THE EFFECT OF PROTEIN SUPPLEMENTED BREADS AS COMPARED WITH A STANDARD WHITE BREAD ON THE GROWTH RATE, TISSUE COMPOSITION, AND LIVER XANTHINE OXIDASE SYSTEM OF THE RAT

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ABSTRACT

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Weanling albino rats were used as experimental animals in assaying the comparative nutritional quality of three protein supplemented breads with that of a standard white bread. The three specialty breads varied with respect to the nature of the protein supplement and quantity of the supplement used in the formula.

In the first experiment, rats were divided into four groups of twenty rats each and fed, ad libitum, the experimental diets consisting of 90 per cent bread. The experimental diets contained the following: I--a standard white bread (12 per cent protein); II--a bread containing a Roman meal baker's mixture (14.1 per cent protein); III--a bread containing a special white diet mix (15.1 per cent protein); and IV--a bread containing ground soybeans (21.0 per cent protein). At two weeks and again at four weeks, ten rats from each group were sacrificed, and both the livers and the carcasses were analyzed for moisture, fat, and nitrogen. The protein efficiency ratio (grams gain per gram of protein

eaten) was used as the criterion in evaluating the diets.

The nutritional quality of the three breads studied did not correlate with the protein content. Roman meal bread contained more protein than the standard white bread; however, the protein efficiency ratio of the rats fed the Roman meal bread was not significantly different from that of the rats fed the control.

The special white diet bread contained only slightly more protein than did the Roman meal bread (15.0 per cent versus 14.1 per cent), but the protein efficiency ratio of the rats fed the special white diet bread was 22 per cent higher than the protein efficiency ratio of the rats fed the Roman meal bread.

The soybean bread contained more protein than did the special white diet bread (21.0 per cent versus 15.0 per cent); however, the protein efficiency ratios of the rats on these diets suggest these two breads were quite similar in quality.

A two week follow-up study was conducted using the two breads with the most similar protein content and the most different growth response (Roman meal and special white diet breads). These breads were studied in iso-protein diets with and without supplements of 0.25 per cent L-lysine·HCl.

The lysine supplements to both breads increased the quality of their respective proteins as exhibited by

significantly higher protein efficiency ratios over those of the unsupplemented breads. The trend in the livers' xanthine oxidase values correlated with the protein efficiency ratios. The addition of lysine had a greater effect on the Roman meal bread than it did on the special white diet bread, suggesting that the latter contained a more nearly complete protein than the Roman meal bread.

The quantity of protein present in a given food cannot be used as the sole criterion in evaluating its nutritive value. As the results of these experiments illustrate, the quality of the protein is as important as is the quantity.

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Teckla Mueller Buelke

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INTRODUCTION

Throughout the ages bread has been the "staff of life." Today, nearly all the people in the United States eat white bread enriched with the B vitamins--thiamine, riboflavin, and niacin; and with the mineral, iron. These factors because they are lost in the milling process are put back into bread by bakers.

The bakers adopted bread enrichment programs in 1941 on a voluntary basis. On October 1, 1943, the government enacted a bread enrichment law to guarantee that the white bread involved in interstate commerce would contain the following as milligrams per pound of bread: thiamine, 1.1 to 1.8; riboflavin, 0.7 to 1.6; niacin, 10.0 to 15.0; and iron, 8.0 to 12.5 (National Research Council, 1944). These enrichment standards have remained unaltered to date (Code of Federal Regulations, 1963).

Now, the interest in further improvement of bread is centered around the protein, or more specifically, the amino acid content.

The National Research Council (1958) recommends from 8 per cent to 11 per cent of the total caloric intake in the human "reference" adult's diet be protein. Proteins supply approximately 13 per cent of the total calories of a standard

white enriched bread which contains 4 per cent milk solids.[1] However, while the total protein content of bread appears to be adequate, the protein is not of high quality. It has been shown that for the albino rat lysine is the most limiting amino acid in wheat products (Osborne and Mendel, 1914 and later workers). The protein of bread (made with 6 per cent non-fat milk solids) contains 2.8 grams of lysine per 16 grams of nitrogen (Block and Bolling, 1951); whereas, a "complete protein," by definition, should contain 5.3 grams of lysine per 16 grams of nitrogen (Howard et al., 1958). These figures indicate about one-half of the bread proteins are not available to the rat when bread is fed as the sole source of nitrogen. It is because of this fact that much research has been done to improve the protein quality of bread.

Many specialty breads are appearing on the market. It has been estimated that these specialty breads constitute 5 per cent to 6 per cent of the total bread market; and of these breads, about one-third carry some statement of protein supplementation on their labels (Friedman, 1959).

Although the percentage of the total calories in the American diet supplied by bread has decreased in recent years, some 84.4 pounds of bread were still consumed per person in

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[1]Calculation made from information in the United States Department of Agriculture Handbook No. 8, Composition of Foods, June 1950.

1958 (United States Department of Agriculture, May 1962). Even though the "high protein" breads represent only a small portion of the total bread market (about 1.4 pounds to 1.7 pounds per person per year), it nevertheless amounts to a considerable volume of business and a large proportion of all advertising in the baking industry. This advertising, implying the need for increased dietary protein and the value of the particular product for satisfying this need, is often misleading (Friedman, 1959).

Friedman (1959) examined various protein specialty breads for protein quality and quantity. The increase in nitrogen content was not always accompanied by an increase in protein quality. For example, one bread had almost twice the nitrogen but only three-fourths the protein quality of the standard white bread.

The studies reported here were devised to determine whether a given protein supplement to a standard white bread substantially improved the nutritive value.

REVIEW OF LITERATURE

Wheat Proteins--Quantity and Quality

In evaluating the protein content of wheat several factors must be considered. First, the amount of protein varies with different portions of the kernel, with different varieties, with the environmental conditions under which the grain is grown, and with the milling process. Second, the value of wheat protein is not only dependent on the quantity but also upon the quality.

The different portions of the wheat kernel, varying in protein content, are: the embryo or germ, about 1.5 per cent of the kernel, situated at one end of the kernel as a small, yellow mass; the endosperm, approximately 83 per cent, forming the greatest part of the kernel; and the bran, about 15 per cent of the kernel, consisting of the outer coats and underlying layer containing the protein cells which cover the entire seed and protect the embryo and endosperm (Osborne and Mendel, 1919).

Morris and co-workers (1946) employed a micro-dissection technique to separate whole wheat kernels into the following fractions: five zones of pure endosperm, two of bran, and one of germ including the attached bran. These fractions of a hard red winter, a soft red winter, and a hard red

spring wheat were analyzed for ash and protein. In all three varieties, the smallest amount of ash and protein was in the central portions of the endosperm fractions, and there was an increasing gradient in ash and protein content from the center of the endosperm outward.

The quantity of protein can be influenced by many factors such as the variety of wheat and the environmental conditions--soil type, fertilizer treatment, and weather--under which it is grown.

Harris and co-workers (1945) conducted a study in which eight varieties of wheat were grown in various parts of North Dakota. They found significant variations in flour protein content existed between varieties and environment, with environmental conditions exerting the greatest influence. The range in protein content for varieties was from 14.1 per cent to 12.9 per cent and for environmental influence was from 14.7 per cent to 11.8 per cent. Similar results were observed in Nebraska during the 1939-40 growing season (Sandstedt and Fortmann, 1944). The protein content of the flour milled from different varieties of wheat planted varied from a low of 12.4 per cent protein in the middle-eastern portion of Nebraska to a high of 16.9 per cent protein in the north-western area of the state. On the other hand, El Gindy and associates (1957) found that variety was a more significant determinant of the protein content of wheat grown in Ohio than were environmental

conditions.

Morris and his associates (1945) noted the percentage of protein in the entire kernel of a hard wheat was 12.8 per cent; whereas, only 9.7 per cent protein was present in the soft wheat kernel. The total endosperm and total bran showed a similar relationship with the hard wheat containing 12.0 per cent and 16.7 per cent protein, respectively, and the soft wheat, 9.3 per cent and 13.7 per cent protein, respectively. These results were corroborated by Jones and Moran (1946).

As these studies have indicated the protein content of wheat is variable, affected by variety and various environmental conditions. However, little is known concerning the relationship of these factors to the relative amino acid composition of the protein.

Miller and his associates (1950) studied the environmental effect on the amino acid content of several varieties of hard red winter wheat. They noted that the mean cystine and methionine content of all the varieties was significantly ($P<0.01$) affected by the environmental conditions (soil type and weather) of the two general districts compared. The mean differences for these two general districts given as per cent of amino acid in protein (N x 5.7) on the dry basis were: 1.39 per cent versus 1.46 per cent methionine, and 2.16 per cent versus 2.38 per cent cystine. The environmental conditions did not cause significant

differences in the mean lysine or glutamic acid content nor in the mean protein values. The protein values for these districts were 14.6 per cent and 14.3 per cent. There were no variations, however, for the cystine, lysine, methionine, or glutamic acid content among the varieties studied.

Animal feeding experiments were used to compare isonitrogenous diets of a high protein flour and a low protein flour. The animals on both of these diets grew at equal rates. These results suggested according to Howard and his co-workers (1958) that the animals had equal lysine intakes, and therefore, the high and low protein flours contained the same amount of lysine per equal amounts of nitrogen. Subsequent chemical analyses supported this hypothesis. These data corroborate those of Miller and co-workers (1950) and Lawrence et al. (1958) that there was no difference in lysine content among varieties. Lawrence and his associates (1958), however, found certain samples of wheat mutants in each variety studied with a lysine content enough higher than the mean of its particular variety to give them reason to hope that breeding wheats of consistently higher lysine content could be possible.

While the variety of wheat appears to play a minor role in determining the amino acid composition, environmental conditions may affect the content of certain amino acids present in wheat. This area demands considerable investigation.

The milling process used in the production of flour exerts a major influence on the protein content. During the first milling separation the grain is split into three fractions: flour, fine particles which contain most of the starch and protein; middlings, intermediate particles made up of endosperm, bran, and the germ; and chop or stock, the coarse particles containing pieces of endosperm and bran (Geddes, 1951). The flour fraction is more completely refined and it is from this fraction that various commercial flours are obtained. Straight flour is a combination of all the flour streams. Patent flours are derived from the more refined streams, and vary considerably in the percentage of total flour represented. Geddes (1951) gave the following approximate percentages: family patent, 70-75 per cent total flour; short patent, 75-80 per cent total flour; long or standard patent, 90-95 per cent total flour. The flour remaining after the patent is removed is called clear flour and it too may be separated into different grades. The flour from the last reduction is called "red dog" because it is dark in color, due to its relatively large amounts of bran and germ.

The commercial white breads, today, are made of a high protein hard wheat patent flour of about 72 per cent extraction flours.[1] Since the patent flours are the most

[1]Personal communication from Norine Condon, Nutrition Education Programmer, American Institute of Baking, September 9, 1963.

refined flours and therefore contain none of the outer layers or germ, they have the lowest protein content of any of the flours. The less refined a flour is and the more bran or germ it contains, the higher the protein content.

A higher amount of milk solids (approximately 3.3 parts to 5.5 parts per 100 parts of flour) than was used before 1956 is now being used in the bakery formulas; therefore, the protein content of these breads is increased. According to Kulp and associates (1956) about 80.4 per cent of the bread on the market contains between 7.5 per cent and 8.5 per cent protein (N x 6.25). A 23 gram slice of white enriched bread (4 per cent non-fat milk solids) contains 2.0 grams, or 8.7 per cent protein (N x 5.7) (United States Department of Agriculture, 1950).

Quantity can not alone determine the nutritive value of the protein contained in a given product; the quality in relation to the quantity of protein is another important factor in evaluation. A protein to be complete and to maintain life and provide for normal growth when used as the sole protein food must contain the essential amino acids in the proper proportion. The value of a protein for tissue synthesis is only as good as its most limiting essential amino acid.

Studies comparing the nutritive value of wheat with other cereal grains, meat, and eggs indicate that the quality of the wheat protein is indeed limited.

The results of Osborne and Mendel's (1920) comparison of four cereals--barley, rye, oats, and wheat--indicated they were not greatly different in their growth promoting efficiency in the weanling rat. However, these researchers felt their method was still crude, and after refinement, other results may be obtained. Later researchers have had different results. The growth promoting values of proteins of whole rye (Jones et al., 1948, and Sure, 1954 and 1955); barley and rolled oats, (Jones et al., 1948, and Sure, 1955); whole yellow corn and brown rice (Jones et al., 1948); polished rice (Sure, 1946, and Jones et al., 1948); peanut meal, soybean meal, non-fat dried milk solids, and dried whole eggs (Sure, 1955) were compared with whole wheat at various levels of protein intake ranging from 4.5 per cent to 12.0 per cent. The results of these rat growth experiments showed oats, rice, rye, non-fat milk solids, and dried whole egg to be superior to wheat at all the protein levels at which they could be compared.

In a rat growth study comparing the proteins of eggs, pork, and whole wheat at the 8 per cent level of intake (N x 6.25), Mitchell and Carmen (1924) found the average biological value for these proteins to be 93, 74, and 67 respectively. In a later study, Mitchell and Block (1946) found the biological value of whole wheat to be 70.

The concept of complete protein has been used as an index of nutritive quality of protein foods. Almost fifty

years ago, Osborne and Mendel (1914) identified lysine as the most limiting amino acid of wheat protein. Since lysine is known to be the most limiting amino acid in wheat and it is assumed that a "complete protein" contains 5.3 grams of lysine per 16 grams of nitrogen, the amount of complete protein in bread can be calculated (Block et al., 1958 and 1959). Block and his co-workers (1958, 1959) found the total protein (N x 6.25) of white bread to be 13.6 per cent and the complete protein to be 7.1 per cent, just a little more than half of the total protein. Whole wheat bread contained 14.2 per cent total protein and only 8.6 per cent complete protein.

The lysine content of the whole wheat kernel and a white bread containing 6 per cent non-fat milk solids is 2.7 grams and 2.8 grams per 16 grams of nitrogen, respectively (Block and Bolling, 1951). The quantity of lysine in whole wheat and white bread is a little more than one-half the amount needed to make them complete protein foods, assuming that the ratio of other amino acids is such that they will not limit the protein.

Whole wheat is 91 per cent digestible (Mitchell and Block, 1946), but lysine in wheat is only 71 per cent available to the white rat (Gupta et al., 1958). This means that even though the lysine content of whole wheat is 2.7 grams per 16 grams of nitrogen, only 1.9 grams are available to the body for metabolism.

The complete protein content of the wheat kernel, flour, and bread varied as processing affected the lysine content at each of these stages. Hepburn and co-workers (1957) carried out microbiological assays for amino acids on four blends of hard red wheat, the flours milled from them, and the breads prepared from the flours. These workers noted a significant decrease in lysine (24 per cent) in the conversion of wheat to flour and a significant increase in lysine (19 per cent) in the conversion of flour into bread. This increase is due to the addition of milk and yeast to the bread formula. These figures indicate the net loss of lysine is 5 per cent. Rosenburg and Rohdenburg (1951) also observed a loss of lysine in bread due to baking. The results of the microbiological assay indicated a 15 per cent loss. These workers also noted that toasting a slice of bread or allowing it to become stale and dry reduced the lysine content by 5 per cent to 10 per cent.

The content of amino acids in hydrolysates of white flour, of bread made from that flour, and of the crust of the bread was determined by ion-exchange chromatography (McDermott and Pace, 1957). Contrary to the results of the two previous studies cited, no significant loss of lysine was found in the conversion of flour to bread. However, these workers observed 15 per cent less lysine present in the bread crust hydrolysate than in the flour hydrolysate. In evaluating these data, the authors pointed out the

difficulty in determining to what extent the loss may be due to the baking process or the destructive action during hydrolysis.

Protein Supplements to Wheat Diets

Because wheat flour, and therefore bread, contains an incomplete protein mixture as the previous studies have indicated, steps have been taken to find substances which will supplement and improve the protein content of bread. These supplements are generally chosen because of their protein or amino acid content. Studies relating to some of these supplements will be discussed here.

Supplement: Non-Fat Milk Solids

Non-fat milk solids can supply lysine and valine to bread; these two amino acids are deficient in wheat. The lysine and valine content of milk protein is 7.5 per cent and 4.5 per cent, respectively (Carlson et al., 1946).

Hove and his co-workers (1945) have devised rat growth experiments in which patent flour alone and patent flour plus a non-fat milk supplement furnished 10 per cent protein in the diets. The researchers found that a supplement of 3 per cent non-fat milk solids to patent flour improved the protein efficiency ratio from 0.84 to 1.00. When 6 per cent non-fat milk solids were added to the patent flour, the protein efficiency ratio was increased to 1.19, only

slightly lower than the value for whole wheat.

The supplemental value of four parts of non-fat milk solids to one hundred parts of high protein wheat flour was studied at three levels of protein intake (10 per cent, 12 per cent, and 15 per cent) and compared to the unsupplemented flour also fed to the weanling rats at the three protein levels (Howard et al., 1958). It was observed that at each protein level, the group of animals on the milk supplemented diets exhibited a far superior growth performance than the animals on the unsupplemented flour diet; and as the protein level of the diets increased, the animals' protein efficiency ratios also increased.

Various experimental breads were incorporated into diets at the 10 per cent protein level and studied in rat pair-feeding experiments. The results of these studies showed the nutritional quality of the white bread containing 3 per cent milk solids to be significantly superior to the control bread with no milk added (Carlson et al., 1946, and Harris et al., 1944). The bread containing 6 per cent milk solids was significantly better than unsupplemented white bread and about the same as unsupplemented whole wheat bread (Carlson et al., 1946).

White breads supplemented with various levels of non-fat milk solids--2 per cent and 6 per cent (Henry et al., 1941); 6 per cent and 12 per cent (Fairbanks, 1938 and 1939); and 3 per cent, 6 per cent, and 12 per cent (Jahnke and

Schuck, 1957)--were studied and compared with non-supplemented white bread in rat growth experiments. With increased addition of milk solids, increased weight gains and body length were noted. Henry et al. (1941) observed an improvement in the biological value of breads supplemented with 2 per cent and 6 per cent non-fat milk solids. Similarily, increased protein efficiency ratios were noted when whole wheat bread diets were supplemented with non-fat dry milk solids as above (Sabistan and Kennedy, 1957). The quality of the white bread containing 6 per cent non-fat dry milk solids was somewhat inferior to that of whole wheat bread containing 3 per cent non-fat dry milk solids (Sabistan and Kennedy, 1957), and equivalent to or better than whole wheat bread with no supplement (Light and Frey, 1943, Riggs et al., 1946, and Sabistan and Kennedy, 1957).

A new white bread containing twenty-five pounds non-fat dry milk solids per one hundred pounds flour was reported by the Eastern Utilization Research Laboratory, Washington, D.C., in 1959. Rodgers and Welton (1959) stated that the added non-fat milk solids increase the protein content about 30 per cent depending on the type of flours used. The biological value of the protein nearly doubled in comparison with the protein in a commercial type bread containing four parts non-fat dry milk solids per one hundred parts of flour. Besides its nutritive improvement, this bread had good consumer acceptability.

Supplement: Wheat Germ

Wheat germ as a protein source contains as much as 30 per cent protein. Many attempts have been made, some quite successfully, to incorporate wheat germ in the baking of bread; but unless the germ is specially treated, a quantity of more than 2 per cent in the flour has a marked detrimental effect on loaf volume and color (Hove and Harrel, 1943).

Hove and Harrel (1943) found that the protein of wheat germ has a high biological value as determined by the rat growth method. The biological value of wheat germ protein varied inversely with the amount of wheat germ protein used in the rat diet. For comparison, the authors listed the biological value of certain animal proteins fed at a 10 per cent level: commercial casein, 2.26; dry skim milk, 2.85; and boiled dry egg white, 2.58. They found wheat germ to be equally as effective a supplement to poor protein diets as casein. Heat processing of wheat germ to make it suitable for human consumption and to give it better keeping quality had no effects on the biological value of the protein.

The effect of the addition of wheat germ at the 2 per cent, 4 per cent, and 6 per cent levels to enriched and non-enriched flour was determined by rat growth experiments (Westerman et al., 1952). The results showed that the addition of wheat germ at the 2 per cent level, the amount found

in the whole wheat grain, did not increase the growth rate above that obtained with enriched flour alone. At the 4 per cent and 6 per cent levels, the growth rate was increased, but at the same rate for both levels. However, this pattern was not the same when supplements were made to non-enriched flour. At all three levels the addition of wheat germ to non-enriched flour produced an increase in growth. The reproduction and lactation performance showed the same trends as the growth test. The females fed enriched flour supplemented with 4 per cent wheat germ produced the largest number of litters and more of these litters survived.

Wheat germ has a protein efficiency value higher than that of any of the other milling fractions. It is about equal to non-fat milk solids in ability to improve the nutritive quality of patent flour. The protein efficiency ratio of patent flour (0.84) was improved to 1.00 and 1.10 by the addition of 3 per cent non-fat milk solids and of 3 per cent wheat germ, respectively (Hove et al., 1945).

Supplement: Wheat Gluten

Wheat gluten, a portion of the actual wheat kernel, has been suggested as a supplement to wheat flour.

According to Munaver and Harper (1959), the digestibility of the protein in wheat gluten was about 95 per cent and this was unaffected by the level of wheat gluten in the diet over the range of 30 per cent to 70 per cent. However,

those amino acids most limiting in wheat are also limiting in wheat gluten (Block and Mitchell, 1946-47). The concentration of these amino acids in whole wheat and wheat gluten are: lysine--2.7 per cent and 2.0 per cent, threonine--3.3 per cent and 2.7 per cent, and valine--4.5 per cent and 4.2 per cent, respectively.

The nutritive value of a wheat gluten supplement to high protein flour was studied using rat growth experiments (Howard et al., 1958). The animals fed the diets in which the flour was supplemented with wheat gluten (two parts per one hundred parts of flour) at the 10 per cent, 12 per cent, and 15 per cent protein levels (N x 6.25) gave protein efficiency ratios of 0.77, 0.99, and 1.01, respectively. Whereas, the animals receiving the unsupplemented flour diet at the same protein level showed protein efficiency ratios of 0.70, 0.89, and 1.04, respectively. The animals on the wheat gluten supplemented flour diets performed no better than the animals on the unsupplemented flour diets. Although, the total protein consumption, at the 15 per cent level of intake, for the gluten supplemented flour diet and the unsupplemented flour was 38.9 grams and 34.4 grams, respectively; this was equivalent to only 14.1 grams and 15.2 grams, respectively, of complete protein. The complete protein of the flour plus gluten diet was less than the unsupplemented flour diet, but the animals on the former diet ate more total protein. From the results of

of this experiment and the data on the amino acid content of wheat gluten, the supplemental value of wheat gluten to bread is of little value.

Supplement: Yeast

The lysine content of yeast is approximately 7 grams to 8 grams per 16 grams of nitrogen; whereas, the whole wheat kernal and wheat flour contain only 2.7 grams and 1.9 grams, respectively, of lysine per 16 grams of nitrogen (Block and Bolling, 1951). The threonine and valine content of yeast is also higher than that of whole wheat. From this information, it would appear that yeast may have supplemental value to wheat diets.

Two white breads--one containing approximately 8 per cent and the other approximately 12 per cent baker's yeast on the dry weight basis--were compared with baker's yeast alone at three levels of protein intake (8.3 per cent up to 12.9 per cent) in weanling rat diets. The supplemented white breads were also compared with the unsupplemented bread at two of the lower protein levels. In both cases, and at all levels of protein intake, the combination of white flour and baker's yeast yielded higher biological values than either the components separately. The authors Kon and Markuze (1931) concluded that a complementary relationship existed between the protein of white flour and that of brewer's yeast when mixed in approximate proportions of seven

to one or eleven to one.

The value of supplementing yeast to augment the protein value of wheat flour diets was studied by replacing 1 per cent, 3 per cent, and 5 per cent of the enriched flour with equivalent amounts of yeast, strain G (Sure, 1947). The diets were adjusted to contain 6 per cent to 7 per cent protein (about 52 per cent to 57 per cent flour). A marked increase in body weight which was entirely out of proportion to the increase in food intake was observed in each of these groups. In other words, a high protein efficiency was exhibited. Even on a 9.03 per cent protein intake, the blending of 80 per cent extraction flour with a small amount (1 per cent or 3 per cent) of this yeast resulted in significant increases in growth, but in a lesser increase in efficiency of food utilization.

In a subsequent rat growth study, Sure (1948a) substituted 1 per cent, 3 per cent, and 5 per cent cultured yeast for an equivalent portion of wheat flour in a basal diet containing 89 per cent wheat four. The optimum growth and greatest protein efficiency were obtained in the diet containing 5 per cent yeast. Sure (1948a, 1950) stated that the increase in biological value of the proteins in enriched wheat flour supplemented with dried food yeast is due to the latter's provision of the amino acid, lysine, and the B complex vitamins.

White bread, containing 3 per cent non-fat dry milk solids, supplemented with 1 per cent or 3 per cent dried, non-viable debittered brewer's yeast when fed to weanling rats at the 10 per cent protein level produced increases in the daily weight gain of 0.34 grams and 0.66 grams, respectively, above the gain (0.55 grams) produced with basic formula white bread (Seeley et al., 1950). The food utilization was also increased from 9.2 grams gain per 100 grams of food intake for white bread without yeast to 12.3 grams and 14.7 grams gain for the bread supplemented with 1 per cent or 3 per cent non-viable, debittered dried brewer's yeast. Similar increases in nutritive value were produced with other combinations of dried yeast and non-fat milk solids. The supplemental value of cultured yeast (G) and brewer's yeast (K) to the proteins in milled wheat flour is equal to that of non-fat milk solids (Sure, 1948b).

Supplement: Soybean Flour

The protein content of soybeans varies with varieties and environmental conditions. Bailey and co-workers (1935) observed a range in protein content (N x 6.25) of 19.6 per cent to 50.3 per cent with an average of 40.0 per cent.

If soybeans are heated, their nutritive value is increased. Wilgus et al. (1936) studied the effect of heating by several oil extraction processes on the relative

protein efficiency,

$$\left(\frac{\text{percentage of protein stored--test diet}}{\text{percentage of protein stored--standard casein diet}} \times 100\right),$$

when the soybeans were fed to chicks. For example, when using the expeller process for extracting the oil the relative protein efficiency was increased from thirty-eight for the raw soybeans to eighty-four for the heated meal. The nitrogen utilization of raw soybean meal fed to rats was 78 per cent; when the meal was cooked the nitrogen utilization increased to 82 per cent (Osborne and Mendel, 1917). Johnson and her co-workers (1939) confirmed previous findings that the nutritive value of soybean protein is improved by heat.

Soybeans have been found to contain a trypsin inhibitor which allows only incomplete intestinal hydrolysis of the soybean protein (Ham and Sandstedt, 1944, and Bowman, 1944). This, therefore, precipitates a deficiency of suboptimal amounts of certain amino acids (cystine and methionine) in soybeans and subsequently limits growth of the animal (Griswold, 1951). This trypsin inhibitor, however, is destroyed upon heating, thereby permitting more complete digestion. Desikachar and De (1947) stated that there may be other toxic growth inhibiting factors in soybeans which are also heat labile. These factors have not been fully studied.

Soybean flour can be used in any proportion up to 30 per cent with flour (white or whole wheat) not only for making bread, but also for other baked products. The addition of relatively small amounts of soybean flour to white flour (up to 10 per cent) may be made without changing the regular baking methods, although better results will be obtained by using a larger proportion of yeast and shortening. The most appetizing, attractive, and acceptable loaf of bread can be obtained if not more than 20 per cent soybean flour is mixed with wheat flour (Bailey et al., 1935).

Bread made from seventy-five parts wheat flour and twenty-five parts soybean flour (containing approximately 43 per cent protein) was fed to rats at the 10 per cent and 15.8 per cent protein level (N x 5.7) (Johns and Finks, 1921). Rats fed a whole wheat bread diet containing 10 per cent protein with no soybean flour supplement grew at one-third to two-thirds of the normal rate; however, rats fed the supplemented diets grew normally on both levels of protein except for a slight initial lag on the 10 per cent protein level. Bread was also made from eighty-five parts wheat flour and fifteen parts soybean flour and fed to rats at the 13.3 per cent protein (N x 5.7) level in the diet. The animals on this diet grew normally. The results of this work indicate that the addition of soybean flour to wheat flour for making bread produces a mixture of proteins which is more nearly complete than the proteins of wheat alone.

These results were corroborated by Kon and Markuze (1931) and Jones and Divine (1944).

Growth studies were carried out by Voltz and her associates (1945) in which weanling albino rats were fed diets containing white bread, white bread with 5 per cent soybean, patent flour, and patent flour with 5 per cent soybean flour. All diets were made of enriched patent flour or bread containing 3 per cent whole milk solids. The white bread contained 13.5 per cent protein and the wheat-soy bread, 14.9 per cent protein (N x 5.7). The protein efficiency ratio increased about 20 per cent when the white bread was supplemented with soybean flour and about 15 per cent when the white flour was similarily supplemented. The biological value of the protein of white bread also increased from 43.3 to 47.7 as a result of the supplementation. In a similar study, Sure (1948b) observed that the protein efficiency ratios of the rats fed diets containing 89 per cent enriched flour supplemented with 2 per cent and 3 per cent soybean flour increased approximately 34 per cent and 51 per cent, respectively.

A 3 per cent soy-bread and 3 per cent milk-bread were found to be approximately equal in quality; a 5 per cent soy-bread and a 6 per cent milk-bread were also approximately equal in quality. All the breads were superior to an unsupplemented white bread (Carlson et al., 1946, and Harris et al., 1944). Harris and his group (1944) continued

their rat growth studies to determine the value of soybean flour supplementation to a white bread containing milk solids. They observed the best growth effects from the following ratios of soybeans:non-fat milk solids supplements to breads--3.0:3.0, 2.5:1.5, and 3.0:2.3. A bread containing equal quantities of soy flour and skim milk solids is superior in protein quality to one containing the same amount of protein added as skim milk solids only.

In an animal growth study, Howard and his associates (1958) noted that rats receiving a diet containing approximately 12 per cent protein (N x 6.25) from flour supplemented with soybean protein (two parts soybean protein to one hundred parts of high protein flour) grew more rapidly than those receiving a diet containing 15 per cent protein from flour alone. Their growth was similar to animals receiving 12 per cent flour diets supplemented with four parts non-fat milk solids to one hundred parts high protein flour.

Crystalline Amino Acid Supplements to Wheat Diets

Crystalline amino acids have also been studied as supplements to improve the protein content of bread.

Supplement: Lysine

Since lysine is the most limiting amino acid in wheat diets, many workers felt the crystalline L-lysine could be used as a wheat flour supplement. Much work has been done concerning the optimum level to be used; some of these studies will be cited here.

Hutchison, Moran, and Pace (1956) in an animal growth study supplemented their basic diet of 80 per cent white bread with increasing amounts of L-lysine·HCl from 0.06 per cent to 1.2 per cent. They observed an increase in growth at each level up to the optimum at 0.25 per cent L-lysine. Yang and his associates (1961) also found 0.25 per cent L-lysine·HCl to be the optimum L-lysine supplement to wheat flour fed to rats at the 10 per cent protein level. The biological value of the supplemented flour diet was 55; whereas, the unsupplemented flour diet was only 34.4.

However, when using DL-lysine·HCl as a supplement to 90 per cent dried commercial white bread, the optimum growth response was obtained with a 0.4 per cent DL-lysine supplement (Rosenburg and Rohdenburg, 1952). Since the rat does not readily use D-lysine this would explain the need for more DL-lysine alone to reach the growth plateau. The animals on the stock diet (21.5 per cent protein) grew as well as those on the bread diet fortified with 0.8 per cent DL-lysine, but the protein efficiency ratio was lower, 1.31 versus 1.91. The authors felt that since the animals on

the 0.8 per cent lysine supplemented bread diet grew as well as or better than animals on the laboratory stock diet, the only important amino acid deficiency in common bread is lysine. The animals on the stock diet did not receive a vitamin supplement as did the animals on the bread diet; and the authors give no indication of the amount of vitamins available from the constituents of this stock diet. This might explain why the animals on the two diets responded similarly when it would be expected that a bread diet would be inferior to a well-formulated stock diet.

Experimental diets consisting of a bread mixture, made from all-purpose flour containing three levels of non-fat milk solids (3 per cent, 6 per cent, and 12 per cent) with each of these levels supplemented with 0.25 parts of lysine, were prepared for rat growth studies (Jahnke and Schuck, 1957). Growth, food efficiency, and nitrogen efficiency were increased with increased total lysine content of the diet. The animals on the diet containing twelve parts non-fat milk solids and the diet containing three parts non-fat milk solids and lysine exhibited similar weight gains and nitrogen efficiency ratios. Since these two diets are so similar and commercial bakers are not likely to use 12 per cent non-fat milk solids in their breads because of the cost, the authors felt that the other formula (three parts non-fat milk solids per one hundred

parts of flour plus 0.25 parts lysine) might be an acceptable method of raising the lysine content of diets when this is desired.

Culik and Rosenburg (1958) compared the reproductive performance of a group of rats fed a diet containing a commercial white bread with a group on a bread diet containing a lysine supplement (0.25 pounds per 100 pounds of flour). Both breads contained approximately 6 per cent non-fat milk solids, and the diets contained all the essential minerals and all the vitamins known to be required by the rat. The results indicated that the addition of lysine to bread improved reproduction and lactation performances considerably and uniformly throughout the seven litters of the parent generation and all successive generations. The performance of the animals maintained on the commercial bread diet was decidedly inferior to the animals on the lysine supplemented bread diet. Culik and Rosenburg (1958) stated:

> Almost all the animals on the commercial bread diet exhibited aside from poor growth, nervousness, irritability, anorexia, and perverted appetite. The animals chewed on feed cups and ate their own hair from the parts of their body they could reach. As the animals grew older and their lysine requirement diminished, this craving for hair subsided and the hair fully regenerated.

The authors also stated that throughout the entire study there were no indications of any adverse effects due to lysine supplementation of the flour used for the bread.

The addition of 0.15 per cent L-lysine to a basal diet (11 per cent protein, N x 5.7) prepared from 90 per cent air-dried wheat bread (containing 3.3 per cent roller-dried skim milk), and ample quantities of vitamins, minerals, and essential fatty acids, doubled the growth rate of the rats and caused a gain in body protein (Ericson and Ovenfors, 1959). However, this growth rate was still only about half that obtained with a stock diet (21 per cent protein, N x 6.25), or a diet containing 12 per cent egg protein (N x 6.25) but the same nitrogen content as the bread diets. A possible explanation the authors offer is that the biological value of the egg protein is better than bread protein. Although, the bread-lysine diets may be somewhat inferior to the more nearly perfect stock diet, this author questions the validity of quality comparison of two diets at such different levels of protein (11 per cent and 21 per cent) as Ericson and Ovenfors have done.

The supplementation of 100 parts of high protein flour with 0.15 parts of L-lysine·HCl was studied by using rat growth experiments. Diets containing the supplemented flour were fed to the animals at three levels of protein intake (10 per cent, 12 per cent, and 15 per cent) (Howard et al., 1958). At each protein level, the protein efficiency ratios of the animals on the supplemented diets were significantly increased over the protein efficiency ratios of the animals on the unsupplemented diets fed at the same levels of

protein intake. Harris and Burress (1959) overcame the lysine deficiency of the bread flour protein by including 0.31 grams and 1.18 grams of L-lysine·HCl per 100 grams of diet containing 8 per cent and 15 per cent wheat protein, respectively. The total lysine content of the protein in these diets was 5 per cent and 7.8 per cent, respectively.

Supplements: Lysine and Other Amino Acids

The nutritive value of bread proteins as measured by the rate of growth of weanling rats is limited, first of all, by the relatively low content of lysine as has been established. This value may be improved by the addition of lysine; however, another amino acid may then become the limiting factor. Studies concerned with the total amino acid balance in bread proteins have been reported.

In rat diets where bread protein contributing about 2.2 per cent nitrogen was supplemented with 0.2 per cent to 0.3 per cent L-lysine, no further improvement in nutritive value was observed by adding L-threonine (Hutchinson et al., 1958 and 1959). However, with bread diets of lower nitrogen content (1.2 per cent to 1.9 per cent) supplemented with 0.25 per cent L-lysine, a further improvement was obtained by the addition of 0.1 per cent L-threonine. Deshpande and co-workers (1957) also noted a combination of a 0.5 per cent L-lysine and a 0.4 per cent DL-threonine supplement to a white flour diet (1.5 per cent nitrogen) improved the

growth response over the 0.25 per cent L-lysine supplement alone. However, further growth improvement was obtained only when seven more essential amino acids were added.

When feeding rats a white bread diet (containing 3 per cent non-fat milk solids) supplemented with L-lysine at a nitrogen level (2.0 per cent) between the two discussed above, Rosenburg et al. (1954) obtained no further growth improvement by adding threonine, valine, or methionine.

Rats fed bread diets containing a higher level of nitrogen (2.2 per cent to 2.3 per cent) and 0.5 per cent added lysine exhibited a further growth improvement when 0.1 per cent L-threonine was also added. The addition to the diet of 0.5 per cent L-lysine alone, however, produced no better growth than did 0.25 per cent of the amino acid.

Sure (1952, 1954) observed that in whole wheat, lysine was the most limiting amino acid, valine the second, and threonine the third most limiting amino acid. Lysine was shown to be the most limiting amino acid, threonine the second, and valine the third in milled wheat flour (Sure, 1953 and 1955). Bender's (1957, 1958) results indicated that lysine was the most limiting amino acid, threonine the second, and methionine the third in a bread diet fed at 1.5 per cent nitrogen level (about 8 per cent protein, N x 5.7) for a ten day period. Whether bread, whole wheat, and milled wheat flour differ in their second and third limiting amino acid has not been clearly established.

The Activity of Liver Xanthine Oxidase with Respect to Dietary Protein

The activity of the liver's xanthine oxidase system is related to the quantity and quality of protein present in the diet.

McQuarrie and Venosa (1945) observed that the measured xanthine oxidase activity of rats' livers decreased approximately 50 per cent when the protein content of the animals' diets was reduced from 25 per cent in a stock diet to 20 per cent in a casein diet. When the protein level was lowered to 10 per cent (soybean oil meal diet or distillers solubles diet), the measurable xanthine oxidase activity was almost, if not completely, lost.

At a level of dietary protein which maintains and supports growth of the adult rat, liver xanthine activity may be appreciably decreased, due to an incomplete assimilation of the amino acids in the protein or a deficiency of a certain essential amino acid (Williams and Elvehjem, 1949, and Williams et al., 1949). These studies suggest that the enzyme activity is related to both the quality and quantity of protein in the diet. Litwack and his group (1952) found this to be true. As the quality of protein fed increased, the activity of xanthine oxidase also increased. These workers also noted that the rate of change of xanthine oxidase activity as related to the concentration of dietary protein leveled off by the ninth or tenth day on the dietary

regime.

A decrease in liver xanthine oxidase is paralleled by a decrease in the content of total nitrogen (Djii et al., 1957). Rat growth and protein efficiency ratios are also methods of determining the quality of a protein. However, the enzyme method is considered more precise than any of the above because interferences such as the hormonal state of the animal, water intake and water balance, and fat deposition will not alter the results (Litwack et al., 1953).

Significance of Supplementation

The many studies of protein and/or amino acid supplementation of wheat flour or bread leads to the question of whether such supplementation is necessary in the human diet.

Leo Friedman (1959) expresses the need to evaluate the problem of protein supplementation:

> Efforts to improve our food supply should always be encouraged, but it is important that enthusiasm in the commercial promotion of new products be tempered by a clear understanding of the facts and their significance. Special caution is needed in the case of proteins because several factors must be considered; namely, quantity, quality, daily intake, and supplementary relationship of the product to the other proteins of the diet.

The nutritional requirements of rats and humans differ, so a direct application of rat studies is not possible. However, one can learn much from the rat studies.

EXPERIMENTAL PROCEDURE

The basic diet used for these experiments was of the following composition:

Dried ground bread	90.00%
Corn oil[1]	5.00%
Mineral salts W[2]	4.00%
Vitamin mixture[3]	0.25%
Choline	0.15%
Sucrose	0.60%

The bread used in the preparation of diet I was a standard white bread and served as control. In diet II, the bread contained 83 per cent white spring wheat clear flour and 17 per cent Roman meal mix (a blend of wheat bran, whole wheat, whole rye, and defatted flaxseed meal). In diet III, the bread contained white Kansas wheat flour and white diet mix (seven parts to one hundred parts of wheat flour). The

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[1] Containing 7.5 mg. a-tocopherol.

[2] Obtained from Nutritional Biochemical Corporation.

[3] Containing 0.5 mg. thiamine, 0.5 mg. riboflavin, 1.0 mg. niacin, 0.25 mg. pyridoxine·HCl, 2.0 mg. calcium pantothenate, 10 mg. inositiol, 0.02 mg. folic acid, 0.002 mg. vitamin B_{12}, 0.01 mg. biotin, 10 mg. vitamin A, 0.18 mg. vitamin D, 0.38 mg. menadione, 1.0 mg. para-amino benzoic acid, and 0.225 gm. sucrose.

manufacturers could send no composition of this mix; however, the label on the packaged loaf indicated that it contained the following: white rye flour, oat flour, soya flour, gluten flour, barley flour; plus dehydrated vegetable flours made from carrots, pumpkins, kelp, lettuce, artichoke, celery, cauliflower, cabbage, and parsley. In diet IV, the bread contained 85 per cent clear flour, 10 per cent ground soybeans, and 5 per cent whole wheat flour. Those specifications which were available from the manufacturer of each bread can be found in the Appendix, page 74.

The breads were dried in a large drying oven at approximately 68° C. Drying time for each bread varied from two hours to four hours depending on the type of bread and thickness of the slices. The dried bread slices were then finely ground in a large Wiley mill using a 20 mesh screen. The ground breads were stored at 3-4° C in large metal cans for subsequent use and analyses.

Samples of each of the dried breads were analyzed for nitrogen, fat, ash, and calorie content. Duplicate one gram samples were used for all analyses, except for the fat determination where duplicate three gram samples were required. Nitrogen content was determined by the macro-Kjeldahl method, and fat content by ether extraction on the Goldfisch apparatus. Samples for ash determination were placed in a muffle furnace which was heated to 500° C. The samples remained in the furnace approximately twenty-

one hours. The Parr Adiabatic Bomb Calorimeter was used for the calorie determination.

Per cent moisture was also determined on fresh samples of each of the breads. The Cenco Moisture Balance containing an infra red light was used for this determination. The thermostat was set at 105 and a ten minute drying period was found to be optimum for bread samples.

Two experimental periods (A and B) were used, one of four weeks duration and the other of two weeks duration. In each period, forty weanling, male albino rats of the Sprague-Dawley strain were divided into four groups of ten each, on the basis of body weight. They were housed in individual wire bottom cages, and allowed both food and water ad libitum. Food intake records were kept, and spilled food was recovered, weighed, and the consumption records corrected accordingly. The animals were each weighed every week.

After the two week period and after the four week period, the ten rats from each group were sacrificed by decapitation. Livers were removed and weighed. The remaining carcasses were frozen until subsequent analyses. Livers were homogenized with water in a Potter Elvehjem homogenizer. The homogenates were quantitatively transferred to weighed evaporating dishes and dried at 90° C for twelve hours. The dried livers were weighed and ground to a fine powder in a small Wiley mill. Per cent moisture in the livers was

calculated.

One gram samples of the dried ground liver were used for the fat determination by ether extraction on the Goldfisch apparatus. The per cent nitrogen was determined on duplicate 0.3 gram samples using the macro-Kjeldahl method.

The carcasses were partially thawed and ground three times in a food mill. To obtain more homogenous samples they were then homogenized in a one quart Waring Blender with 100 milliliters of water. Twenty gram samples of homogenate were placed into weighed evaporating dishes. These samples were dried at about 95° C for fourteen hours. The dried samples and dishes were weighed and the per cent moisture subsequently determined.

Thirty or forty gram samples of the homogenates were lyophilized to be used for fat and nitrogen determination. The freeze-dried samples were broken into fine flakes and stored in covered glass bottles at room temperature for subsequent analyses. Per cent fat and per cent nitrogen were determined using the same procedures used on the livers.

A two week follow-up study (Experiment C) was performed using the two breads with the most similar protein content and most different growth response. These two breads were the bread containing the Roman meal mix and the bread containing the white diet mix (groups II and III, Experiment A). The per cent of ground dried bread used in the diet was adjusted in the case of the latter bread to

obtain iso-protein diets labeled II and V, respectively. Each of these iso-protein diets was supplemented with 0.25 per cent L-lysine·HCl (Diets VI and VII, respectively). Lysine supplements to the diets were made at the expense of sucrose.

Forty weanling, male, albino rats of the Sprague-Dawley strain were divided into four groups of ten rats for this experiment. Again, the animals were allowed both food and water ad libitum, and food consumption and growth records were kept as previously.

After a two week period the animals were sacrificed by decapitation and the livers were removed. One gram samples were immediately removed and homogenized in five milliliters of 0.039 M $NaKPO_4$ buffer, pH 7.3, for the determination of liver xanthine oxidase. The remainder of the livers were weighed and frozen, as were the carcasses, for subsequent analyses. The carcasses and the remainder of the livers, combined as necessary in groups to get a large enough sample, were analyzed as in the previous experiments.

Xanthine oxidase activity was determined by the manometric procedure using the Warburg apparatus. A modification of the method of Axelrod and Elvehjem (1941) was used in this study. The procedure was altered as follows: 1) the substrate was pipetted into the main compartment instead of into the side arm of the Warburg flask, and 2) the time allowed for temperature equilibration was decreased from ten

minutes to five minutes. These changes were made to help eliminate timing errors and loss of substrate which occurs when tipping the flask to get the substrate from the side arm into the main compartment. Since there is quite a long lag phase (about forty minutes) before xanthine oxidase activity begins, there is no danger of loss of activity. These changes reduced the number of manipulations necessary and shortened the time of the operation, thereby increasing the accuracy of the procedure. The Warburg flasks were incubated in a 37^{o} C water bath for the entire determination. Readings of activity were taken at ten minute intervals, beginning after the five minutes equilibration time. The xanthine oxidase activity was expressed as microliters of oxygen uptake per hour per gram of liver.

Standard errors of the mean were calculated for all data. The student "t" test was used as a measure of significance.

RESULTS

The results of this study are summarized in Tables 1 through 8.

Bread Analyses Data (Table 1)

Results of bread analysis, other than moisture content, are reported on the dry weight basis. The protein content (N x 5.7)[1] for the standard white bread (diet I) was 12.2 per cent. An increase in protein content of the other breads varied from 15.6 per cent more protein in the Roman meal bread (diet II) to 72.1 per cent more protein in soybean bread (diet IV). The special white diet bread (diet III) contained 23.0 per cent more protein than the standard white bread. Thus, the manufacturers' protein supplements, added to these breads, indeed increased the protein content as determined by chemical analyses.

Along with the increase in the protein levels of these supplemented breads a similar increase was noted in the fat and ash content. The fat content ranged from 1.88 per cent for the standard white bread to 3.02 per cent for the soybean bread and the per cent ash ranged from 2.81 per

[1]Association of Official Agricultural Chemists, 1960.

cent to 4.34 per cent for the same breads.

The breads were quite similar in moisture content, averaging 38.8 per cent. The caloric value of the breads was also very similar; the mean was 4.41 calories per one gram sample.

Results of Experiment A and B

Food Consumption and Growth Data (Table 2)

After two weeks and four weeks on the regime, no significant difference was observed in the food intake of the rats fed the Roman meal bread (group II) as compared to the rats fed the standard white bread (group I). However, the rats fed the Roman meal bread gained slightly more weight than did the rats fed the standard white bread. This difference was significant at the 1 per cent level ($P < 0.01$). But, in both experimental periods, the protein efficiency ratios of the rats fed the Roman meal bread (group II) were not significantly different from those of the rats fed the standard white bread (group I).

The rats fed the special white diet bread (group III) consumed more food and gained significantly ($P < 0.01$) more weight than the rats fed the standard white bread during both the two week and four week experimental periods. The protein efficiency ratios for both of these experimental periods were also significantly greater ($P < 0.01$) for the rats fed the

special white diet bread as compared to the rats fed the standard white bread.

The food consumption and weight gain of the rats fed the soybean bread (group IV) was significantly greater at the 1 per cent level ($P < 0.01$) than that of the rats fed the standard white bread (group I). This observation was true for both the two week and four week experimental periods. Even though the rats fed the soybean bread exhibited almost triple the weight gain of the rats on the standard white bread (group I), their protein efficiency ratio following the two week feeding period was only a little greater ($P < 0.05$) than the control Group I. However, after four weeks on the diet, the protein efficiency ratio of the rats fed the soybean bread was significantly greater at the 1 per cent level ($P < 0.01$) than that of the animals on the standard white bread.

Liver Analyses Data (Table 3)

The per cent nitrogen in the livers of the rats fed Roman meal bread (group II) was not significantly different from that of the rats fed the standard white bread (group I) following the two week experimental period. However, after four weeks on the diets, the livers of the rats fed Roman meal bread contained slightly more nitrogen than those of the control group. This was significantly different at the 5 per cent level ($P < 0.05$). No significant differences

were observed in the liver fat content of the animals fed the Roman meal bread as compared to those fed the standard white bread for both the two week and four week experimental periods. The moisture content of the livers of the rats fed the Roman meal bread was slightly lower ($P < 0.05$) than those of the rats fed the standard white bread for the two week period. After four weeks on the experimental diets no significant difference was noted in the moisture content of the livers of these two groups.

The livers of the rats fed the special white diet bread (group III) responded in a similar manner as the livers of the rats fed the Roman meal bread. For the two week period, the nitrogen content of the livers of the rats fed the special white diet bread and the rats fed the standard white bread were not significantly different. However, after four weeks on the diet, the per cent nitrogen in the livers of the rats fed the special white diet bread was significantly higher at the 1 per cent level ($P < 0.01$) than in those fed the standard white bread. The per cent liver fat of these two groups was not significantly different for both the two week and four week experimental periods. The per cent liver moisture was slightly lower for the rats fed the special white diet bread for the two week period, but was not significantly different from that of the control group following four weeks on the diet.

The nitrogen content of the livers of the rats fed the soybean bread (group IV) was significantly higher at the 1 per cent level ($P < 0.01$) than those of the rats fed the standard white bread (group I) and those of the rats fed the Roman meal bread (group II) for both the two week period and the four week period. For the four week period the nitrogen content of group IV was also significantly higher ($P < 0.01$) than that of the rats fed the special white diet bread (group III). The liver fat content of the rats fed the soybean bread was significantly lower than that of all the other groups for both the two week and four week experimental periods. These differences were all significant at the 1 per cent level ($P < 0.01$) except when the soybean fed group was compared to the control group for the two week period. These were significantly different at only the 5 per cent level ($P < 0.05$). The liver moisture content was significantly higher ($P < 0.05$) than that of the rats fed the special white diet bread for the two week period and significantly lower ($P < 0.05$) than that of the rats fed the Roman meal bread for the four week period.

The wet weight of the livers of the rats fed the soybean bread (group IV) averaged higher than the liver weights of the rats of the other groups.

Carcass Analyses Data (Table 4)

The data from the carcass analyses recorded in this section were obtained using carcasses from which livers had been removed.

The per cent nitrogen, per cent fat, and per cent moisture of the carcasses of the rats fed the Roman meal bread (group II) were not significantly different from those of the rats fed the standard white bread.

A significantly higher fat content was noted in the carcasses of the rats fed the special white diet bread (group III) as compared with the rats fed the standard white bread for the two week period. The difference was significant at the 5 per cent level ($P < 0.05$). However, there was no significant difference in the fat content of the carcasses of both of these groups following the four week experimental period. There were also no significant differences noted in the per cent nitrogen and per cent moisture of the carcasses of both of these groups for the two week and four week experimental periods.

No significant differences were observed in nitrogen content of the carcasses of the rats fed the soybean bread (group IV) when compared with those of the rats fed the standard white bread for both the two week and four week experimental periods. The carcass fat content of these groups for the two week feeding period was not significantly different. The per cent fat of the carcasses of the rats

fed the soybean bread for the four week period, however, was almost 16 per cent higher than that of the rats fed the standard white bread. This difference was significant at the 1 per cent level ($P < 0.01$). The carcass moisture of the rats fed the soybean bread for two weeks was slightly higher than that of the rats fed the standard white bread. The difference was significant at the 5 per cent level ($P < 0.05$). However, no significant difference in carcass moisture was observed between the two groups fed their respective diets for four weeks.

Results of Experiment C

Food Consumption and Growth Data (Table 5)

The rats in the group fed the lysine supplemented Roman meal bread (group VI) consumed about thirteen grams more food per week than did the group of rats fed the unsupplemented Roman meal bread (group II). This difference was significant at the 1 per cent level ($P < 0.01$). Rats on the lysine supplemented special white diet bread consumed approximately eleven grams more food per week than rats on the unsupplemented bread (group V). This was significantly different only at the 5 per cent level ($P < 0.05$).

Rats fed the lysine supplemented Roman meal bread (group VI) grew approximately 2-1/2 times faster than the rats fed the unsupplemented Roman meal bread (group II) and

the protein efficiency ratio was about twice that of the rats fed the unsupplemented bread ($P < 0.01$).

The weight gains and the protein efficiency ratios of the rats fed the lysine supplemented special white diet bread (group VII) were almost twice those of the rats fed the unsupplemented special white diet bread ($P < 0.01$).

Liver Analyses Data (Table 6)

No significant differences were noted in the per cent nitrogen, per cent fat, or per cent moisture of the rat livers when the groups receiving the lysine supplemented bread were compared to the groups receiving the unsupplemented breads.

Liver Xanthine Oxidase Data (Table 7)

The average activity of the liver xanthine oxidase system of the animals fed the Roman meal bread (group II) was 127 microliters of oxygen uptake per hour per gram liver, whereas when the animals received the Roman meal bread supplemented with L-lysine·HCl (group VI) the xanthine oxidase activity increased to 174 microliters of oxygen uptake per hour per gram of liver. These activities, however, were significantly different at the 5 per cent level ($P < 0.05$) only. The livers of the rats receiving the special white diet bread (group V) were observed to have a xanthine oxidase activity of 129 microliters of oxygen uptake per hour per gram of liver. Adding L-lysine·HCl to this diet

(group VIII) caused an apparent increase of the liver xanthine oxidase activity to 193 microliters of oxygen uptake per hour per gram liver. However, these activities were not significantly different from one another. The failure to obtain significance between means was undoubtedly due to the high standard errors; this enzyme system proved to be extremely variable from animal to animal.

Carcass Analyses Data (Table 8)

The data from the carcass analyses recorded in this section were obtained using the carcass from which the liver had been removed.

The per cent nitrogen in the carcasses of the rats fed the Roman meal bread plus lysine (group VI) was significantly less at the 5 per cent level ($P < 0.05$) than that of the rats fed the unsupplemented Roman meal bread (group II). These same rat carcasses contained significantly more fat than those of the rats fed the unsupplemented Roman meal bread. This difference was also significant at the 5 per cent level ($P < 0.05$). There was no significant difference between these groups with respect to carcass moisture content.

No significant differences were observed in the per cent nitrogen, per cent fat, or per cent moisture of the carcasses of the rats fed the lysine supplemented special white diet bread (group VII) when compared to those of the rats fed the unsupplemented bread (group II).

DISCUSSION AND CONCLUSIONS

The results of this study indicate that increasing the protein content of a commercial bread does not necessarily result in a more nutritious product for the albino rat.

In this study, several commercial breads were studied with respect to their nutritional quality in relation to their protein quantity; these breads contained protein supplements of various quality and quantity. It would have defeated the purpose of this study to devise isonitrogeneous diets in experiments A and B; as a result, choosing a criterion for comparing data from the four groups of rats in these first two experiments was somewhat difficult. In order to take into account the differences in diet composition, food intake, and weight gain; the use of the protein efficiency ratio, calculated over a four week period, was chosen as the criterion in evaluating the various diets. A. E. Bender (1956), after making numerous comparative studies, feels that 'protein efficiency ratio gives a measure of nutritive value which is as useful as net protein utilization' (biological value times digestibility). Use of the protein efficiency ratio as a method of evaluating protein quality has been recommended as the official method of the Association of Official Agricultural Chemists for materials

containing in excess of 9.09 per cent protein (N x 6.25) (Derse, 1960).

The Roman meal bread contained more protein than the standard white bread (14.1 per cent versus 12.2 per cent). Although the rats on this diet consumed more protein and gained more weight than the control animals, the protein efficiency ratio of the rats fed the Roman meal bread was not significantly different from that of the rats fed the standard white bread. No differences in tissue composition between these two groups were observed. The nutritive value of the Roman meal bread was not different from that of the standard white bread despite the increase in protein quantity. This additional protein did not provide the amino acids lacking in the standard white bread.

The special white diet bread contained 24 per cent more protein than the standard white bread (15.0 per cent versus 12.2 per cent). As a result of this increase in protein, the protein efficiency ratio increased to 1.34 from the 1.18 of the standard white bread. The carcass composition of these groups was not different from each other. Although the special white diet bread contained only 6 per cent more protein than the Roman meal (15.0 per cent versus 14.1 per cent), the performance of the animals on this diet was much better. The animals on the special white diet bread gained 50 per cent more weight than the animals on the Roman meal bread and the protein efficiency ratio was 22

per cent higher. In contrast to the Roman meal bread, the protein supplement used in the special white diet bread significantly increased the nutritive value of the bread for the white rat.

The soybean bread contained almost twice the protein of the standard white bread and the protein efficiency ratio was significantly increased to 1.43 from the 1.18 of the standard white bread.

Although, the soybean bread contained 40 per cent more protein than the special white diet bread, the protein efficiency ratios of the rats fed these diets suggested these two breads were quite similar in quality. However, the liver fat of the group of rats fed the soybean was significantly lower and the liver nitrogen was significantly higher than that of all the other groups. This probably occurred because the animals fed the soybean bread consumed more protein than the animals on the other diets.

Although marked differences in protein efficiency ratio and growth were observed, results of carcass analyses were quite similar between groups. This suggests the stimulation in growth by any of these diets as compared with the control did not favor a specific tissue component, i.e., fat or nitrogen. The increased growth observed in the rats fed the special white diet bread or the soybean bread was reflective of an increased quantity of total tissue deposited.

The data collected during the two week experimental period agree qualitatively, but not quantitatively, with those collected during the four week experimental period. The rats on the four week study were consistently smaller at comparative periods to the rats on the two week regime. The inability to obtain quantitative reproducibility is due to factors such as genetic differences and environmental conditions, since the two experiments were conducted at different times during the year and with different litters of rats.

In experiment C, the two breads (Roman meal and special white diet) which had the most similar protein contents and yet produced the most different responses in the albino rat were chosen for further comparison in iso-protein diets and with a lysine supplement. The lysine supplement was added to these breads to determine if the protein quality could be further improved. Although the Roman meal bread and special white diet bread were made up in iso-protein diets, the rats on the special white diet bread exhibited better growth than the rats on the Roman meal bread as shown by the protein efficiency ratios. This again supports the observation made in experiments A and B, and suggests the special diet bread contains a more nearly complete protein than the Roman meal bread.

The L-lysine·HCl supplements to the Roman meal bread diet and the special white diet bread diet increased the quality of the respective proteins as exhibited by significantly

higher protein efficiency ratios over that of the unsupplemented breads. However, the addition of lysine had a greater effect on the Roman meal bread than it did on the special white diet bread. The lysine supplement stimulated a 92.6 per cent increase in the protein efficiency ratio over the unsupplemented Roman meal bread diet and 52.7 per cent increase in the protein efficiency ratio over the unsupplemented special white diet bread. The breads were more nearly similar in protein quality as a result of the supplement, which again indicates the special white diet bread contained a better amino acid pattern.

Because the results of the liver and carcass analyses were quite similar for each group, this again suggests the quality of the diet did not affect the deposition of any tissue component but rather the quantity of total tissue deposited.

The liver xanthine oxidase system which is sensitive to small changes in protein quality, showed definite trends. The xanthine oxidase activity of the livers of the rats fed the lysine supplemented breads increased over that of the rats fed the unsupplemented breads. Even though, these differences are not significant by calculation, the trend toward a correlation between liver xanthine oxidase activity and nutritive value of the diet is definitely present. The large standard errors of the mean are no doubt a factor causing such low significance and this in turn is probably because

the activity of this enzyme system was so erratic that reproducible results were often difficult to obtain. It is possible that the animals had already begun adapting to these diets before xanthine oxidase determinations were initiated (Litwack et al., 1952).

The results of these experiments again substantiate the concept that total quantity of protein (as determined chemically) in a given food stuff is practically worthless in determining the value of the protein. In order to properly evaluate the quality of a given protein, or mixture of proteins, some knowledge of the amino acid composition must be available.

SUMMARY

Weanling albino rats were used as experimental animals in assaying the comparative nutritional quality of three protein supplemented breads with that of a standard white bread. The three specialty breads varied with respect to the nature of the protein supplement and quantity of the supplement used in the formula.

In the first experiment, rats were divided into four groups of twenty rats each and fed, ad libitum, the experimental diets consisting of 90 per cent bread. The experimental diets contained the following: I--a standard white bread (12 per cent protein); II--a bread containing a Roman meal baker's mixture (14.1 per cent protein); III--a bread containing a special white diet mix (15.1 per cent protein); and IV--a bread containing ground soybeans (21.0 per cent protein). At two weeks and again at four weeks, ten rats from each group were sacrificed, and both the livers and the carcasses were analyzed for nitrogen, fat, and moisture. The protein efficiency ratio (grams gain per gram of protein eaten) was used as the criterion in evaluating the diets.

The nutritional quality of the three breads studied did not correlate with the protein content. Roman meal

bread contained more protein than the standard white bread; however, the protein efficiency ratio of the rats fed the Roman meal bread was not significantly different from that of the rats fed the control diet.

The special white diet bread contained only slightly more protein than did the Roman meal bread (15.0 per cent versus 14.1 per cent), but the protein efficiency ratio of the rats fed the special white diet bread was 22 per cent higher than the protein efficiency ratio of the rats fed the Roman meal bread.

The soybean bread contained more protein than did the special white diet bread (21.0 per cent versus 15.0 per cent); however, the protein efficiency ratios of the rats on these diets suggest these two breads were quite similar in quality.

A two week follow-up study was conducted using the two breads with the most similar protein content and the most different growth response (Roman meal and special white diet breads). These breads were studied in iso-protein diets with and without supplements of 0.25 per cent L-lysine·HCl.

The lysine supplements to both breads increased the quality of their respective proteins as exhibited by significantly higher protein efficiency ratios over those of the unsupplemented breads. The trend in the livers' xanthine oxidase values correlated with the protein efficiency ratios.

The addition of lysine had a greater effect on the Roman meal bread than it did on the special white diet bread, suggesting that the latter contained a more nearly complete protein than the Roman meal bread.

The quantity of protein present in a given food cannot be used as the sole criterion in evaluating its nutritive value. As the results of these experiments illustrate, the quality of the protein is as important a factor as is the quantity.

TABLE 1

BREAD[1] ANALYSES DATA

Bread[2]	Protein (Nx5.7) (% dry wt)	Fat (% dry wt)	Moisture (%)	Ash (% dry wt)	Calories (per 1 gm sample)
I	12.2	1.88	38.6	2.81	4.32
II	14.1	2.46	38.0	3.18	4.42
III	15.0	2.84	38.3	4.02	4.38
IV	21.0	3.02	40.4	4.34	4.52

[1]Those specifications which were available from the manufacturer of each bread may be found in the appendix, page 74.

[2] I. Standard white bread--control
II. Roman meal bread
III. Special white diet bread
IV. Soybean bread.

TABLE 2

FOOD CONSUMPTION AND GROWTH DATA
(Experiment A and B)

Group[1]	Food Intake (gm/wk)	Weight Gain (gm/wk)	Protein[2] Efficiency Ratio
	Four Weeks		
I	58.6 ± 1.7[3]	8.4 ± 0.2[3]	1.18 ± 0.03[3]
II	63.8 ± 2.3	10.0 ± 0.4	1.11 ± 0.02
III	74.3 ± 1.7	15.0 ± 0.5	1.34 ± 0.02
IV	98.4 ± 3.0	29.6 ± 0.9	1.43 ± 0.02
	Two Weeks		
I	60.2 ± 2.0[3]	12.9 ± 0.4[3]	1.77 ± 0.06[3]
II	65.9 ± 5.4	15.2 ± 0.4	1.62 ± 0.06
III	80.9 ± 3.1	25.5 ± 1.2	2.10 ± 0.04
IV	89.2 ± 3.4	36.2 ± 1.8	1.92 ± 0.03

[1]Group I--Standard white bread
Group II--Roman meal bread
Group III--Special white diet bread
Group IV--Soybean bread.

[2]Grams of weight gain per gram of protein eaten.

[3]Standard error of the mean.

TABLE 3

LIVER ANALYSES DATA
(Experiment A and B)

Group[1]	Liver Weight (gm wet wt)	Nitrogen (% wet wt)	Fat (% dry wt)	Moisture (%)
		Four Weeks		
I	3.42	2.35 ± 0.04[2]	18.7 ± 1.5[2]	71.4 ± 0.5[2]
II	3.72	2.53 ± 0.05	16.0 ± 0.7	72.5 ± 0.1
III	4.43	2.57 ± 0.06	16.6 ± 0.5	72.1 ± 0.2
IV	6.06	3.07 ± 0.10	11.5 ± 0.6	71.7 ± 0.3
		Two Weeks		
I	2.80	2.52 ± 0.06[2]	15.7 ± 1.2[2]	70.9 ± 0.2[2]
II	3.16	2.57 ± 0.02	16.3 ± 0.9	70.3 ± 0.2
III	4.06	2.88 ± 0.05	17.2 ± 1.3	69.9 ± 0.3
IV	5.02	2.78 ± 0.04	12.0 ± 0.7	70.9 ± 0.3

[1]Group I--Standard white bread
Group II--Roman meal bread
Group III--Special white diet bread
Group IV--Soybean bread.

[2]Standard error of the mean.

TABLE 4

CARCASS ANALYSES DATA[1]
(Experiment A and B)

Group[2]	Carcass Weight (gm wet wt)	Nitrogen (% wet wt)	Fat (% dry wt)	Moisture (%)
		Four Weeks		
I	81	2.62 ± 0.04[3]	35.9 ± 1.4[3]	62.7 ± 0.5[3]
II	88	2.60 ± 0.05	37.1 ± 1.2	63.3 ± 0.4
III	109	2.61 ± 0.04	38.2 ± 1.4	63.4 ± 0.5
IV	165	2.68 ± 0.04	41.6 ± 1.0	61.5 ± 0.5
		Two Weeks		
I	70	2.70 ± 0.13[3]	38.2 ± 1.2[3]	61.5 ± 0.8[3]
II	76	2.58 ± 0.06	39.4 ± 1.3	63.5 ± 0.6
III	97	2.44 ± 0.04	44.3 ± 1.9	62.0 ± 0.8
IV	118	2.55 ± 0.05	40.3 ± 1.7	63.7 ± 0.5

[1]Analyses on carcass from which livers had been removed.

[2]Group I--Standard white bread
Group II--Roman meal bread
Group III--Special white diet bread
Group IV--Soybean bread.

[3]Standard error of the mean.

TABLE 5

FOOD CONSUMPTION AND GROWTH DATA
(Experiment C)

Group[1]	Food Intake (gm/wk)	Weight Gain (gm/wk)	Protein[2] Efficiency Ratio
II	51.6 ± 2.6[3]	9.0 ± 0.7[3]	1.33 ± 0.06[3]
VI	65.2 ± 2.9	22.0 ± 1.3	2.56 ± 0.07
V	67.2 ± 3.0	16.0 ± 0.8	1.97 ± 0.02
VII	78.8 ± 3.7	29.2 ± 1.7	3.01 ± 0.09

[1]Group II--Roman meal bread
Group VI--Roman meal bread + 0.25% L-lysine·HCl
Group V--Special white diet bread
Group VII--Special white diet bread + 0.25% L-lysine·HCl.

[2]Grams of weight gain per gram of protein eaten.

[3]Standard error of the mean.

TABLE 6

LIVER ANALYSES DATA
(Experiment C)

Group[1]	Liver weight (gm wet wt)	Nitrogen (% wet wt)	Fat (% dry wt)	Moisture (%)
II	2.38	2.26 ± 0.04[2]	15.4 ± 0.5[2]	73.4 ± 0.8[2]
VI	3.75	2.28 ± 0.02	14.1 ± 1.2	71.7 ± 0.4
V	3.05	2.30 ± 0.04	16.3 ± 0.6	70.8 ± 0.5
VII	4.24	2.25 ± 0.04	14.6 ± 0.6	70.8 ± 0.4

[1]Group II--Roman meal bread
Group VI--Roman meal bread + 0.25% L-lysine·HCl
Group V--Special white diet bread
Group VII--Special white diet bread + 0.25% L-lysine·HCl.

[2]Standard error of the mean.

TABLE 7

LIVER XANTHINE OXIDASE DATA
(Experiment C)

Group[1]	ul O_2/hr/gm liver
II	127 ± 17[2]
VI	174 ± 11
V	129 ± 20
VII	193 ± 22

[1]Group II--Roman meal bread
Group VI--Roman meal bread + 0.25% L-lysine·HCl
Group V--Special white diet bread
Group VII--Special white diet bread + 0.25% L-lysine·HCl.

[2]Standard error of the mean.

TABLE 8

CARCASS ANALYSES DATA[1]
(Experiment C)

Group[2]	Carcass Weight (gm wet wt)	Nitrogen (% wet wt)	Fat (% dry wt)	Moisture (%)
II	62	2.99 ± 0.06[3]	28.4 ± 1.3[3]	64.0 ± 0.7[3]
VI	88	2.77 ± 0.05	33.9 ± 1.8	64.2 ± 0.5
V	76	2.70 ± 0.04	35.6 ± 1.8	63.7 ± 0.6
VII	103	2.74 ± 0.04	35.8 ± 1.6	64.6 ± 0.6

[1]Analyses on carcass from which livers had been removed.

[2]Group II--Roman meal bread
Group VI--Roman meal bread + 0.25% L-lysine·HCl
Group V--Special white diet bread
Group VII--Special white diet bread + 0.25% L-lysine·HCl.

[3]Standard error of the mean.

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APPENDIX

A SUMMARY OF THE INFORMATION AVAILABLE FROM THE MANUFACTURER OF EACH BREAD

Composition of the Breads:

	Bread II (Roman meal bread)	Bread III (special white diet bread
Protein (%)	10.0 (average)	11.5
Fat (%)	3.1	1.18
Carbohydrate (%)	47.8	50.13
Moisture (%)	38.0	
Calories (per pound)	1150.0	1186.8
Ash (%)	2.27	2.13

All breads contained yeast and yeast foods and all except the soybean bread contained dried milk solids. The standard white bread and the Roman meal bread were enriched according to government standards with thiamine, riboflavin, niacin, iron, and calcium.

The bread used in diet II contained 83 per cent white spring wheat clear flour (minimum protein 14-1/2 per cent) and 17 per cent Roman meal baker's mixture. The Roman meal baker's mixture contained selected wheat bran, whole wheat, whole rye, defatted flaxseed meal. The bread wrapper bore this statement ". . . made from high protein wheat."

In diet III, the bread contained white Kansas wheat flour and a white diet mix (seven parts to one hundred parts of wheat flour). The label on the packaged loaf of bread indicated that the white diet mix contained the following: white rye flour, oat flour, soya flour, gluten flour, barley flour; plus dehydrated vegetable flours made from carrots, pumpkins, kelp, lettuce, artichoke, celery, cauliflower, cabbage, and parsley. The label from this bread bore the statement "Extra Protein."

The bread used in diet IV contained 85 per cent clear flour, 10 per cent ground soybeans, and 5 per cent whole wheat flour.

ROOM USE ONLY