanling male Wistar rats were purchased from Canadian Breeder Laboratories, St. Constant, Quebec. The body weight of the rats at the time of assigning to the reference diet ranged from 37 to 63 gm.

PRE-EXPERIMENTAL HANDLING OF RATS

The rats were housed 8 - 10 per cage (75 x 42 cm.) and fed rat-chow[1] for one day. They were then randomly allotted to individual screen-bottom cages (20 x 25 cm.) and fed the 8%-reference protein diet for a two-day adjustment period.

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[1] Purina Laboratory Rat Chow.

TABLE 1

DESCRIPTION OF CEREALS EVALUATED

Cereal	Nitrogen %	Source
Rice		
IR480-59	1.616	Dr. Bienvenido Juliano
IR8	1.488	International Rice
Intan	0.922	Research Station,
BPI-76-1	2.058	Phillipines
Wheat -		
Grown in Manitoba		Dr. L. E. Evans,
Neepawa	2.752	Department of Plant Science
Glenlea	2.599	University of Manitoba
Lemli	1.0904	Winnipeg, Manitoba
Wheat -		
Grown in Nebraska		Dr. Paul Mattern
A-73-3	2.875	Department of Agronomy
A-73-1	2.726	University of Nebraska
73-5916	2.042	Lincoln, Nebraska
Wheat Flour -		
From Nebraska Wheat		Dr. Paul Mattern
A-73-3	2.405	Department of Agronomy
A-73-1	2.538	University of Nebraska
73-5916	1.732	Lincoln, Nebraska
Triticale - Rosner		
Grown in:		Dr. E.N. Larter and
Manitoba	2.285	Dr. P. Gustafson
Tule Lake, California	2.256	Department of Plant Science
Davis, California	2.395	University of Manitoba
		Winnipeg, Manitoba
Triticale		
Composite[1] 18% Prot.	3.272	Dr. P. C. Williams
Composite[1] 10% Prot.	1.880	Grain Research Laboratory
		Canadian Grains Commission
		Winnipeg, Manitoba

[1]Composite samples prepared to provide 18 and 10% protein levels.

ANRC casein[1] was the reference protein except in experiments 1 and 2 where vitamin-free casein[2] was used. No difference was found between the two sources of reference protein although the PAG has recommended ANRC casein as the reference protein. Room temperature was thermostatically controlled at 24°C. Lighting consisted of a 12-hour light and dark cycle. Diets and water were given ad-libitum.

DIETS

The basal diet (Table 2) which was the same for all experiments, served as the 0% protein diet.

The cereals (Table 1) and casein were added to the basal diet, at the expense of starch, to provide 0.32%, 0.80%, and 1.28% nitrogen. Intan rice, because of its low protein content, was fed at 0.32%, 0.48%, and 0.80% nitrogen.

The effect on growth, of lysine supplementation of wheat gluten[3] and enriched wheat flour,[4] was studied. Lysine, as lysine-monohydrochloride, was added to the wheat gluten diets at levels of 146.5, 97.7, and 48.8 mg./100 gm. of nitrogen in experiment 1 (Table 3), and to wheat flour diets at levels of 195.3 and 97.7 mg./100 gm. of nitrogen in experiments 2 and 3 (Table 4 and Table 5). In experiment 3, the effect of supple-

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[1]ANRC High nitrogen casein, Sheffield Chem. Co., Norwich, N.Y.

[2]Vitamin Test Casein, Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.

[3]Vital Wheat Gluten - Industrial Grain Products Ltd., Montreal.

[4]Robin Hood, Enriched Wheat Flour.

TABLE 2

COMPOSITION OF BASAL DIET

Ingredients	% of Diet
Wheat Starch	81.0
Mineral Mix[1]	2.7
Vitamin Mix[2]	1.0
Alphacel[3]	5.3
Corn Oil[4]	10.0

[1]Supplies per Kg. of diet: 6.0 gm. calcium, 5.0 gm. phosphorus, 500 mgm. sodium, 1.8 gm. potassium, 500 mgm. chlorine, 400 mgm. magnesium, 50 mgm. manganese, 25 mg. iron, 12 mgm. zinc, 5 mgm. copper, 0.15 mgm. iodine, 1.0 gm. sulfate.

Levels recommended by: Beare-Rogers, J.L., Nera, E.A.

[2]Supplies per Kg. of diet: 10 mgm. thiaminhydrochloride, 10 mgm. riboflavin, 10 mgm. pyridoxine hydrochloride, 30 mgm. calcium pantothenate, 500 mgm. inositol, 50 mgm. niacin, 100 mgm. para-amino benzoic acid, 0.2 mgm. biotin, 0.02 mgm. vitamin B_{12}, 2.0 gm. choline bitartrate, 1.5 mgm. vitamin A, 0.025 mgm. vitamin D_2, 91.0 mgm. dl-L-tocopherol, 5.0 gm. menadione, 2.0 mgm. folic acid.

Levels recommended by: Bernhart, F.W., Tomarelli, R.M.

[3]I.C.N. - Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A.

[4]Mazola Corn Oil.

menting wheat with threonine and lysine was studied. Threonine was added to the 8% protein diet at a level of 62.5 mg./gm. nitrogen (Table 6).

TABLE 3

DETAILS OF LYSINE SUPPLEMENTATION
EXPERIMENT 1

Protein Source	Diet (% Protein)	Amount of Lysine.HCl. Added to Diet mg./100 gm.
Wheat Gluten	2.0	15.62
	2.0	31.25
	2.0	46.87
	5.0	39.00
	5.0	78.00
	5.0	117.50
	8.0	62.50
	8.0	125.00
	8.0	187.50

TABLE 4

DETAILS OF LYSINE SUPPLEMENTATION
EXPERIMENT 2

	Protein in Diet			
		Analyzed[1]		
Protein Source	Calculated %	Replic.1 %	Replic.2 %	Amt. of Lysine.HCl. Added to Diet mg./100 gm.
Wheat Flour	2.0	2.06	2.27	31.25
	2.0	2.04	2.25	62.50
	5.0	5.75	5.29	78.13
	5.0	5.69	5.36	156.25
	8.0	8.64	7.83	125.00
	8.0	8.72	8.10	250.00

[1]Analyzed with addition of lysine.

TABLE 5

DETAILS OF LYSINE SUPPLEMENTATION
EXPERIMENT 3

Protein Source	Protein in Diet: Calculated %	Protein in Diet: Analyzed[1] Replic.1 %	Protein in Diet: Analyzed[1] Replic.2 %	Amt. of Lysine.HCl. Added to Diet mg./100 gm.
Wheat Flour	2.0	2.39	2.48	31.25
	2.0	2.45	2.46	62.50
	5.0	5.47	5.56	78.13
	5.0	5.53	5.63	156.25
	8.0	8.44	8.77	125.00
	8.0	8.53	8.84	250.00

[1]Analyzed with addition of lysine.

TABLE 6

DETAILS OF LYSINE AND THREONINE SUPPLEMENTATION
EXPERIMENT 3

Protein Source	Protein in Diet: Calc. %	Protein in Diet: Analyzed[1] Replic.1 %	Protein in Diet: Analyzed[1] Replic.2 %	Amount of Lysine.HCl. Added to Diet mg./100 gm	Amount of Threonine Added to Diet mg./100 gm.
Wheat Flour	8	8.57	8.63	-	80.0
	8	8.70	8.87	125.00	80.0
	8	8.93	8.99	250.00	80.0

[1]Analyzed with addition of lysine and threonine.

The nitrogen content of all diets was established using the Kjeldahl procedure. The AOAC method (1965) for total nitrogen was modified as follows: the mercuric oxide and potassium sulphate were replaced by 2 gm. of a pre-mixed catalyst.[1]

EXPERIMENT 1

Following the 2-day adjustment period on the 8% protein (1.28% N) casein reference diet, the rats were assigned to groups of 4 animals each, such that mean weights were similar among groups. The mean initial weight of the groups was 48.4 gm. with a range in mean weight from 47.4 to 49.4 gm. These outcome groups were randomly assigned to the various diets (Table 7). Feed and water were supplied ad libitum. Feed consumption and weight gain were measured on days 3, 7, 10 and 14. Spilled feed was collected daily from papers placed under the cages and, unless soiled, returned to the feeders.

TABLE 7

DESCRIPTION OF DIETS USED
EXPERIMENT 1

Protein Source	Protein in Diet		Amount in the Diet	
	Calculated %	Analyzed %	Protein Source[1] %	Starch %
Wheat Gluten	2.0	2.31	2.4	78.6
	5.0	5.08	6.1	74.9
	8.0	7.79	9.7	71.3

[1]Added to basal diet at expense of starch.

[1]#4 Kel-pak - Canadian Laboratory Supplies Ltd.

Regression co-efficients of weight gain on nitrogen intake over a 14-day experimental period were calculated for each cereal. In addition, multiple regression coefficients were calculated for initial weight, nitrogen intake, and weight gain, using the statistical package for the social sciences SPSSH - Version 5.01

EXPERIMENTS 2 and 3

Experimental procedures were identical to experiment 1 except that the rats were assigned two per group in each of two replicates. The cereals tested are listed in Table 8 and Table 9. Statistical analysis was based on the combined data in each experiment. The mean initial weight of the groups in experiment 2 was 61.2 gm. with a range in mean weight from 59.4 to 63.2 gm. In experiment 3 the mean initial weight of the groups was 56.3 gm. with a range in mean weight from 55.0 to 57.6 gm.

EXPERIMENT 4

Experimental procedure was identical to experiment 1 except for the cereals tested (Table 10). The mean starting weight of all groups was 53.0 gm. with a range from 53.4 to 52.5 gm.

EXPERIMENT 5

Protein digestibility was tested in the triticale samples by feeding diets containing 8% protein, in an effort to determine if digestibility contributed to the observed variation in apparent protein quality of triticale grown at different

TABLE 8

DESCRIPTION OF DIETS USED
EXPERIMENT 2

Protein Source	Protein in Diet			Amount in the Diet	
		Analyzed			
	Calc. %	Replic.1 %	Replic.2 %	Protein Source[a] %	Starch %
Wheat Flour	2.0	1.93	2.26	16.33	64.67
	5.0	5.66	5.21	40.80	40.20
	8.0	8.51	7.82	65.30	15.70
Triticale-Rosner Grown in Davis California	2.0	2.25	2.35	13.35	67.65
	5.0	5.31	5.20	33.40	47.60
	8.0	7.76	8.11	53.44	27.56
Grown in Tule Lake California	2.0	2.29	2.35	14.19	66.81
	5.0	5.27	5.25	35.46	45.54
	8.0	8.14	7.92	56.74	24.26
Grown in Manitoba	2.0	2.32	2.42	14.00	67.00
	5.0	5.34	5.32	35.00	46.00
	8.0	8.27	8.00	56.00	25.00
Rice IR480-5-9	2.0	2.19	2.29	19.80	61.20
	5.0	5.14	5.19	49.50	31.50
	8.0	8.66	8.36	79.30	1.70
IR 8	2.0	2.45	2.39	21.50	59.50
	5.0	5.28	5.42	53.80	27.20
	8.0	7.77	7.72	81.00	-
Intan	1.5	2.26	2.41	34.60	46.40
	2.0	3.17	3.15	50.60	30.40
	5.0	4.74	4.79	81.00	-
BPI-7-6-1	2.0	2.56	2.58	15.60	65.40
	5.0	5.82	6.06	38.90	42.10
	8.0	9.42	9.10	62.20	18.80
Casein	2.0	2.07	2.54	2.40	78.60
	5.0	5.09	5.29	5.90	75.10
	8.0	8.34	8.97	9.50	71.50
Basal Diet	0.0	0.32	0.26	-	81.00

[a]Added to basal diet at expense of starch.

TABLE 9

DESCRIPTION OF DIETS USED
EXPERIMENT 3

Protein Source	Protein in Diet			Amount in the Diet	
		Analyzed			
	Calc. %	Replic.1 %	Replic.2 %	Protein Source[a] %	Starch %
Wheat Flour	2.0	2.35	2.43	16.33	64.67
	5.0	5.44	5.58	40.80	40.20
	8.0	8.45	8.63	65.30	15.70
Manitoba Wheat					
Glenlea	2.0	2.35	2.27	12.31	68.69
	5.0	5.11	5.31	30.77	50.23
	8.0	7.91	8.01	49.23	31.77
Neepawa	2.0	2.31	2.33	11.63	69.37
	5.0	5.20	5.12	29.07	51.93
	8.0	7.99	8.27	46.51	34.49
Lemli	2.0	2.25	2.36	16.81	64.19
	5.0	5.10	5.38	42.02	38.98
	8.0	8.05	8.23	67.23	13.77
Nebraska Wheat					
A73-1	2.0	2.28	2.41	11.74	69.26
	5.0	5.13	5.31	29.34	51.66
	8.0	7.96	8.25	46.95	34.05
A73-3	2.0	2.24	2.33	11.13	69.87
	5.0	5.19	5.42	28.07	52.93
	8.0	8.31	8.52	44.51	36.48
A73-5916	2.0	2.29	2.32	15.67	65.33
	5.0	5.08	5.25	39.19	41.81
	8.0	8.08	8.19	62.69	18.31
Casein Vitamin Free					
Casein	2.0	2.50	2.50	2.40	78.60
	5.0	5.48	5.61	5.90	75.10
	8.0	8.35	8.80	9.50	81.00
ANRC Casein	2.0	2.41	2.38	2.25	78.75
	5.0	5.28	5.33	5.55	75.45
	8.0	8.09	8.61	8.89	72.11
Basal Diet	0.0	0.33	0.25	-	81.00

[a]Added to basal diet at expense of starch.

TABLE 10

DESCRIPTION OF DIETS USED
EXPERIMENT 4

Protein Source	Protein in Diet		Amount in the Diet	
	Calculated %	Analyzed %	Protein Source[a] %	Starch %
Triticale 18	2	2.48	9.78	71.22
	5	5.58	24.45	56.55
	8	8.61	39.12	41.88
Triticale 10	2	2.39	17.02	63.98
	5	5.43	42.54	38.46
	8	8.11	68.07	12.93
Nebraska Wheat Flour A73-1	2	2.39	12.61	68.39
	5	5.47	31.52	49.48
	8	8.49	50.43	30.57
A73-3	2	2.41	13.31	67.69
	5	5.59	33.27	47.73
	8	8.66	53.23	27.77
A73-5916	2	2.40	18.48	62.52
	5	5.45	46.19	34.82
	8	8.61	73.89	91.17
ANRC Casein	2	2.39	2.25	78.75
	5	5.59	5.55	75.45
	8	8.55	8.89	72.11
Basal Diet	0	0.32	-	81.00

[a]Added to basal diet at expense of starch.

locations.

Rats which had been fed diets containing 8% protein in the second replicate of experiment 3, were assigned at the completion of the experiment to groups of 4 each, such that mean weights were similar among groups. The mean weight of all groups was 64.9 gm. with a range from 64.0 to 65.5 gm. The outcome groups were randomly assigned to the various triticale diets (Table 11). The diets were fed ad libitum for 8 days, followed by a protein-free diet for 6 days. Feces were collected during the last 5 days of the test diet and during the last 3 days of the protein-free regimen. Feed consumption was recorded for the 5-day collection period. Weight change was recorded for the 3-day pre-collection and 5-day collection period on the test diet and for the 6 days on the protein-free regimen.

TABLE 11

DESCRIPTION OF DIETS USED
EXPERIMENT 5

Protein Source	Protein in Diet		Amount in the Diet	
	Calculated %	Analyzed %	Protein Source[a] %	Starch %
Triticale Grown in Manitoba	8.0	8.55	56.00	25.00
Grown in Davis, Calif.	8.0	8.04	53.44	27.56
Grown in Tule Lake, Calif.	8.0	8.05	56.74	24.26
ANRC Casein	8.0	8.45	8.89	72.11

[a]Added to basal diet at expense of starch.

Feces were collected daily and dried in a force-air draft oven at 100°C for 24 hours. Daily fecal collections from each rat were combined for each period. The feces were then ground with an intermediate Wiley mill to pass a #40 screen. A representative sample was analyzed for total nitrogen by the Kjeldahl procedure.

True digestibility was calculated using the formula:

$$\text{True Digestibility} = \frac{I - (F - Fo)}{I} \times 100$$

where I = daily nitrogen intake

F = daily fecal nitrogen excretion on test diet

Fo = daily fecal nitrogen excretion on protein-free diet

EXPERIMENT 6

The experimental procedure was the same as in experiment 5, except that the study was run simultaneous with experiment 4. The animals were placed on the protein-free regimen at the completion of experiment 4. The triticale samples tested are listed in Table 10.

RESULTS

EFFECT OF INITIAL WEIGHT

The weights of the animals used in the present experiments ranged from 37 to 63 gms. at the time that they were assigned to the reference protein diet, in spite of the fact that the purchase order specified a 10 gm. weight range. Thus multiple regressions[1] were calculated to determine the effect of initial weight, as well as nitrogen intake on the growth response. In addition, the data was subjected to simple regression[2] analysis where the effect of nitrogen intake only on weight gain was evaluated. Correlation coefficients of nitrogen intake on weight gain (Table 12) were higher for all cereals when tested by multiple regression than by simple regression. The difference between the correlation coefficient for the multiple and simple regression was significant ($P < 0.05$) for 25 of the 32 dietary treatments. These results suggest that initial weight affected growth response and efficiency of protein utilization, and therefore could affect the results when growth is used to evaluate protein quality.

In addition to improving the fit of the regression line,

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[1] $Y = \text{constant} + B_1 X_1 + B_2 X_2$
where Y = weight gain, X_1 = nitrogen intake,
X_2 = initial weight

[2] $Y = B_o + B_1 X$
where Y = weight gain, X = nitrogen intake

TABLE 12

EFFECT OF INITIAL WEIGHT ON THE REGRESSION COEFFICIENT

Exp.	Protein Source	Simple Regression: Regression Line	Simple Regression: R^1	Multiple Regression: Regression Line	Multiple Regression: R^1
1.	Wheat Gluten	$Y = -8.6 + 12.6X$	0.83	$Y = 0.7 + 13.1X_1 + (-0.2X_2)$	0.91
	Wheat Gluten & 0.62% Lysine[2]	$Y = -8.3 + 13.4X$	0.89	$Y = -0.6 + 14.1X_1 + (-0.2X_2)$	0.94
	Wheat Gluten & 1.25% Lysine[2]	$Y = -8.9 + 18.5X$	0.97	$Y = -5.5 + 18.7X_1 + (-0.1X_2)$	0.97
	Wheat Gluten & 1.88% Lysine[2]	$Y = -9.6 + 18.8X$	0.88	$Y = 0.9 + 19.0X_1 + (-0.2X_2)$	0.92
2.	Casein	$Y = -14.0 + 29.5X$	0.96	$Y = 15.5 + 29.6X_1 + (-0.5X_2)$	0.97
	Rosner Triticale				
	Grown in Man.	$Y = -13.7 + 20.0X$	0.93	$Y = 13.5 + 20.4X_1 + (-0.4X_2)$	0.98
	Tule Lake, Calif.	$Y = -13.9 + 20.9X$	0.89	$Y = 12.1 + 21.0X_1 + (-0.4X_2)$	0.95
	Davis, Calif.	$Y = -14.9 + 22.6X$	0.89	$Y = 15.3 + 22.7X_1 + (-0.5X_2)$	0.96
	Wheat Flour	$Y = -13.4 + 15.9X$	0.87	$Y = 4.4 + 16.1X_1 + (-0.3X_2)$	0.93
	Wheat Flour & 1.25% Lysine[2]	$Y = -13.4 + 20.6X$	0.96	$Y = 2.2 + 21.1X_1 + (-0.3X_2)$	0.98
	Wheat Flour & 2.50% Lysine[2]	$Y = -13.9 + 20.2X$	0.96	$Y = 0.8 + 20.6X_1 + (-0.2X_2)$	0.98
	Rice IP480-59	$Y = -13.0 + 23.2X$	0.98	$Y = 2.5 + 23.1X_1 + (-0.2X_2)$	0.99
	Rice IR8	$Y = -14.1 + 24.9X$	0.97	$Y = 1.0 + 25.3X_1 + (-0.2X_2)$	0.98
	Rice BPI 76-1	$Y = -12.6 + 19.8X$	0.96	$Y = -0.5 + 19.7X_1 + (-0.2X_2)$	0.96
	Rice Intan	$Y = -15.3 + 32.2X$	0.93	$Y = -1.3 + 32.3X_1 + (-0.2X_2)$	0.95

[1] Correlation Coefficient

[2] Based on % of protein

continued

TABLE 12 - Continued

Exp.	Protein Source	Simple Regression Regression Line	Simple Regression R^1	Multiple Regression Regression Line	Multiple Regression R^1
3.	Casein	$Y = -12.9 + 30.2X$	0.97	$Y = 14.0 + 29.9X_1 + (-0.5X_2)$	0.98
	ANRC-Casein	$Y = -11.8 + 31.6X$	0.95	$Y = 20.0 + 31.9X_1 + (-0.6X_2)$	0.97
	Wheat Flour	$Y = -10.5 + 14.5X$	0.84	$Y = 5.2 + 14.6X_1 + (-0.3X_2)$	0.91
	Wheat Flour & 1.25% Lysine[2]	$Y = -12.0 + 19.5X$	0.94	$Y = 1.3 + 19.7X_1 + (-0.2X_2)$	0.95
	Wheat Flour & 1.88% Lysine[2]	$Y = -12.1 + 20.5X$	0.93	$Y = 2.3 + 20.7X_1 + (-0.3X_2)$	0.95
	Wheat - Lemli	$Y = -11.3 + 17.9X$	0.96	$Y = 0.9 + 18.1X_1 + (-0.2X_2)$	0.98
	Wheat - Glenlea	$Y = -11.5 + 17.6X$	0.90	$Y = 1.9 + 17.9X_1 + (-0.2X_2)$	0.94
	Wheat - Neepawa	$Y = -12.1 + 18.3X$	0.77	$Y = 8.5 + 18.7X_1 + (-0.4X_2)$	0.87
	Nebraska Wheat A73-1	$Y = -10.9 + 16.5X$	0.79	$Y = 17.8 + 16.6X_1 + (-0.5X_2)$	0.92
	A73-3	$Y = -11.9 + 16.9X$	0.89	$Y = 7.2 + 16.9X_1 + (-0.3X_2)$	0.93
	A73-5916	$Y = -11.4 + 18.6X$	0.85	$Y = 10.4 + 19.0X_1 + (-0.4X_2)$	0.90
4.	ANRC Casein	$Y = -10.1 + 28.5X$	0.98	$Y = 0.4 + 28.7X_1 + (-0.2X_2)$	0.98
	Nebraska Flour A73-1	$Y = -9.8 + 13.2X$	0.90	$Y = -0.6 + 13.2X_1 + (-0.2X_2)$	0.91
	A73-3	$Y = -9.8 + 13.5X$	0.93	$Y = 0.8 + 13.6X_1 + (-0.2X_2)$	0.95
	A73-5916	$Y = -9.4 + 13.8X$	0.94	$Y = 0.9 + 13.9X_1 + (-0.2X_2)$	0.96
	Triticale 10% Protein	$Y = -10.3 + 18.6X$	0.98	$Y = -0.9 + 18.7X_1 + (-0.2X_2)$	0.99
	Triticale 18% Protein	$Y = -10.4 + 17.9X$	0.97	$Y = -9.4 + 17.9X_1 + (-0.0X_2)$	0.97

[1]Correlation Coefficient

[2]Based on % of protein

adjustment for initial weight in the calculation of the regression, also reduced the variances for the regressions as tested by Bartlett's Test of homogeneity of variance. The variances for the multiple regressions were statistically similar for the regressions within each group of cereals (i.e., samples of rice, triticale, Manitoba wheat, Nebraska wheat, etc.), although the variances were not necessarily similar among the different types of cereals tested (i.e., rice compared to the Nebraska flour). The only exception to this general pattern was for the wheats grown in Manitoba where the variances among Lemli, Glenlea and Neepawa wheat (experiment 3) were significant ($P < 0.05$). On the other hand, the variances for the simple regressions were different not only for the Manitoba wheats, but also for the casein group and the wheat gluten diets. Since adjustment for initial weight improved the correlation coefficients and reduced the within group variance of the regressions, the present data supports the recommendation of the PAG of the U.N. that variation in the initial weight of the rats used in the growth slope assay should not be greater than 6 gms. In view of the wide range in initial weights of rats in the present study however, these recommendations may not always be practical.

As mentioned earlier, the variances for the simple regressions were different for the casein diets. There were significant differences between the variances for ANRC-casein in experiments 3 and 4 and between the variances for the vitamin-free test casein diets in experiments 2 and 3. Thus

the differences in the variances of animals fed the casein diets in experiments 2, 3 and 4 could not be attributable to casein source. Since the 4 casein diets were fed under the same experimental conditions, it would appear that initial weight or some other differences among the various lots of rats account for the differences in the variances among the casein groups. This explanation is not entirely adequate, however, since the variances for other treatment groups such as Rice 1R8 and 1R480-5-9 did not differ even though the range in initial weight of the rats was as great as that of rats fed the casein diets (Table 13). Equivalent growth rates are expected among animals in different experiments when the protein sources are the same in otherwise comparable diets. The variability in growth response observed in the present study points up a distinct problem in the growth-slope assay as well as in other growth assays such as PER. The effect of initial weight on growth rate per unit of protein generally is not considered in the PER assay (NRC 1963). Thus PER values may be influenced by the weight of the animals used.

Statistical analysis of multiple regressions is very complex and time-consuming. Although the data from the present studies indicated that initial weight influenced growth per unit of protein, the limited data available from the present experiments did not seem to warrant the time required for multiple regression analysis. Consequently all further comment is based on simple regression analysis of weight gain on nitrogen intake.

TABLE 13

INITIAL AND FINAL WEIGHTS FOR ANIMALS RECEIVING 4 PROTEIN SOURCES

Exp. No.	Protein Source	2% Dietary Protein		5% Dietary Protein		8% Dietary Protein	
		Initial Weight gm.	Final Weight gm.	Initial Weight gm.	Final Weight gm.	Initial Weight gm.	Final Weight gm.
2	Casein	61.5	58.5	64.5	69.0	63.0	103.5
		67.0	56.0	65.0	65.5	72.0	110.5
		56.0	50.0	50.0	67.5	54.0	98.5
		57.0	52.0	62.0	82.0	59.0	118.5
3	Casein	49.5	44.5	55.5	74.0	54.5	98.0
		61.5	59.0	57.0	71.0	57.0	93.0
		58.5	51.0	58.5	60.5	60.5	104.0
		59.0	52.0	58.5	60.0	53.0	104.0
2	Rice 1R8	62.5	55.5	64.0	67.0	62.0	80.5
		68.5	61.5	71.0	80.5	68.5	92.0
		57.0	50.0	50.5	58.5	51.0	75.5
		57.0	47.0	63.0	70.5	67.5	101.5
2	Rice 1R480-59	62.0	54.0	64.5	73.0	63.0	99.0
		70.5	65.0	69.5	69.0	65.5	101.0
		55.0	50.5	51.5	58.0	52.0	80.5
		52.5	45.5	60.0	65.5	59.0	81.5

EQUALITY OF SLOPES

The slope of the regression line calculated for weight gain per unit of nitrogen intake reflects the quality of the protein in the test diet. The higher the quality of the protein in the cereal, the greater the growth per unit of nitrogen and thus the greater the slope value for the regression of weight gain on nitrogen intake. Thus differences in quality of protein can be determined by assessing the equality of the slopes of the regression lines.

For purposes of analysis and comparisons, the cereals were grouped as follows:

a) Wheats grown in Manitoba and Nebraska.

b) Flour milled from wheats grown in Nebraska.

c) Triticale - Rosner, grown in Manitoba; Tule Lake, California; and Davis, California;
- 2 composite samples designed to contain 10 and 18% protein, grown in Colorado.

d) Rice - Intan, BPI, 1R480-5-9, 1R8.

e) Enriched wheat flour (duplicate samples from experiments 2 and 3) supplemented with 1.25% lysine (78 mg. lysine/gm. nitrogen) and 2.50% lysine (156 mg. lysine/gm. nitrogen).

f) Wheat gluten supplemented with 0.62% lysine (39 mg. lysine/gm. nitrogen), 1.25% lysine (78 mg. lysine/gm. nitrogen) and 1.88% lysine (117 mg. lysine/gm. nitrogen).

g) Casein - 2 samples of ANRC-Casein and 2 samples of vitamin-free test casein.

Since the within group variances of the regressions (Bartlett's test) were not different for the wheats grown in Manitoba and Nebraska, the flours from Nebraska wheats, rice diets and the enriched wheat flour diets, the equality of the

slopes within each of these 4 groups were tested as outlined in Appendix Table 6. Protein quality was similar ($P < 0.05$) for the wheats grown in Manitoba and Nebraska and for the flours from the Nebraska wheats, as reflected by the similar slopes for each of the protein sources within these two groups. Protein quality was not similar for the rice samples and for the enriched wheat flour diets. To determine which of the protein sources were different the slopes within each of these two latter groups were compared using the formula:

$$\hat{B}_1^{(1)} - \hat{B}_2^{(2)} \pm t_{[df]}\alpha/2 \sqrt{\frac{SS(Res_1) + SS(Res_2)}{df}\left[\frac{1}{\sum_i x_{1i}^2} + \frac{1}{\sum_i x_{2i}^2}\right]}$$

accept Ho $\sigma_1^2 = \sigma_2^2 = \sigma_3^2 \ldots . \sigma_a^2$

C.I. 95%

Since the differences of the variances in the regressions for the groups fed the casein diets, triticale diets, and wheat gluten diets were significant, the equality of the slopes could not be tested with the method outlined in Appendix Table 6. Instead the slopes within each of these 3 groups were compared using the formula:

$$\hat{B}_1^{(1)} - \hat{B}_2^{(2)} \pm t_{[df]}\alpha/2 \sqrt{\frac{MS(Dev_1)}{\sum_i x_{1i}^2} + \frac{MS(Dev_2)}{\sum_i x_{2i}^2}}$$

reject Ho $\sigma_1^2 = \sigma_2^2 = \ldots . \sigma_a^2$

C.I. 95%

It must be recognized that this is an approximate test since it is not known to what extent the variability of the regres-

sion lines differed within each of these treatment groups. In addition to the above mentioned groups, the slopes for the wheats grown in Nebraska and the respective flours milled from these wheats also were compared using the approximate test.

The application of these tests is most appropriately discussed by looking at casein and each group of cereals or cereal products separately.

Casein

The ANRC-high nitrogen and vitamin-free test caseins were each used as the reference protein in two experiments. Although the PAG of the U.N. has recommended ANRC-high nitrogen casein as the reference protein, there were no differences ($P < 0.05$) in protein quality among the casein diets (Table 14). Thus the data for the casein groups was pooled and all further comparisons and comments are based on the regression of the pooled data.

TABLE 14

SLOPES OF THE REGRESSION LINES FOR THE CASEIN DIETS

	Experiment			
	2	3	3	4
	Test Casein	Test Casein	ANRC-Casein	ANRC-Casein
Slope	29.5_a	30.2_a	31.7_a	28.5_a

Different letters indicate significant difference ($P < 0.05$)

Rice

The test for slope comparisons indicated that there was a difference ($P < 0.05$) in the protein quality of the various rice samples, and that the protein in Intan rice is superior to the protein in the other 3 rice samples (Table 15). Each of the slopes of the regression lines for the rice diets was also compared to the slopes of the regression line for the casein diets. The results (Table 16) suggested that the protein in Intan rice is superior even to that of casein. However, total growth was poorer for Intan rice than for any of the other rice samples tested (Appendix Table 2). Due to the low protein content (5.8%), Intan rice supplied a maximum of only 4.7% dietary protein, even when it made up 81% of the diet. Hegsted and Juliano (1974) also found that the slopes for Intan and 1R8 rice were "above expectations". They concluded, although none of the regression lines showed any significant degree of curvature, that the levels of protein tested for Intan and 1R8 rice were too low, and that the points fell on the curvilinear portion of the growth response line. They based their conclusion on the fact that the weight loss of the animals receiving the lowest protein level studied, was not different from the weight loss of the animals receiving a protein-free diet. As a consequence, Hegsted and Juliano (1974) calculated the regression for Intan and 1R8 rice using only the data from the two groups of animals receiving the highest levels of protein and obtained slopes which were "seemingly more reasonable". In the present study, the weight

TABLE 15

PROTEIN CONTENT OF THE RICE CEREALS, AND SLOPES OF THE REGRESSION LINES FOR THE RICE DIETS EXPERIMENT 2

Cereal	% Protein	Slope
Intan	5.8	32.2_a
1R8	9.3	24.9_b
1R480-59	10.1	23.2_b
BPI-76-1	12.9	19.8_c

Different letters indicate significant difference ($P < 0.05$)

TABLE 16

SLOPES OF THE REGRESSION LINES FOR THE RICE AND CASEIN DIETS

	Casein	Intan	1R8	1R480-5-9	BPI-76-1
Slope	29.8_a	32.2_b	24.9_c	23.2_c	19.8_d

Different letters indicate significant difference ($P < 0.05$)

change of the rats fed the lowest protein level of Intan rice did not coincide with the weight loss of the group fed the protein-free diet, and since the regression was essentially linear, it did not seem reasonable to exclude this group from the calculation. Nevertheless it is highly questionable that the protein in Intan rice is superior to casein. Thus the growth-slope assay appears to present distinct problems when testing the quality of low protein material such as rice.

Bressani and Juliano (1971) and Eggum and Juliano (1973) found that the quality of the protein in rice tended to decline as the protein content of the rice was increased by genetic or other means. The present data corroborate these findings in that the slope of the growth-response line was lower for the rice samples containing the higher protein levels (Table 15). In view of the apparent difficulties in evaluating the quality of protein in rice samples, these findings might simply reflect the limitations of the assays.

Wheat Flour

The effect of lysine supplementation on the growth response of rats fed diets based on enriched wheat flour was studied in experiments 2 and 3. The improved growth with lysine supplementation (Table 17) was in agreement with the finding by other workers such as Kies and Fox (1970), who have found lysine limiting in wheat. No further improvement in growth was obtained when supplementation was increased from 1.25% lysine (78 mg./gm. nitrogen) to 2.50% lysine (156 mg./gm. nitrogen), which suggests that lysine was no longer the

first limiting amino acid. The addition of 1% threonine (62 mg./gm. nitrogen) to the diet, supplemented with 2.50% lysine, resulted in a further improvement in growth (Table 18), which bears out the fact that lysine was no longer the first limiting amino acid in this diet.

TABLE 17

SLOPES OF THE REGRESSION LINES FOR THE ENRICHED WHEAT FLOUR DIETS EXPERIMENTS 2 and 3

	Wheat Flour$_{(2)}$	Wheat Flour$_{(3)}$	Wheat Flour$_{(2)}$ & 1.25% Lysine	Wheat Flour$_{(3)}$ & 1.25% Lysine	Wheat Flour$_{(2)}$ & 2.50% Lysine	Wheat Flour$_{(3)}$ & 2.50% Lysine
Slope	15.9_{bc}	14.6_{b}	20.5_{a}	19.5_{ac}	20.2_{a}	20.5_{a}

Numbers (2) and (3) indicate experiments 2 and 3.

Different letters indicate significant difference ($P < 0.05$).

TABLE 18

EFFECT ON GROWTH OF LYSINE AND THREONINE SUPPLEMENTATION TO THE 8% PROTEIN-WHEAT FLOUR DIETS - EXPERIMENT 3

Diet	Nitrogen Intake[1] gm.	Weight Gain[1] gm.
Wheat Flour	1.04	4.0
Wheat Flour + 1.0% Threonine	1.02	3.2
Wheat Flour + 1.25% Lysine	1.22	11.7
Wheat Flour + 1.25% Lysine + 1.0% Threonine	1.44	15.0
Wheat Flour + 2.50% Lysine	1.11	11.0
Wheat Flour + 2.50% Lysine + 1.0% Threonine	1.58	26.1

[1]Mean for 4 animals.

Wheat Gluten

The data for the studies with wheat gluten (experiment 1) support the results of the wheat flour study, which showed lysine was limiting in wheat. The addition of 1.25% lysine again appeared adequate for optimum growth (Table 19), since no further improvement occurred when 1.88% lysine was added. These results suggest that lysine was no longer the first limiting amino acid.

TABLE 19

SLOPES OF THE REGRESSION LINES FOR THE WHEAT GLUTEN DIETS - EXPERIMENT 1

	Wheat Gluten	Wheat Gluten & 0.62% Lysine	Wheat Gluten & 1.25% Lysine	Wheat Gluten & 1.88% Lysine
Slope	12.6_a	13.4_a	18.5_b	18.8_b

Different letters indicate significant difference ($P < 0.05$).

Wheat Grown in Manitoba and Nebraska

There were no differences among the slopes of the regression lines for the wheat samples grown in Manitoba and Nebraska (Table 20) which suggests that the quality of the protein was similar for all of the wheat samples assayed. The protein quality of the flour milled from the three wheats grown in Nebraska also was similar (Table 21). The slopes of the regressions for the three flours were lower than for the corresponding wheat diets, but the difference was significant only for the variety A73-5916 (Table 21). These results would suggest that milling does not necessarily reduce the quality of the

protein in wheat.

TABLE 20

SLOPES OF THE REGRESSION LINES FOR WHEAT GROWN IN NEBRASKA AND MANITOBA - EXPERIMENT 3

	Nebraska			Manitoba		
	A73-1	A73-3	A73-5916	Neepawa	Glenlea	Lemli
Slope	16.9_a	16.5_a	18.6_a	18.3_a	17.6_a	17.9_a

Different letters indicate significant difference ($P < 0.05$).

TABLE 21

SLOPES OF THE REGRESSION LINES FOR WHEAT GROWN IN NEBRASKA, AND FLOUR FROM THE NEBRASKA WHEAT EXPERIMENTS 3 and 4

	Wheat			Flour		
	A73-3	A73-1	A73-5916	A73-3	A73-1	A73-5916
Slope	16.9_{ab}	16.5_{ab}	18.6_a	13.5_b	13.2_b	13.7_b

Different letters indicate significant difference ($P < 0.05$).

Triticale

No differences were found among the slopes of the regression lines for the three samples of Rosner triticale (Table 22) grown in Manitoba, Tule Lake, California, and Davis, California. These findings suggest that protein quality was similar for the three samples and that it was not influenced by growing location or growing season. Although all three samples were harvested in the same year, the Manitoba and Tule Lake samples were spring plantings whereas the Davis sample was grown during the winter.

TABLE 22

SLOPES OF THE REGRESSION LINES FOR TRITICALE DIETS

	Rosner Triticale			Composite Samples	
	Manitoba	Tule Lake Calif.	Davis Calif.	18% Prot.	10% Prot.
Slope	20.1_{ab}	20.9_{ab}	22.6_{a}	17.9_{b}	18.5_{ba}

Different letters indicate significant difference ($P < 0.05$).

In addition to the Rosner triticales, two composite samples of triticale designed to contain approximately 10 and 18% protein were made up from triticale grown in the Colorado region. In spite of the considerable difference in protein content, the quality of the protein in the two samples was similar (Table 22). The quality of the protein in the two composite samples was also similar to the three Rosner triticale samples, except for the Davis sample which was superior to the protein quality of the 18% protein sample.

LINEARITY

There has been debate over the range of dietary protein levels which will produce a linear relationship between growth and nitrogen intake. Bressani et al. (1974) reported that generally protein quality is overestimated when the dietary protein levels studied are in the lower range (1, 2 and 3%) and underestimated when the dietary levels are in the higher range (3, 4 and 5%). Young and Taylor (1973) suggested that amino acids are conserved to varying degrees when they are in short

supply. Samonds and Hegsted (1974) believed that when amino acids are conserved, which would be the situation at low levels of protein intake, the dose response line is not necessarily linear. They suggest that if the data from animals receiving low levels of protein intake is included in the calculation of the growth response line, the slope will be altered, and the intercept will predict a higher weight gain at zero protein intake than that observed with the animals fed a protein-free diet. In spite of these theoretical concepts, Hegsted and Chang (1965) found regression lines to be essentially linear for lactalbumin, casein, soy protein, and wheat gluten, although the lowest protein levels studied were 2.2, 3.4, 8.5 and 11.4 respectively. In addition, Hegsted and Juliano (1974) found the regression lines to be essentially linear for a variety of rice samples where protein levels of the test diets were: 1.6, 3.3 and 4.9% for Intan; 2.2, 4.4 and 6.5% for IR8; and 3.3, 6.6 and 9.9% for IR480-5-9.

All regressions of weight gain on nitrogen intake for the present studies were essentially linear as indicated by the fact that the correlation coefficients were high and ranged from 0.77 to 0.98 (Table 23). Furthermore, the correlation coefficients were not altered appreciably when the regression lines were calculated, excluding the group of rats fed a protein-free diet (Table 24). These findings would suggest that growth response was essentially linear for the dietary protein levels studied (0, 2, 5 and 8%). Samonds and Hegsted (1974) found that the predicted weight loss at zero

TABLE 23

ESTIMATES OF RELATIVE PROTEIN VALUE

Exp.	Protein Source	Individual Intercept	Regression Lines Slope	Correlation Coefficient	RPV %
	Casein[a]	-12.1	29.8	0.96	100
1.	Wheat Gluten	-8.6	12.6	0.83	42
	Wheat Gluten + 0.62% Lysine	-8.3	13.4	0.89	45
	Wheat Gluten + 1.25% Lysine	-8.9	18.5	0.97	62
	Wheat Gluten + 1.88% Lysine	-9.6	18.8	0.88	63
2.	Rosner Triticale				
	Grown in Man.	-13.7	20.0	0.93	67
	Tule Lake, Cal.	-13.9	20.9	0.89	70
	Davis. Calif.	-14.9	22.6	0.89	76
	Rice-1R480-5-9	-13.0	23.2	0.98	78
	1R8	-14.1	24.9	0.97	84
	BPI 76=1	-12.6	19.8	0.96	67
	Intan	-15.3	32.2	0.93	108
	Wheat Flour	-13.4	15.9	0.85	53
	Wheat Flour + 1.25% Lysine	-13.4	20.6	0.96	69
	Wheat Flour + 2.50% Lysine	-13.9	20.2	0.96	68
3.	Wheat Flour	-10.5	14.5	0.85	49
	Wheat Flour + 0.10% Lysine	-12.0	19.5	0.94	65
	Wheat Flour + 0.20% Lysine	-12.2	20.5	0.93	69
	Wheat Grown in Nebraska				
	A73-3	-11.9	16.9	0.89	57
	A73-1	-10.9	16.5	0.79	55
	A73-5916	-11.4	18.6	0.83	63
	Wheat Grown in Manitoba				
	Neepawa	-12.1	18.3	0.77	61
	Glenlea	-11.5	17.6	0.90	59
	Lemli	-11.3	17.9	0.96	60
4.	Composite Triticale Samples				
	18% Protein	-10.4	17.9	0.97	60
	10% Protein	-10.3	18.6	0.98	62
	Flour From Nebraska Wheat				
	A73-3	-9.9	13.5	0.93	45
	A73-1	-9.8	13.2	0.90	44
	A73-5916	-9.4	13.8	0.95	46

[a]Based on combined data from 4 casein groups.

TABLE 24

EFFECT OF PROTEIN-FREE GROUP
ON THE REGRESSION LINES

Exp.	Protein Source	Individual Regression Lines: Includ. Zero Protein Intercept	Slope	R^1	Exclud. Zero Protein Intercept	Slope	R^1
1.	Wheat Gluten	-8.6	12.6	0.83	-10.4	15.1	0.85
	Wheat Gluten + 0.62% Lysine	-8.3	13.4	0.89	-9.3	14.8	0.89
2.	Casein	-13.2	29.1	0.94	-14.0	29.5	0.95
	Wheat Flour	-13.3	15.9	0.87	-12.1	14.5	0.85
	Rice						
	Intan	-15.3	32.2	0.93	-15.9	33.4	0.92
	IR480-59	-13.0	22.2	0.98	-11.5	22.2	0.98
	BPI 76-1	-12.6	19.8	0.96	-10.7	18.6	0.96
	IR8	-14.1	24.9	0.97	-13.4	24.4	0.97
3.	Casein	-13.7	30.7	0.97	-12.9	30.1	0.97
	ANRC-Casein	-11.6	31.5	0.93	-11.8	31.7	0.95
	Wheat Flour	-10.5	14.5	0.85	-9.2	12.9	0.79
	Nebraska Wheat						
	A73-1	-10.9	16.5	0.79	-10.1	15.3	0.69
	A73-3	-11.9	16.9	0.89	-12.2	17.3	0.87
	A73-5916	-11.3	18.6	0.85	-10.9	18.2	0.80
	Manitoba Wheat						
	Lemli Wheat	-11.3	17.9	0.96	-10.9	17.5	0.96
	Neepawa Wheat	-12.1	18.3	0.77	-12.6	18.9	0.71
4.	ANRC-Casein	-9.1	27.9	0.97	-10.1	28.5	0.98
	Triticale						
	18% Protein	-10.4	17.9	0.97	-10.1	17.6	0.96
	Triticale						
	10% Protein	-10.3	18.6	0.98	-9.8	18.2	0.98

[1]Correlation Coefficient

protein intake ranged from 7.5 to 14.7 gm. for wheat gluten and from 7.1 to 12.8 gm. for wheat flour, compared to the observed value which ranged from 14.9 to 28.5 gm. Similarly, Hegsted and Chang (1965) observed a weight loss of 16 gm. for rats fed a protein-free diet compared to a predicted weight loss at zero protein intake of 11.2 gm. for soy flour and 14.9 gm. for wheat gluten. Hegsted and Juliano (1974) contend that "the degree to which the dose-response line deviates from the assumed linear line depends on the amino acid which is most limiting and is particularly marked with protein limiting in lysine or methionine - the amino acid believed to be most limiting in many diets". In the present study, lysine was limiting in the wheat gluten and wheat flour diets, yet the predicted weight loss at zero protein intake did not differ greatly from the actual weight loss of the animals receiving a protein-free diet. For wheat gluten, the predicted weight loss was 8.6 gm. compared to the observed weight loss of 7.1 gm., while the predicted values for the wheat flour diets in experiments 2 and 3 were 13.4 and 10.5 gm. respectively compared to the observed values of 14.0 and 10.2 gm. for the animals receiving a protein-free diet. Lysine supplementation improved the quality of the protein in wheat gluten and wheat flour but did not alter the intercept for wheat flour in experiment 2. The addition of 1.88% lysine to the wheat gluten diet changed the intercept from -8.6 to -9.6, while the addition of 1.25 and 2.5% lysine to the wheat flour diet in experiment 3 changed the intercept from -10.5 to -12.0.

These changes were not significant. Thus it appears that even when lysine was the limiting amino acid, the regression lines were not curvilinear.

Hegsted and Juliano (1974) and Samonds and Hegsted (1974) have suggested that if the predicted weight loss at zero protein intake is less than the observed value, the growth response line is curvilinear at the lower levels of protein intake. Since, in the present study, the predicted weight loss for all cereals appeared to be similar to the observed value, and the correlation coefficients for nitrogen intake on weight gain were high, it would seem that the growth response lines were linear.

EFFECT OF PROTEIN-FREE GROUP ON THE REGRESSION LINE

Groups recommending adoption of the growth-slope assay, such as the PAG of the U.N., have not made a recommendation concerning the inclusion or the exclusion of the group of animals fed the protein-free diet. Bressani et al. (1974) and Hegsted and Juliano (1974) recommended inclusion of the animals fed a protein-free diet. On the other hand, based on the collaborative study, Hegsted (1974) reported that slopes of the regression lines excluding the values for rats fed the protein-free diet appeared to be the "most legitimate measure of protein quality". McLaughlin and Campbell (1974) also have suggested that exclusion of the group fed the protein-free diet gives a more accurate measure of protein quality.

In the present study, exclusion of the group fed the protein-free diet appeared to have a negligible effect on the slope, intercept and linearity of the regression lines (Table 24). These findings suggest that the regression lines for all cereals were essentially linear over the range of dietary proteins tested (0, 2, 5, 8%).

The correlation coefficients for weight gain on nitrogen intake did not change appreciably when the group fed the protein-free diet was excluded, except for wheat flour, Nebraska wheat A73-1 and Neepawa wheat in experiment 3 (Table 24). The correlation coefficients changed from 0.85 to 0.79, from 0.79 to 0.69 and 0.77 to 0.71 respectively, when the group fed the protein-free diet was not included in the calculation of the regressions. These lower values may be attributable to the variability of the animals receiving the 8% protein diets. One rat receiving the 8% protein wheat flour diet consumed 0.97 gm. nitrogen but lost 1.0 gm. of weight, whereas another rat fed the same diet consumed 1.1 gm. of nitrogen and gained 7.5 gm. Similarly for the Nebraska wheat, one rat receiving the 8% protein diet consumed 0.84 gm. nitrogen and lost 3 gm. of weight while another rat consumed 0.8 gm. nitrogen and gained 5.5 gm., and for the Neepawa wheat one rat consumed 0.84 gm. nitrogen and gained 7.5 gm. whereas another rat in the same group consumed 0.84 gm. nitrogen and gained only 1 gm. of weight. By removing the less variable data, namely that of the group receiving the protein-free diet, the effect of the highly variable and generally incon-

sistent data for the group receiving the 8% protein diet was magnified and reduced the goodness of fit of the regression line.

Changes observed in the slope of the growth-response line when the group fed a protein-free diet was excluded, were small and inconsistent. The largest change observed was the increase in slope value from 12.6 to 15.1 for wheat gluten when the group fed the protein-free diet was excluded. On the other hand, the slope for wheat flour decreased from 15.9 to 14.5 and from 14.5 to 12.9 in experiments 2 and 3 respectively. Similarly the slope of the regression line for the Nebraska wheat A73-1 decreased from 16.5 to 15.3, while the slope for A73-3 increased from 16.9 to 17.3 when the group fed a protein-free diet was excluded from the regression calculation. Thus, the changes observed in the regression equations and in the correlation coefficients were small and inconsistent, and it appears that in the present study, the data from the group of rats receiving the protein-free diet, did not affect appreciably the outcome of the assay.

RELATIVE PROTEIN VALUE

Groups such as the IUNS Subcommittee on the Biological and Clinical Evaluation of Protein Foods, and the Protein-Calorie Advisory Group of the U.N. presently regard the relative protein value (RPV) assay as the preferred biological assay for the assessment of the nutritional quality of dietary proteins. The basis of the assay is description of

the protein quality of a test material relative to that of a reference protein such as casein or lactalbumin.

Relative protein values were calculated for each of the protein sources tested in the present study (Table 1). The slope of the regression lines for weight gain on nitrogen intake reflected the protein qualities but not potencies of the test cereals relative to a standard. The quality of the protein in wheat gluten, for example, was inferior to the quality of protein in Neepawa wheat as reflected by the much lower slope value of 12.5 compared to 18.3 for the Neepawa wheat (Table 23). These slope values, however, provided no information about the true biological value of the protein. Hegsted and Chang (1965) have suggested that this limitation with the growth-slope assay can be overcome by expressing the growth response per unit of nitrogen intake relative to that of a reference protein. They stressed the importance of the relative nature of the RPV assay, otherwise the information gained is limited simply to the fact that the protein sources within a particular test differ in nutritive value. That is to say, the protein in wheat gluten is inferior to the protein in Neepawa wheat but whether both are relatively high or relatively low biological proteins is not known. The slopes of the regressions for weight gain on nitrogen intake in the present study were expressed relative to the slope of the regression for casein. Two methods of expression were tried; either as a percent of regression for the casein group in the same experiment or as a percent of the regression for

the combined data from all four casein groups (Table 25). Wheat gluten had a relative protein value of 42 compared to 61 for the Neepawa wheat when expressed in terms of the combined data for casein, which suggests that neither are particularly high biological value proteins.

Wheat flour and wheat gluten contain a lower lysine content than wheat due to the absence of the bran portion of the kernel (Hulse and Laing, 1974). The lower lysine content of these wheat products was reflected by the lower RPV's for wheat gluten and wheat flour samples than for wheat. RPV's for wheat flours ranged from 44 to 53 compared to 55 to 63 for the Manitoba and Nebraska wheats. RPV's for enriched wheat flour were increased from a range of 44 to 53 to a range of 65 to 69 when supplemented with 0.10% lysine, which suggests that at this level of lysine the protein quality of wheat flour was superior to that of whole wheat. Similarly, the higher RPV's for Rosner triticale of 67 to 76 compared to 55 to 63 for wheat and 65 to 69 for lysine supplemented wheat flour, may be attributable to the higher lysine content (Hulse and Laing, 1974), and perhaps greater availability of lysine in triticale than in wheat. Knipfel (1969) found the PER adjusted to a value of 2.5 for casein, was 1.55 for Rosner triticale, but only 1.03 for wheat. The PER assay thus suggests the protein quality of triticale was approximately 30% greater than that of wheat compared to only 14% difference observed with the RPV assay in the present study. The observed difference with the RPV assay tends to be more consistent with

TABLE 25

ESTIMATES OF THE RELATIVE PROTEIN VALUES
BASED ON THE REFERENCE PROTEIN CASEIN

Exp. No.	Protein Source	Relative Protein Value: Within Experiment Casein Reference %	Relative Protein Value: Combined Casein Reference %
1.	Wheat Gluten		42
	Wheat Gluten + 0.62% Lysine		45
	Wheat Gluten + 1.25% Lysine		62
	Wheat Gluten + 1.88% Lysine		63
2.	Rosner Triticale grown in Manitoba	68	67
	Tule Lake, Calif.	71	70
	Davis, Calif.	77	76
	Rice - 1R480-5-9	79	78
	1R8	84	84
	BPI 76-1	67	67
	Intan	109	108
	Wheat Flour	54	53
	Wheat Flour + 1.25% Lysine	70	69
	Wheat Flour + 2.50% lysine	69	68
3.	Wheat Flour	47	49
	Wheat Flour + 0.10% Lysine	63	65
	Wheat Flour + 0.20% Lysine	66	69
	Nebraska Wheat		
	A73-3	55	57
	A73-1	53	55
	A73-5916	60	63
	Manitoba Wheat		
	Neepawa	59	61
	Glenlea	57	59
	Lemli	58	60
4.	Triticale 18% Protein	63	60
	10% Protein	65	62
	Nebraska Flour		
	A73-3	47	45
	A73-1	46	44
	A73-5916	48	46

the relative lysine levels of triticale (196 mg./gm. total nitrogen) and wheat (179 mg./gm. total nitrogen) (Hulse and Laing, 1974) than the PER values reported by Knipfel.

The relative protein value assay, not unlike other assays, is not entirely satisfactory. Intan rice had a RPV of 108 compared to casein set at 100. It is highly questionable that the protein in Intan rice is superior to that of casein. Intan rice, due to its low protein content of 5.8%, provided only 5% protein in the diet, even when it replaced 100% of the cornstarch in the basal diet. At the 5% dietary protein level, the weight gain per gram of nitrogen intake was 12.1 gm. for Intan rice compared to 12.8 gm. for casein. When the regression line of weight gain on nitrogen intake for casein was calculated using only the groups of animals receiving the 0, 2 and 5% dietary protein levels, the slope of the line was 34 compared to only 29 when the group receiving the 8% protein diet was included. Thus when the regression lines for Intan rice and casein diets were calculated over comparable dietary protein levels, the RPV of Intan rice was 94. These findings suggest that the test protein and reference protein should be assayed over comparable dietary protein levels.

In a recent collaborative study, (Samonds and Hegsted, 1974), utilizing a series of test proteins, it was found that nutritive values expressed simply as the regression coefficients varied appreciably among laboratories. Inclusion of a reference protein such as lactalbumin or casein, and expres-

sion of the slopes of the regressions for the test proteins in terms of the reference protein (RPV), greatly reduced this variation among laboratories. Inclusion of the reference protein thus provided a more accurate comparison among laboratories. However, expressing the nutritive value of the test protein relative to the reference protein did not alter the ranking of protein sources within laboratories.

A valid assay should be reproducible, and this could only be assessed for the RPV assay in a few instances; namely, for casein in the case of experiments 2, 3 and 4, and for wheat flour and wheat flour supplemented with lysine in experiments 2 and 3. When the regressions for wheat flour diets and for wheat supplemented with 1.25% lysine were expressed relative to the casein group in each experiment, there were differences between the two experiments of about 7 in the RPV's for both diets (Table 25). Expressed relative to the combined data from the four casein groups the differences in RPV between the experiments were about 4 for both diets (Table 25). Thus the observed differences between the two experiments were greater when the RPV was expressed relative to the casein in the experiment, than when the RPV was expressed relative to the combined data for the casein groups.

Correcting results with an internal standard is only as useful as the reproducibility of the standard or reference protein. The PAG of the U.N. recommends ANRC-high nitrogen casein as the reference protein, nevertheless, the suitability of casein might be questioned. Samonds and Hegsted (1974)

observed that "the variability in the casein groups was very high, and for this reason it was a poor standard, although we have no evidence that this is a general characteristic of casein". Although the slopes of the growth response lines were similar for the four casein groups, there were significant differences ($P < 0.05$) among the variances for the regression lines. There is no satisfactory explanation for these differences. Initial weight of the animals did not account for this variation among rats in their response to casein, although initial weight had an effect on the overall growth response. The variances for the regression lines for the casein and for the test protein also differed within experiments, which raises the question of the validity and reliability of the RPV assay. It is not completely valid statistically to compare the slopes of regression lines when there is a difference in the variances of these lines. Samonds and Hegsted (1974) suggest that one way of overcoming this problem is to include the variances for both slopes in the error term of the ratio.

There appear to be some fundamental problems with the RPV assay which need to be resolved; such as the reproducibility, the range of protein levels assayed, and the suitability of the reference protein. Nevertheless, the RPV assay appeared to distinguish among the various cereals and cereal products on the basis of protein quality.

DIGESTIBILITY

Rosner triticale was grown in Manitoba, Tule Lake California, and Davis California, to determine if protein quality is affected by growing location or season. In addition to the three Rosner triticale samples, two composite samples of triticale grown in the Colorado area were designed to provide 18 and 10% protein. Prior to statistical analysis, the growth-response lines appeared to be different for the various triticale samples (Table 14), and digestibility studies were carried out to determine if the apparent differences in protein quality were due to digestibility (Appendix Table 6 and Table 7).

Subsequent statistical analysis indicated that there were no differences in protein quality for the 5 triticale samples, except that the quality of the protein in the triticale from Davis California, was superior to the protein in the 18% protein composite sample ($P > 0.05$, Table 22). Analysis of variance indicated that the true digestibility was similar for all the triticale samples. Thus digestibility did not contribute to the difference in quality between the protein from the Davis-grown triticale and from the 18% protein composite sample.

The digestibility for the casein protein was slightly higher ($P > 0.05$) than for all the triticale samples.

SUMMARY AND CONCLUSION

The purpose of the present study was to evaluate the growth-slope assay for the determination of protein quality in cereals. The main assumption made in the growth-slope assay is that weight gain be linear with nitrogen intake. That this assumption was true in the present study was reflected by the relatively high correlation coefficients (0.77 to 0.98) between weight gain and nitrogen intake. In addition, the intercepts of the regression lines, or the predicted weight loss at zero protein intake, were similar to the actual weight loss of the rats fed a protein-free diet and the exclusion of the protein-free treatment group in the calculation of the regression line did not appreciably alter the correlation coefficient. Although the regression lines were essentially linear, goodness of fit for the regression lines was improved slightly when the initial weight of the animals was taken into consideration by multiple regression analysis. However, weight gain was essentially linear with nitrogen intake for all cereals by both simple and multiple regression, and in general, dietary protein levels of 2, 5 and 8% were satisfactory for the evaluation of protein quality of cereals using the growth slope assay. The upper level of 8% dietary protein in the test diet was selected, since the protein level in many cereals is too low to provide more than 8% dietary protein in the test diet. Even a level of 8% protein

could not be achieved with Intan rice in the present study due to its low protein content. Intan rice provided a maximum of only 5% protein even when it constituted 80% of the test diet. Nevertheless, the regression line for Intan rice appeared to be linear, although it has been suggested that if there are only a few points on the regression line and they fall over a narrow range, the line might simply appear linear. The assay therefore may not be satisfactory for the determination of protein quality in cereals which provide less than 8% protein in the diet, although additional research is necessary before definite conclusions can be made. In fact the whole question of the range of protein intakes over which weight is linear and the effect of protein source on this relationship appears to require further investigation.

Inclusion of the protein-free treatment group in the calculation of the regression line for weight gain on nitrogen intake did not affect the observed protein quality of the materials tested in the present study. The intercept, slope and correlation coefficients for the regression lines were similar whether the protein-free group was included or excluded in the calculation of the regression line. These findings are contrary to those of Bressani et al. (1974) and Samonds and Hegsted (1974) who found that inclusion of the protein-free treatment group did affect the slope of the regression of weight gain on nitrogen intake and in turn the estimate of protein quality. Recommendations have been made both for the inclusion and the exclusion of data for the protein-free

group in the calculation of the regression line. However, results of the present study indicated that weight changes for the protein-free group had little effect on the observed protein quality of cereals or cereal products.

The slopes of the regression lines for weight gain on nitrogen intake were assumed to reflect the quality of the protein in the cereals tested in the present study. Thus differences in protein quality among the various cereals and cereal products were determined by assessing the equality of the slopes of the regression lines. Tests for equality of slopes were restricted to comparisons within the various groups of cereals tested; namely, wheats, flours prepared from wheat, wheat gluten, rice, and triticale. The test for equality of slopes is only approximate if the variances of the regression lines are not similar within the groups being compared as was the case for the wheat gluten, triticale, and casein groups. Tests for equality of slopes indicated that protein quality differed among the rice and triticale samples tested in the present study, although marked differences in quality occurred only among the samples of rice.

Slopes of the regression lines for weight gain on nitrogen intake reflected differences in protein quality among the materials assayed in a particular test, but they gave no indication of the relative potency or true biological values of the proteins in these test materials. To overcome this limitation, the slope of the regression line for each of the protein sources tested was expressed as a percent of the slope (RPV),

of the regression line for the reference protein casein. The validity and reliability of the RPV might be questioned however, since the variances of the regression lines for the test proteins were not always similar to the variances of the regression line for the reference protein. In addition, the suitability of casein as the reference protein might also be questioned since the variances of the regression lines for the casein group differed significantly among experiments. However, the RPV's for the cereals and cereal products tested in this study appeared to correspond to the nutritive values reported for these cereals and cereal products. The RPV based on the combined data for the four casein groups, was 42 for wheat gluten compared to 63 for wheat gluten supplemented with 0.10% lysine. The RPV's for wheat flour varied from 44 to 53 compared to values between 65 and 69 for wheat flour supplemented with 1.25% lysine. Similarly, the RPV's ranged from 55 to 62 for wheat, from 60 to 75 for triticale, and from 66 to 108 for rice. Thus the RPV assay appears to be satisfactory for the determination of protein quality in cereals in spite of a number of questions which need to be resolved.

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APPENDIX

TABLE 1

RECORD OF GROWTH AND FOOD INTAKE[a]
EXPERIMENT 1

Cereal Source	Dietary Protein Calculated %	Dietary Protein Analyzed %	Initial Weight[b] gm.	Final Weight[b] gm.	Food Intake[b] gm.
Wheat Gluten	2	2.31	48.6	41.2	55.7
	5	5.08	49.1	46.5	67.0
	8	7.79	49.4	52.9	70.7
Wheat Gluten + 0.62% Lysine	2	2.32	47.9	41.1	53.7
	5	5.12	47.4	45.0	59.5
	8	7.85	49.0	54.0	74.2
Wheat Gluten + 1.25% Lysine	2	2.34	48.1	42.2	53.5
	5	5.16	47.9	46.5	57.5
	8	7.91	48.7	60.2	84.5
-[c]	0	0.047	47.9	40.7	50.0

[a]14-day period.

[b]Mean for four animals.

[c]Basal diet.

TABLE 2

RECORD OF GROWTH AND FOOD INTAKE[a] - EXPERIMENT 2

Cereal Source	Dietary Protein Calculated %	Dietary Protein Analyzed %	Initial Weight[b] gm.	Final Weight[b] gm.	Food Intake[b] gm.
-[c]	0	0.29	62.0	48.0	59.5
Casein	2	2.30	60.4	54.1	65.7
	5	5.19	60.4	71.0	96.0
	8	8.65	62.0	107.7	147.0
Rice 1R8	2	2.42	61.2	53.5	61.7
	5	5.35	62.1	69.1	96.5
	8	8.65	62.2	87.4	126.7
Rice BPI 76-1	2	2.57	62.1	55.2	58.5
	5	5.94	61.6	70.7	101.7
	8	9.26	59.6	86.9	132.2
Rice 1R480-5-9	2	2.24	60.0	53.7	70.0
	5	5.16	61.4	66.4	88.0
	8	8.51	59.9	90.5	138.7
Rice Intan	2	2.33	60.2	51.9	72.2
	3	3.16	60.0	57.7	73.5
	5	4.76	59.7	69.0	98.5
Wheat Flour[d]	2	2.08	61.8	51.1	56.7
	5	5.44	60.2	59.2	70.7
	8	8.16	61.0	64.5	88.7
Wheat Flour[d]	2	2.16	60.8	52.4	59.5
+ 0.1% Lysine	5	5.52	59.7	59.1	68.0
	8	8.23	61.5	79.0	111.2
Wheat Flour[d]	2	2.14	60.7	50.7	50.0
+ 2.5% Lysine	5	5.52	61.5	60.0	67.7
	8	8.41	62.2	77.7	106.7
Rosner Triticale					
Grown in					
Tule Lake Calif.	2	2.32	61.6	52.1	59.7
	5	5.26	63.2	64.7	77.2
	8	8.03	61.1	73.6	100.7
Davis Calif.	2	2.30	62.4	52.9	65.5
	5	5.26	62.9	65.7	93.0
	8	7.93	59.4	78.2	110.5
Manitoba	2	2.37	59.5	50.7	57.5
	5	5.33	62.0	62.5	81.0
	8	8.13	60.6	76.0	110.7

[a] 14-day period.

[b] Mean for 4 animals.

[c] Basal diet.

[d] Robin Hood Enriched Wheat Flour.

TABLE 3

RECORD OF GROWTH AND FOOD INTAKE[a] - EXPERIMENT 3

Protein Source	Dietary Protein Calculated %	Dietary Protein Analyzed %	Initial Weight[b] gm.	Final Weight[b] gm.	Food Intake[b] gm.
-[c]	0	0.29	56.9	45.6	55.2
Casein	2	2.50	57.1	51.7	62.2
	5	5.54	57.4	66.5	87.2
	8	8.57	56.2	99.7	135.0
ANRC Casein	2	2.39	55.0	50.9	63.5
	5	5.30	56.6	73.1	105.0
	8	8.35	56.1	96.6	123.2
Wheat Flour[d]	2	2.39	56.4	48.6	51.2
	5	5.51	55.5	55.6	67.0
	8	8.04	56.0	60.0	76.0
Wheat Flour + 1.25% Lysine	2	2.43	57.0	48.2	58.2
	5	5.51	57.0	55.6	57.2
	8	8.65	56.6	68.2	88.5
Wheat Flour + 2.50% Lysine	2	2.45	55.7	47.0	56.0
	5	5.58	56.6	58.7	76.5
	8	8.68	55.4	66.4	79.5

[a] 14-day period.

[b] Mean for 4 animals.

[c] Basal Diet.

[d] Robin Hood Enriched Wheat Flour.

Continued

TABLE 3 Continued

RECORD OF GROWTH AND FOOD INTAKE[a] - EXPERIMENT 3

Protein Source	Dietary Protein Calculated %	Dietary Protein Analyzed %	Initial Weight[b] gm.	Final Weight[b] gm.	Food Intake[b] gm.
Wheat Grown in Nebraska					
A73-5916	2	2.30	55.9	46.5	50.0
	5	5.16	55.1	55.7	68.7
	8	8.13	57.2	67.1	87.2
A73-1	2	2.34	55.7	48.1	49.0
	5	5.22	56.9	56.4	70.0
	8	8.10	55.2	59.6	73.7
A73-3	2	2.28	57.5[c]	48.5[c]	53.0[c]
	5	5.30	55.1	51.1	56.5
	8	8.41	56.4	63.0	79.2
Wheat Grown in Manitoba					
Lemli	2	2.30	56.5	49.5	59.0
	5	5.24	57.6	55.6	62.5
	8	8.14	56.7	67.2	92.7
Neepawa	2	2.32	55.7	45.4	48.2
	5	5.16	56.4	54.5	60.7
	8	8.13	56.5	60.5	67.5
Glenlea	2	2.31	56.1	47.2	48.0
	5	5.21	56.0	54.5	61.7
	8	7.96	56.2	63.6	85.2

[a]14-day period.

[b]Mean for 4 animals.

[c]Mean for 3 animals.

TABLE 4

RECORD OF GROWTH AND FOOD INTAKE[a] - EXPERIMENT 4

Protein Source	Dietary Protein Calculated %	Analyzed %	Initial Weight[b] gm.	Final Weight[b] gm.	Food Intake[b] gm.
-[c]	0	0.32	53.2	43.0	57.2
ANRC Casein	2	2.39	53.0	50.1	75.2
	5	5.59	52.9	75.1	116.0
	8	8.55	53.2	98.4	143.7
Triticale 10% Protein	2	2.39	53.1	47.0	62.2
	5	5.43	53.5	57.2	81.7
	8	8.11	53.0	73.0	120.0
Triticale 18% Protein	2	2.48	52.7	46.4	66.7
	5	5.58	52.7	56.7	79.5
	8	8.61	53.5	70.7	115.2
Flour From Nebraska Wheat A73-5916	2	2.40	53.1	47.7	62.7
	5	5.45	52.7	53.4	75.7
	8	8.61	52.9	61.2	95.5
A73-1	2	2.39	53.4	46.2	62.5
	5	5.47	53.2	53.4	83.2
	8	8.49	53.2	58.6	91.5
A73-3	2	2.41	53.0	46.1	61.5
	5	5.59	53.4	52.7	64.7
	8	8.66	52.5	59.0	89.7

[a]14-day period.

[b]Mean for 4 animals.

[c]Basal diet.

SUMMARY OF CALCULATIONS FOR TESTING EQUALITY OF SLOPES ($\beta_1^{(i)}$) - Table 5

Condition (i)	d.f.	Σx^2	Σxy	Reg SS = $\frac{(\Sigma xy)^2}{\Sigma x^2}$	Σy^2	$SS(Dev)_i$	d.f.
1	n_1-1	$\underset{j}{\Sigma} x_{1j}^2$	$\underset{j}{\Sigma} x_{1j}y_{1j}$	$\frac{(\underset{j}{\Sigma} x_{1j}y_{1j})^2}{\underset{j}{\Sigma} x_{1j}^2}$	$\underset{j}{\Sigma} y_{1j}^2$	$SS(Dev)_1$	n_1-2
2	n_2-1	$\underset{j}{\Sigma} x_{2j}^2$	$\underset{j}{\Sigma} x_{2j}y_{2j}$	$\frac{(\underset{j}{\Sigma} x_{2j}y_{2j})^2}{\underset{j}{\Sigma} x_{2j}^2}$	$\underset{j}{\Sigma} y_{2j}^2$	$SS(Dev)_2$	n_2-2
3	n_3-1	$\underset{j}{\Sigma} x_{3j}^2$	$\underset{j}{\Sigma} x_{3j}y_{3j}$	$\frac{(\underset{j}{\Sigma} x_{3j}y_{3j})^2}{\underset{j}{\Sigma} x_{3j}^2}$	$\underset{j}{\Sigma} y_{3j}^2$	$SS(Dev)_3$	n_3-2
Deviations from Individual Regressions						$SS(Dev)_P = \underset{i}{\Sigma} SS(Dev)_i$	$\underset{i}{\Sigma} n_i-6$
Totals when $\beta_1^{(i)}$'s equal	$\underset{i}{\Sigma} n_i-3$	$\underset{ij}{\Sigma\Sigma} x_{ij}^2$	$\underset{i\ j}{\Sigma}(\Sigma x_{ij}y_{ij})$		$\underset{i\ j}{\Sigma}(\Sigma y_{ij}^2)$	$SS(Dev)_{H_{02}} = \underset{i\ j}{\Sigma}(\Sigma y_{ij}^2) - \frac{[\underset{i\ j}{\Sigma}(\Sigma x_{ij}y_{ij})]^2}{\underset{ij}{\Sigma\Sigma} x_{ij}^2}$	$\sum_{i=1}^{3} n_i-3-1$
Differences among $\beta_1^{(i)}$'s						$SS(Dev)_{H_{02}} - SS(Dev)_P$	3 - 1

To test H_{02}, use $F_{3-1,\ \underset{i}{\Sigma} n_i-6} = \frac{[SS(Dev)_{H_{02}} - SS(Dev)_P]/(3-1)}{SS(Dev)_P/(\underset{i}{\Sigma} n_i-6)}$

TABLE 6

DIGESTIBILITY STUDY - EXPERIMENT 5

Protein Source	Dietary[1] Protein %	Nitrogen Consumed[2] gm./day	Fecal Nitrogen Excreted on Test Diet[2] gm./day	Fecal Nitrogen[2] Excreted on Protein Free Diet gm./day	Digestibility %
ANRC-Casein	8.45	0.115	0.014	0.006	93
Rosner Triticale Manitoba	8.55	0.081	0.013	0.005	90
Tule Lake California	8.05	0.088	0.015	0.005	89
Davis California	8.04	0.097	0.018	0.006	97

[1]Based on analysis.

[2]Mean for 4 animals.

TABLE 7

DIGESTIBILITY STUDY - EXPERIMENT 6

Protein Source	Dietary[1] Protein %	Nitrogen Consumed[2] gm./day	Fecal Nitrogen Excreted on Test Diet[2] gm./day	Fecal Nitrogen[2] Excreted on Protein Free Diet gm./day	Digestibility %
ANRC-Casein	8.55	0.168	0.015	0.008	95
Triticale 18% Protein	8.61	0.136	0.022	0.006	88
Triticale 10% Protein	8.11	0.134	0.025	0.007	86

[1]Based on analysis.

[2]Mean for 4 animals.