

THE NUTRITIONAL VALUE OF TWO SUPPLEMENTED
BREADS COMPARED WITH THAT OF A
STANDARD WHITE BREAD

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
Dorothy June Walworth
1960

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THE NUTRITIONAL VALUE OF TWO SUPPLEMENTED BREADS COMPARED
WITH THAT OF A STANDARD WHITE BREAD

By

Dorothy June Walworth

AN ABSTRACT

Submitted to the College of Home Economics
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE

Department of Foods and Nutrition

1960

Approved

Dorothy Anata

ABSTRACT

The nutritional value of the protein in each of 2 breads, advertised as containing more protein per unit weight than the average bread, was compared with a standard white bread. One bread contained Roman meal. The other bread contained a blend of wheat flours supplemented with lysine. Each bread was analyzed for nitrogen.

Thirty three weanling, male, albino rats were divided into 3 equal groups and fed 90 per cent bread diets. Food and water were allowed ad libitum throughout the 2 week experimental period. Food intake and weight records were kept. At the close of the experimental period, animals were decapitated, and livers were removed and analyzed for xanthine oxidase activity, nitrogen, fat, and moisture. Carcasses were analyzed for nitrogen, fat, and moisture.

The bread containing Roman meal provided approximately $1/3$ more protein (dry weight) than did the standard white bread. However, rats fed the bread containing Roman meal did not grow at a rate greater than the control, thus, had lower protein efficiency ratios than did rats fed the standard white bread.

The xanthine oxidase system in the livers from rats fed bread containing Roman meal was not significantly more active per unit weight of liver nitrogen than this enzyme

system in the livers from rats fed the standard white bread. No significant differences were observed between these 2 groups with respect to the composition of the carcass, or the liver with one exception. Livers from rats fed bread supplemented with Roman meal contained a greater per cent of nitrogen than did the livers from rats fed the standard white bread. This difference was small, but significant.

Therefore, while the bread containing Roman meal provided more protein than did the standard white bread, as determined by chemical analysis, the quality of this protein was not improved; the proportions of amino acids supplied by the protein of Roman meal did not complement those of white flour. The additional cost of Roman meal bread (10 cents more per pound) was not warranted in terms of nutritional value.

The second bread studied presented an entirely different picture. The amount of protein provided by the lysine supplemented bread was approximately twice that provided by the standard white bread as determined by chemical analysis. Significantly more growth and significantly higher protein efficiency ratios were observed in rats fed the lysine supplemented bread than in rats fed the standard white bread.

The xanthine oxidase system was significantly more active in livers from rats fed the lysine supplemented bread than in livers from rats fed the standard white

bread. Rats fed the lysine supplemented bread had significantly larger livers, which contained a greater per cent of nitrogen and a smaller per cent of fat, than did rats fed the standard white bread. The only significant difference observed between these 2 groups, with respect to carcass composition, was a greater per cent of nitrogen in the carcasses of rats fed the lysine supplemented bread.

In addition to providing more protein, the lysine supplemented bread provided a better balanced amino acid pattern, thus, the protein was more efficiently utilized than that of the standard white bread. As a result, the nutritional value of the protein in the lysine supplemented bread was markedly superior to that of the standard white bread. The superior nutritional value of the protein in the lysine supplemented bread may be worth the additional cost of .7 cents per pound for individuals consuming sub-optimum protein. However, it is recognized that the majority of people in the United States consume adequate protein.

Liver xanthine oxidase activity was an excellent measure of the nutritional value of the protein in bread. Significant differences in the activity of this enzyme in the livers from young rats were observed after an experimental feeding period of only 14 days, therefore, it is a sensitive, fast, and economical assay method.

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ACKNOWLEDGMENTS

The author gratefully acknowledges the encouragement and guidance given her throughout this project by Dr. Dorothy Arata. Thanks are also extended to Dr. Catherine Carroll for her interest and help.

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INTRODUCTION

INTRODUCTION

In recent years, the emphasis on protein and on weight reduction has increased the popularity of "high protein--low calorie" foods. Manufacturers have supplemented bread with a variety of foods, and emphasized the protein content of the bread in their labeling. These breads constitute approximately 2 per cent of the total bread market (Friedman, 1959). When applied to a population of approximately 170 million people, who consume approximately 120 pounds of bread per person per year (Block and Mandl, 1958), this represents a considerable volume of business.

Advertising claims concerning "high protein" breads present vague information, which implied a need for increasing dietary protein, and emphasizes the value of a particular product for meeting this need. Labeling claims refer to "more," "better," or "improved" protein in the bread. A few breads are advertised as containing protein, "equivalent to meat, milk, and eggs." When a variety of these special breads were examined, the quantity of protein ranged from approximately equal to twice that of a standard bread, while the quality of protein varied from $3/4$ to twice that of a standard bread (Friedman, 1959). Thus, claims and label declarations, with respect to protein, are often misleading.

The majority of these special breads, advertised as containing more or better quality protein than the standard bread, are supplemented with natural foods. However, recently bread has been supplemented with a chemical compound, lysine, the amino acid most deficient in wheat. Although lysine supplements have improved the nutritional value of the protein of wheat, most investigators question the necessity of supplementing cereal products with lysine in the United States, where the majority of the people consume an adequate intake of high quality protein.

This experiment was undertaken to study the nutritional value of the protein contained in 2 commercially produced breads, advertised as "high protein" breads, as compared to that of a standard white bread. One bread contained a supplement composed of cereal grains. The other bread contained a supplement of lysine. The cost of these breads was also considered.

Measurements used in this study included growth, protein efficiency ratio, per cent of nitrogen, fat, and moisture in the livers, and per cent of nitrogen, fat, and moisture in the carcasses of young rats. Since the activity of the liver enzyme, xanthine oxidase, is affected by dietary protein, and has been used successfully in measuring the nutritional value of other proteins (Litwack et al., 1952; Dju et al., 1957), this criterion was also used in this study.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In the later half of the nineteenth century experimenters believed the value of a protein food depended upon the amount of nitrogen it contained. After the discovery of some of the amino acids, variations in the nutritive value of protein foods were explained in terms of differences in their amino acid composition. The present concept of the nutritive value of protein indicates that dietary protein must supply eight "essential" amino acids, which can not be synthesized by man in adequate amounts, in addition to supplying nitrogen for body synthesis of other compounds. The amount of protein nitrogen, and the proportion of the essential amino acids supplied by a food determine the nutritional value of the protein it contains.

THE PROTEIN OF WHEAT

Quantity of Protein: The quantity of protein in whole wheat is not absolute, but depends upon environment and variety of wheat (Harris et al., 1945), and on soil conditions and fertilizers (El Gindy et al., 1957). These experimenters found the protein content of whole wheat ranged between 10 per cent and 17 per cent.

On an equal weight basis, the protein content of fractions of the wheat grain varies in the following order,

approximately 26 per cent of the germ, 14 per cent of the bran, and 11 per cent of the endosperm, while the intact grain contains approximately 12 per cent protein (Morris et al., 1946). Although the germ and bran contain more protein than does the endosperm on an equal weight basis, they constitute only a small portion of the total wheat kernel. The total wheat kernel contains approximately 2 per cent germ, 14 per cent bran, and 84 per cent endosperm (Lowe, 1950).

The protein content of flour depends upon the protein content of the whole grain, and the extraction of the bran and germ during milling. Whole wheat flour, which contains the germ and bran, provides more protein than does patent flour, which contains only the heart of the endosperm. Generally, white flour represents between 72 per cent and 75 per cent extraction. The protein content of "standard grade" flour (the flour most widely used) varies from 9 per cent to 12 per cent (Morris et al., 1946).

The protein content of bread depends on the amount of protein present in the flour and in the other ingredients used in making the bread. Kulp et al. (1956) analyzed 255 samples of enriched white bread from various locations throughout the United States, and noted an average protein content of 8.6 per cent. This agrees with the figure released by the United States Department of Agriculture (1950), i.e. 8.5 per cent protein in white bread containing 4 per cent nonfat milk solids. Commercial

bakers use between 3 and 4 per cent nonfat milk solids in the preparation of the standard white bread.

"Although bread flour contains sufficient total protein (13 per cent of the calories), less than one-half of this protein is available to the animal for the formation of new tissue. The recognized protein inadequacy of wheat flour is therefore not the result of a lack of total protein per calorie but due solely to the low biological value of the wheat proteins." (Howard et al., 1958)

Quality of Protein: During the early part of the twentieth century, the poor quality of wheat protein was demonstrated by Osborne and Mendel (1912, 1914, 1920). Other investigators substantiated their results. When compared with other foods, wheat grains contain a poorer quality protein than do oats (Jones et al., 1948), rye (Kon and Markuze, 1931; Sure, 1954, 1955), meat, eggs, or milk (Mitchell and Carman, 1924; Mitchell and Block, 1946; Mitchell, 1947).

The poor quality of the protein in wheat is attributed to its amino acid composition. When the amino acids present in the protein in whole wheat were compared with those in egg, which supplies an amino acid mixture almost completely utilizable in animal metabolism, the protein in whole wheat was approximately 63 per cent deficient in lysine (Mitchell and Block, 1946). In order for a protein to supply the optimal proportion of amino acids for utilization by the animal, it must contain 5.3

grams of lysine per 16 grams of nitrogen (Howard et al., 1958; Block and Mandl, 1958). Since the lysine content of the protein in whole wheat ranges between 2.5 and 2.7 grams per 16 grams of nitrogen (Block and Weiss, 1956; Hepburn et al., 1957), the deficiency of this amino acid limits the utilization of the protein in whole wheat.

The proteins contained in various fractions of the whole wheat grain differ in quality as well as quantity. Wheat germ and wheat bran promoted higher protein efficiency ratios in young rats than did whole wheat. Of the three, wheat germ contained the highest quality protein (Hove et al., 1945). The better quality of the protein in wheat germ and wheat bran as compared with whole wheat is due, in part, to a difference in lysine content. The protein of wheat germ contains approximately 5.5 grams of lysine per 16 grams of nitrogen (Block and Weiss, 1956), which is equivalent to the lysine content of an ideally balanced protein mentioned previously. The protein of wheat bran contains approximately 3.8 grams of lysine per 16 grams of nitrogen (Block and Weiss, 1956), which is more than the lysine content of the protein in whole wheat.

Standard white flour, which consists primarily of wheat endosperm, contains a poorer quality protein than does whole wheat. The protein in white flour contains approximately 2.0 to 2.2 grams of lysine per 16 grams of nitrogen (Block and Mandl, 1958). Of the essential amino acids contained in whole wheat, only lysine is decreased

as a result of milling (Hepburn et al., 1957). On the other hand, milling increases the digestibility of white flour as compared with whole wheat, thus, more of the protein contained in white flour is available to the animal for growth and metabolism. The increase in digestibility partially compensates the loss of lysine during milling.

The quality of protein in bread depends on the proportions of amino acids supplied by the protein in flour and other ingredients. Ingredients, which contain a well-balanced protein or supply an extra quantity of lysine, improve the protein quality of the bread. Although a small loss of lysine occurs during the baking process, this does not significantly affect the protein quality of bread (Rosenberg and Rohdenburg, 1951). The protein of standard white bread contains approximately 2.5 to 2.8 grams of lysine per 16 grams of nitrogen (Block and Weiss, 1956; Block and Mandl, 1958). Thus the ingredients used in making bread have added 0.5 to 0.6 grams of lysine per 16 grams of nitrogen above that supplied by white flour.

The evidence presented supports the fact that whole wheat bread contains a higher quality protein than does standard white bread. The protein of whole wheat bread produced higher biological values in both young and adult rats (Mitchell, 1947), and in adult human subjects (Murlin et al., 1941) than did the protein of white bread.

Analysis of the amino acid composition (Block and Mandl, 1958) and growth in young rats (Block et al., 1959) indicated the protein of whole wheat bread was better balanced and more efficiently utilized than the protein of white bread.

In conflict with these data, French and Mattill (1935) found the biological value of white bread equal to that of whole wheat bread. The difference in their results and those of other investigators may be due to the fact that 10 per cent of the nitrogen in their experimental diets came from sources other than bread.

PROTEIN SUPPLEMENTS TO WHEAT DIETS

Supplements of Nonfat Milk Solids: Application of the supplementary relationship between the proteins of wheat and milk resulted in the improvement of the protein of bread. Hove et al. (1945) added 3, 6, or 20 per cent nonfat milk solids to a diet of white flour, which supplied 10 per cent protein. They observed an increase in the growth and protein efficiency ratios of young rats as the amount of milk solids in the diet increased. In a similar study, Carlson et al. (1946) noted an increase in the biological value and protein stored in the bodies of young rats, as the amount of milk solids in the diet increased from 3 to 6 per cent. Corresponding results were obtained when a diet of whole wheat bread, which was supplemented with either 3, 6, or 12 per cent nonfat milk solids, was fed to young

rats at the 11.5 per cent protein level (Sabiston and Kennedy, 1957).

Jahnke and Schuck (1957) suggested that the addition of more than 6 per cent nonfat milk solids adversely affects the taste and texture of bread. However, Welton et al. (1959) have added 25 per cent nonfat milk solids to bread, which increased the nutritional value of the protein to approximately 2.5 time that of the standard white bread, as measured by rat growth. This bread was similar in appearance to standard white bread, and had excellent flavor.

Supplements of Yeast: The protein of yeast may effectively supplement the protein of wheat, because the concentration of lysine in yeast protein is comparable to that of whole egg (Block and Weiss, 1956). The addition of 5 per cent dry yeast to white bread, at the 12.5 per cent protein level, increased the growth of young rats (Light and Frey, 1943). Similarly, when young rats were fed a diet of enriched flour, which contained 1, 3, or 5 per cent yeast and supplied 9 per cent protein, growth increased with increased amounts of yeast in the diet (Sure, 1948). Sure stated, "The increased biological value of the proteins of milled enriched wheat flour with dried food yeast is due to the yeasts' provision of lysine and possibly other dietary essentials."

The addition of 3 per cent yeast to a white bread diet promoted more growth in young rats than did the addition of 3 per cent nonfat milk solids. However, the addition of both yeast and nonfat milk solids to a white bread diet, at the 12 per cent protein level, promoted better growth than did the addition of either alone (Seeley et al., 1950).

Supplements of Wheat Germ: Improvement of the protein of white flour with the addition of wheat germ was suggested by the previous discussion relative to the lysine contained in wheat germ protein (p. 6). Hove et al. (1943) noted that wheat germ, skim milk, or beef muscle promoted equal growth and protein efficiency in young rats when fed at the 10 per cent protein level. The addition of either 4 or 6 per cent wheat germ to a mixed diet, which contained 48 per cent white flour, significantly increased the growth rate of young rats (Westerman et al., 1952). When either 3, 6, or 20 per cent wheat germ was added to a white flour diet, which supplied 10 per cent protein, the growth and protein efficiency ratios of young rats increased with increased amounts of wheat germ in the diet (Hove et al., 1945). Equal amounts of either wheat germ or nonfat milk solids added to the diet promoted equal results. They concluded wheat germ and nonfat milk solids are of equal value for improving the nutritive value of flour.

Supplements of Wheat Gluten: The value of wheat gluten for supplementing the protein of bread is dubious,

since it contains less lysine than whole wheat, wheat germ, or wheat bran (Block and Weiss, 1956). The addition of 2 per cent wheat gluten to a white flour diet at either the 10 per cent or 15 per cent protein level did not affect the growth rate or the per cent of nitrogen deposited in the carcasses of young rats (Howard et al., 1958). They concluded, "The futility of adding wheat gluten to bread from the point of view of protein nutrition is apparent."

CRYSTALLINE AMINO ACID SUPPLEMENTS TO WHEAT DIETS

Supplements of Lysine: Small supplements of the amino acid, lysine, improved the protein of wheat. The amount of supplemental lysine necessary for optimum nutritional value depended on the amount of lysine supplied by the protein of the wheat, and other ingredients in the product. Hutchinson et al. (1956) observed that a supplement of 0.25 per cent L-lysine was better than lower quantities of this amino acid, and equal to greater quantities for promoting growth in young rats fed a white flour diet which supplied 12.5 per cent protein. At this level of supplementation the total lysine in the diet was 0.5 per cent. When the total lysine in the diet was more than 0.5 per cent, the nitrogen efficiency was reduced.

Rosenberg and Rohdenburg (1952) supplemented a commercial white bread, which contained 3 per cent nonfat milk solids, with various levels of lysine. The experimental bread diets supplied 12.5 per cent protein. A

supplement of 0.2 per cent L-lysine supported better growth in young rats than did smaller amounts of this amino acid. At the 0.2 per cent L-lysine level of supplementation the total lysine content of the diet was 0.5 per cent, a quantity identical with that used by Hutchinson et al. (1956). Rats fed diets containing larger amounts of lysine grew faster initially but at maturity were equal in weight to animals fed the diet supplemented with 0.2 per cent L-lysine. They suggested fortifying bread with 0.2 parts lysine per 100 parts of flour.

Jahnke and Schuck (1956, 1957) noted a supplement of 3 per cent nonfat milk solids and 0.25 per cent L-lysine equaled a supplement of 12 per cent nonfat milk solids for promoting growth in young rats fed a diet equivalent to unbaked white bread, which supplied approximately 12 per cent protein. They suggested that commercial bakers are not likely to use 12 per cent nonfat milk solids in bread, therefore, the possibility of supplementing bread with 0.25 per cent L-lysine should be given consideration for raising the lysine content of diets where this appears to be desirable.

These experimenters (Jahnke and Schuck, 1957) also measured the rate at which fat was deposited in the livers of the animals, since this is affected by the amino acid balance of the diet when the protein content of the diet is low. The 0.25 per cent L-lysine supplement had no affect on the amount of fat deposited in the livers of the

animals when the diet contained either 3, 6, or 12 per cent nonfat milk solids. The total lysine content of the diets ranged from 0.44 to 0.63 per cent.

Culik and Rosenberg (1958) observed five generations of rats fed a commercial white bread which contained 6 per cent nonfat milk solids, and supplied 12 per cent protein, either with or without a supplement of 0.25 per cent L-lysine. The total lysine content of the supplemented bread diet was 0.51 per cent. The reproduction and lactation performance of rats fed the lysine supplemented bread was superior to that of rats fed the unsupplemented bread, and equal to that of rats fed a stock ration of mixed foods, which supplied 22 per cent protein. The lysine supplemented bread supported normal reproduction and lactation of rats when fed as the sole source of dietary protein.

Experimenters disagree concerning the extent to which lysine supplementation improves the protein of wheat. Flodin (1956) suggested that the nutritional value of the protein in lysine supplemented wheat products is comparable to that of proteins from animal sources. On the other hand, Bender (1958) suggested that lysine supplementation of wheat products increases the utilization of the protein only by approximately 10 per cent.

Supplements of Lysine Plus Other Amino Acids: The possibility of further improving the nutritional value of the protein of wheat with supplements of other amino acids in addition to lysine has been studied.

Desphande et al. (1957) observed the growth and amount of fat deposited in the livers of young rats fed a white flour diet, which supplied 9.5 per cent protein. The diet was supplemented with various amounts of lysine and/or threonine, ranging from 0.25 to 0.90 per cent and 0.20 to 0.60 per cent respectively. A supplement of 0.25 per cent L-lysine promoted more growth than did the unsupplemented diet. The addition of either 0.20 per cent DL-threonine or larger amounts of lysine did not further improve the growth. However, maximum growth was attained when the diet was supplemented with 0.50 per cent L-lysine plus 0.40 per cent DL-threonine.

Sure, (1952, 1954a, 1955a, 1957) conducted a series of experiments to determine the value of adding lysine, threonine, valine, or methionine to diets of whole wheat flour, or white flour, which supplied approximately 8 per cent protein. In every study, the diets supplemented with amino acids produced more growth and higher protein efficiency ratios in young rats than did the unsupplemented diets. A supplement of 0.25 per cent L-lysine and 0.20 per cent DL-threonine was better than a supplement of 0.25 per cent L-lysine only (Sure, 1952, 1954a), and equivalent to a supplement of 0.25 per cent L-lysine, 0.20 per cent DL-threonine, and 0.50 per cent DL-valine for promoting growth and protein efficiency in young rats fed a whole wheat flour diet (Sure, 1954). However, a supplement of

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0.40 per cent L-lysine combined with 0.20 per cent DL-threonine, and 0.50 per cent DL-valine produced the most growth and highest protein efficiency ratios in young rats fed a whole wheat flour diet (Sure, 1954). The nutritional adequacy of this diet was further improved, as measured by growth and protein efficiency ratio, by substituting 0.40 per cent DL-methionine for the 0.50 per cent DL-valine used in the previous study (Sure, 1957). The inclusion of methionine in the amino acid supplement significantly reduced the amount of fat in the livers of the rats as compared with those of rats fed the unsupplemented white flour diet. Supplements of lysine alone, or in combination with threonine, and/or valine did not appreciably affect the amount of fat deposited in the livers of the rats (Sure, 1957).

A bread diet identical to one described previously (Rosenberg and Rohdenburg, 1952) was supplemented with various amounts and combinations of lysine, valine, threonine, and methionine (Rosenberg et al., 1954). Lysine alone was equivalent to any combination of lysine, valine, threonine, or methionine for promoting growth and protein efficiency in young rats. Thus, they concluded that the only amino acid deficiency in commercial white bread is lysine. The difference between their results and those of other investigators may be due to the 12.5 per cent protein level of their experimental diet, or the 3 per cent nonfat milk solids contained in the bread.

It is difficult to compare the studies available because of the variations in experimental procedures. Perhaps further investigation will clarify the details that at present are obscure.

The Practical Aspects of Supplementing Foods with Amino Acids: Any commercial supplementation of foods with amino acids should be contingent on the cost, safety, effectiveness, and necessity of the program.

The economic aspect of supplementing bread with lysine has been discussed extensively by Flodin (1953, 1958). He suggests that lysine can be made available at a cost of 1 to 3 cents per gram (1953). "The cost of amino acid fortification, at least in the case of lysine, is potentially in the range of very low-cost protein additives," (Flodin, 1958). However, the economic interests held by DuPont in this program should not be ignored.

It appears doubtful that adverse effects would result from the supplementation of bread with small amounts of lysine under normal conditions. Adverse effects resulting from lysine supplementation of wheat have been observed only when an excessive amount of the supplementary amino acid was added to the diet (Hutchinson et al., 1956). For economic reasons, a food manufacturer would not add an excess of lysine to bread, thus the possibility of an excess quantity of lysine in bread is slim.

The effectiveness of supplementing bread with lysine is questionable, and difficult to determine, when

considered in relation to a diet of mixed foods, because of the supplementary relationship of various proteins, and the variability of individuals in their choice of foods. Westerman et al. (1957) noted that rats fed diets containing 37 per cent white flour and 12 per cent meat, milk, and eggs, grew as well as rats fed this diet supplemented with 0.3 per cent L-lysine. When the meat was omitted, lysine supplementation improved growth, however, when meat and milk were both omitted the flour equaled 49 per cent of the diet, growth was slow even with a supplement of lysine. The value of any amino acid supplementation program will not be realized unless the diet is adequate with respect to other nutrients, particularly the vitamins involved in the metabolism of amino acids.

In the United States there is no need to improve the protein in the average diet. In 1955 the average diet provided 103 grams of protein per person per day. In this country, 92 per cent of the population met the National Research Council recommended allowances for protein. (United States Department of Agriculture, 1957). Of the total protein consumed in 1957, 65 per cent was from animal sources and 20 per cent from flour and cereal products (United States Department of Agriculture, 1958).

The present information available indicates that the daily lysine requirement of young men (Rose et al., 1955) and young women (Jones et al., 1956) is low and is

more than adequately met in the average American diet. Clark et al. (1957) expressed the opinion, "The possibility of a general inadequacy of lysine in diets consumed by the adult population in this country seems remote."

Nevertheless, it should be acknowledged that a small percentage of people in the United States are consuming a diet suboptimum in protein. In two independent surveys Williams (1945) and Jean et al. (1952) observed the average quantity of protein consumed by pregnant women was below that recommended by the National Research Council. It has also been suggested that the protein nutrition of children between the ages of 2 and 6 should be improved (Jeans, 1950; Lynch and Snively, 1955). Morgan (1959) compiled recent surveys of the nutritional status of the United States population. According to this report, the protein consumed by adolescent girls and women over the age of 70 was significantly lower than the National Research Council recommended allowances. One way the protein requirements of these individuals may be more adequately met is by commercially supplementing bread with lysine.

The Food and Nutrition Board of the National Research Council has examined the possibility of supplementing bread with lysine. Their conclusions are summarized in the following statements:

The committee recognizes the potential offered by amino acid supplementation in human nutrition, but no convincing evidence of a need for such supplementation for the individual eating an average mixed diet in the United States has as yet been presented. The

possibility exists, of course, that certain segments of the population could benefit by supplementation of certain food items in the diet. (National Research Council, 1959)

In other countries, however, severe protein malnutrition is prevalent. Sufficient protein is not available, and sources of high quality protein are limited. The grams of animal protein available per person per day in China, India and Indonesia were 4.9, 5.8, and 4.7 respectively (Phillips, 1951). The possibility of improving the dietary protein with supplements of mixed foods locally available, and with supplements of amino acids, is being examined in Mexico (Gomez et al., 1958), Central American and Panama (Behar et al., 1958), and French West Africa (Senecal, 1958). Each of these investigators suggested the present knowledge of amino acid supplementation is inadequate, and recommended further investigation.

THE RELATIONSHIP OF LIVER XANTHINE OXIDASE TO DIETARY PROTEIN

The liver xanthine oxidase enzyme system is very sensitive to the quantity and quality of protein present in the diet. The activity of this system is affected by the availability of the essential amino acids (Williams and Elvehjem, 1949), thus, it is a suitable criterion for judging the protein value of the diet. An advantage of using this criteria for measuring protein quality is the speed with which this enzyme system responds to the protein

adequacy of the diet. Since the xanthine oxidase system responds maximally to dietary conditions in approximately ten days (Litwack et al., 1952), it provides a more economical method of measuring the value of dietary protein than does growth studies. The results obtained by using the activity of this enzyme as a measure of protein quality agree with the results obtained by long-term growth studies (Litwack et al., 1953).

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

The diets used in this experiment were of the following composition: 90 per cent dried, ground bread; 5 per cent corn oil¹; 4 per cent mineral salts²; 0.25 per cent vitamin mixture³; 0.15 per cent choline; and 0.60 per cent sucrose.

The bread used in the preparation of diet I was a standard white bread and served as the control. In diet II, the bread contained 5/6 white flour and 1/6 Roman meal (a blend of whole wheat, whole rye, bran, and flax-meal). In diet III, the bread contained a blend of gluten, whole wheat, and unbleached flours supplemented with 1.7 grams of L-lysine monohydrochloride per pound. Those specifications, which were available from the manufacturer of each bread may be found in Appendix, page 1.

The breads were purchased at local markets. They were oven dried at 50 degrees centigrade for 3 hours,

¹Containing 7.5 mg α -tocopherol acetate and 0.38 mg menadione.

²Salts IV obtained from Nutritional Biochemicals, Inc.

³Containing 0.5 mg thiamine, 0.5 mg riboflavin, 1.0 mg niacin, 0.25 mg pyridoxine HCl, 2.0 mg calcium pantothenate, 10 mg inositol, 0.02 mg folic acid, 0.002 mg vitamin B₁₂, 0.01 mg biotin, 10 mg vitamin A, 0.18 mg vitamin D, and 0.225 g sucrose.

finely ground in the Hobart grinder, and stored in covered metal containers. Nitrogen was determined in duplicate on 1.0 gram samples of the dried, ground bread by the Macro-Kjeldahl procedure.

Thirty three weanling, male, albino rats of the Sprague-Dawley strain were divided into 3 groups of 11 each. The average weight per group was 43 grams. The rats were housed in separate, wire mesh cages and allowed food and water ad libitum for a period of 14 days. Food intake and weight records were kept. Between 14 and 19 days the animals were decapitated (2 animals from each group per day), the livers removed, weighed, and homogenized and the activity of the xanthine oxidase system determined. The remainder of the liver homogenates were stored frozen for the determination of moisture, nitrogen, and fat.

The contents of the gastrointestinal tract were washed out, and the carcasses were weighed and frozen for the determination of carcass moisture, nitrogen, and fat.

Liver xanthine oxidase activity was determined by the use of manometric procedures. A modification of the method of Axelrod and Elvehjem (1941) was used in this study. For each determination, the substrate (0.2 milliliters of 0.038 molar xanthine in 0.05 molar sodium hydroxide) was pipetted into the side arm of a Warburg flask. The center well contained 0.2 milliliters of 10 per cent potassium hydroxide. A folded piece of filter paper was inserted into the well to provide a greater surface area

for the adsorption of carbon dioxide produced. The flask was chilled in crushed ice.

After the flask was prepared, the rat was stunned by a sharp blow on the head and decapitated. The liver was quickly removed from the decapitated animal and immediately chilled in ice. The excess moisture was removed by blotting with filter paper. A portion of the liver was weighed and homogenized with five volumes of cold, distilled water, in a Potter-Elvehjem homogenizer. One milliliter of this homogenate, which contained 0.167 gram of tissue, was pipetted into the main compartment of the chilled Warburg flask. The total volume contained in the flask was brought to 2.2 milliliters by adding chilled, distilled water. The flask was seated on the manometer, placed in a water bath maintained at 37 degrees centigrade, and equilibrated for a period of 10 minutes. The substrate was tipped into the main compartment from the side arm, and the stopcock was closed. The manometer reading was recorded every 10 minutes for a period of 200 minutes.¹ Duplicate determinations were run for each rat.

Endogenous activity was determined similarly except for the omission of the xanthine substrate from the flask. Data were corrected for endogenous activity and changes in atmospheric pressure. A 30 minute period, within the log phase of the enzyme activity curve, was chosen to calculate

¹For the first rat in each group readings were recorded only 120 minutes.

the activity of the xanthine oxidase system. Enzyme data were calculated and reported per 10 milligrams of nitrogen, per gram of fresh weight liver, and per total weight of the liver.

When the enzyme determination was completed, the remaining liver, which had been weighed, homogenized, and stored in a frozen state, was thawed, placed into a weighed evaporating dish, and dried at 90 degrees centigrade for 12 hours. The dry liver homogenate was cooled to room temperature in a dessicator, weighed, finely ground, and stored in a covered bottle. The per cent of moisture in the liver was calculated.

The amount of fat in the liver was determined by continuous ether extraction in the Goldfish apparatus. The sample of dried, ground liver was weighed to the nearest milligram and consisted of the entire amount available, approximately 0.5 gram per rat in groups I and II, and approximately 1.0 gram per rat in Group III. The per cent of fat in the liver was calculated on a dry weight basis.

The nitrogen content of a 0.25 gram sample of dried, ground, fat free liver was determined by the Macro-Kjeldahl method. A single determination was run for each rat in group I, because of the small amount of sample available. Duplicate determinations were run for each rat in groups II and III. The per cent of nitrogen in the total liver, which included the fat, was calculated.

The frozen carcass was sawed into chunks and ground three times to obtain a homogeneous mixture. A 20 gram sample from each carcass was oven dried at 90 degrees centigrade to a constant weight, then pulverized in a mortar. Atmospheric moisture collected during pulverizing was removed by oven drying. The sample was then placed in a covered container and stored in a dessicator. The per cent of moisture in the total carcass, including the liver, was calculated.

The amount of fat present in each carcass was determined in duplicate on 1.0 gram samples of dried, pulverized carcass by continuous ether extraction in the Goldfish apparatus. The per cent of fat in the total carcass, which included the liver, was calculated on a dry weight basis.

The amount of nitrogen present in each carcass was determined in duplicate on 0.5 gram samples of dried, pulverized carcass by the Macro-Kjeldahl method. The per cent of nitrogen in the total carcass, including the liver, was calculated.

All individual data reported was averaged per group, and the standard error of the mean was calculated. "Student's" t test was used as a measure of significance.

RESULTS

RESULTS

The results of this study are summarized in Tables I, II, III, and IV; data for individual animals are presented in the Appendix, p. ii through p. xiii.

PROTEIN CONTENT OF BREAD

The protein content ($N \times 5.7$)¹ of the breads used in this experiment, reported on a dry weight basis, was 12.5 per cent in the standard white bread (diet I), 16.3 per cent in the bread containing Roman meal (diet II), and 25.0 per cent in the lysine supplemented bread (diet III).

FOOD CONSUMPTION AND GROWTH DATA (Table I)

No significant differences were observed in the food intake and growth data of rats fed the bread containing Roman meal (group II) as compared with rats fed the standard white bread (group I). Since the bread containing Roman meal supplied $1/3$ more protein than did the standard white bread on a dry weight basis, the animals in group II had lower protein efficiency ratios than did the control rats. This difference was small, but significant at the 5 per cent level ($P < 0.05$).

¹Association of Official Agricultural Chemists, 1950.

The rats fed the lysine supplemented bread (group III) consumed approximately 1 1/2 times the food and grew approximately 5 times as fast as rats fed the standard white bread (group I). As a result, the protein efficiency ratios of the rats fed the lysine supplemented bread were approximately twice those of the rats fed the standard white bread ($P < 0.01$).

LIVER ANALYSIS DATA (Table II)

The per cent of nitrogen stored in the livers from rats fed the bread containing Roman meal (group II) was greater than that in the livers from rats fed the standard white bread (group I). This difference was significant at the 1 per cent level ($P < 0.01$). No significant differences were noted in the per cent of fat, per cent of moisture, or dry weight of the livers of rats fed the bread containing Roman meal (group II) as compared with rats fed the standard white bread (group I).

Livers from rats fed the lysine supplemented bread (group III) contained a significantly greater per cent of nitrogen and a significantly smaller per cent of fat than did the livers from rats fed the standard white bread (group I). These differences were significant at the 1 per cent level ($P < 0.01$). The dry weight of the livers from rats fed the lysine supplemented bread (group III) was approximately 2 1/2 times that of the livers from rats fed

the standard white bread (group I). No significant difference in the per cent of moisture was observed between these 2 groups.

LIVER XANTHINE OXIDASE DATA (Table III)

When the xanthine oxidase activity was calculated per unit weight of nitrogen, no significant difference was observed between rats fed bread containing Roman meal (group II) and rats fed the standard white bread (group I). When the xanthine oxidase activity was calculated per gram of fresh weight liver, or per total weight of the liver, this enzyme system in the livers from rats in group II was more active than in the livers from rats in Group I. These differences were small, but significant at the 1 per cent level ($P < 0.01$).

The xanthine oxidase system was significantly more active in livers from rats fed the lysine supplemented bread (group III) than in livers from rats fed the standard white bread (group I), regardless of the units used in calculating. These differences were significant at the 1 per cent level ($P < 0.01$).

Since rats fed bread containing Roman meal and rats fed lysine supplemented bread deposited significantly more nitrogen in the liver than did rats fed the standard white bread (table II), the xanthine oxidase activity expressed in terms of unit weight of liver nitrogen is probably more appropriate. With this unit of measurement,

only the rats fed the lysine supplemented bread had significantly more active xanthine oxidase systems in the livers than did rats fed the standard white bread.

CARCASS ANALYSIS DATA (Table IV)

There were no significant differences in the per cent of nitrogen, per cent of fat, per cent of moisture, or dry weight of the carcasses of rats fed the bread containing Roman meal (group II) as compared with those of rats fed the standard white bread (group I).

However, the carcasses of rats fed the lysine supplemented bread (group III) contained a greater per cent of nitrogen than did the carcasses of rats fed the standard white bread (group I). This difference was significant at the 1 per cent level ($P < 0.01$). The dry weight of the carcasses of rats fed the lysine supplemented bread was twice that of the rats fed the standard white bread ($P < 0.01$). There were no significant differences between these 2 groups in the per cent of carcass fat or moisture.

DISCUSSION AND CONCLUSIONS

DISCUSSION AND CONCLUSIONS

Data presented in this paper emphasize the importance of quality as well as quantity of protein supplied by a food.

Chemical analysis of the bread containing Roman meal supported the advertising claim, "contains about 25 per cent more protein than most white breads." The small but significant increase in the per cent of nitrogen contained in the livers from rats fed the bread containing Roman meal as compared with those of rats fed the standard white bread, was probably due to the greater quantity of protein supplied by the Roman meal bread.

Although the bread containing Roman meal supplied approximately $1/3$ more protein, the quality of this protein was no better than that of the standard white bread when measured by biological assay. No significant difference was observed in the growth of rats fed the bread containing Roman meal and that of rats fed the standard white bread, thus rats fed the bread containing Roman meal had lower protein efficiency ratios. Since growth studies are usually of longer duration, it is possible that these data were influenced by the experimental period of 14 to 19 days.

Measurement of the activity of the liver xanthine oxidase system, which responds maximally to dietary

conditions in approximately 10 days (Litwack et al., 1952), indicated that the quality of the protein provided by bread containing Roman meal was not improved as compared to that of the standard white bread. The activity of the liver xanthine oxidase system in rats fed bread containing Roman meal was not significantly different than the activity of this enzyme system in livers from rats fed the standard white bread, when measured per unit weight of liver nitrogen. Therefore, it is suggested that Roman meal, composed of whole wheat, whole rye, bran, and flax-meal, did not provide an amino acid pattern that complemented that of white flour.

For individuals consuming diets restricted with respect to the quantity and quality of protein, substituting a bread containing a supplement of Roman meal for a standard white bread is not warranted. This is especially true in view of the increased cost of this supplemented bread; approximately 10 cents more per pound.

The nutritional value of the protein in a lysine supplemented bread, which was advertised as containing, "53 per cent more protein than the same weight of regular white bread," was also studied. Chemical analysis performed in this laboratory indicated that the lysine supplemented bread provided approximately twice the quantity of protein, which was provided by the standard white bread.

Biological assay demonstrated a marked improvement in the quality as well as the quantity of the protein

provided by the lysine supplemented bread. More growth, higher protein efficiency ratios, and a greater per cent of nitrogen in the livers and carcasses, were observed in rats fed the lysine supplemented bread as compared with rats fed the standard white bread. The activity of the liver xanthine oxidase system was in agreement with this data. Livers from rats fed the lysine supplemented bread had significantly more xanthine oxidase activity than did livers from rats fed the standard white bread. It is suggested that the blend of wheat flours and the lysine supplement improved the amino acid balance of the protein in this bread as compared with that of the standard white bread.

This study did not clarify the metabolic processes involved, which resulted in the significantly smaller per cent of fat in the livers of rats fed the lysine supplemented bread. Nevertheless, the relationship of amino acid balance in dietary protein to the deposition of fat in the liver of experimental animals supports the conclusion that the amino acid balance of the protein in the lysine supplemented bread was improved as compared with that of the standard white bread.

For individuals consuming suboptimum protein the superior nutritional value of the lysine supplemented bread may be worth the additional cost of 7 cents per pound. However, it is recognized that in the United States there

is no need for improving protein consumption by the average person.

This study indicated that the use of liver xanthine oxidase activity provided an excellent technique for determining the protein value of bread diets. Significant changes were noted after an experimental feeding period of only 14 days. Thus, the use of this enzyme system as a criterion of protein quality is both fast and economical.

The assay methods used in this study made necessary the use of weanling rats as experimental subjects. However, the results may contribute additional information concerning the nutritional value of protein in supplemented breads for human consumption.

SUMMARY

SUMMARY

The nutritional value of the protein in each of 2 breads, advertised as "high protein" breads, was compared with that of a standard white bread. One bread contained Roman meal. The other bread contained a blend of wheat flours supplemented with lysine.

Thirty three weanling, male, albino rats (3 groups of 11) were fed 90 per cent bread diets. Rats in group I were fed a standard white bread. Rats in group II were fed a bread containing Roman meal. Rats in group III were fed a lysine supplemented bread. Food and water were allowed ad libitum for a period of 2 weeks. Records of food intake and weight gain were kept. At the end of the experimental period the rats were killed, and the livers were analyzed for xanthine oxidase activity, nitrogen, fat, and moisture. The carcasses were analyzed for nitrogen, fat, and moisture. Each bread was analyzed for nitrogen.

The bread containing Roman meal provided approximately 1/3 more protein (dry weight) by chemical analysis than did the standard white bread. However, no significant difference was observed in the growth of young rats fed the supplemented bread as compared with those fed the standard white bread. As a result, rats fed the bread containing Roman meal had significantly lower protein efficiency ratios.

No significant difference was noted in the xanthine oxidase activity per unit weight of liver nitrogen between the livers from rats fed the bread containing Roman meal and those from rats fed the standard white bread. The only significant difference observed between these 2 groups, with respect to the composition of the carcass or the liver, was a greater per cent of nitrogen in the livers from rats fed the bread containing Roman meal. This difference was small, but significant, and in view of other data, probably reflects the greater quantity of protein supplied by the Roman meal supplemented bread.

While the bread containing Roman meal provided more protein, the quality of this protein was not improved as compared with that of the standard white bread. Therefore, the amino acid composition of Roman meal did not complement that of white flour. The additional cost of Roman meal bread (10 cents more per pound), combined with a biological value for protein which is no better than the standard white bread, makes this an unsatisfactory product.

The second bread studied was supplemented with lysine and provided approximately twice the amount of protein, by chemical analysis, than was provided by the standard white bread. Rats fed the lysine supplemented bread had significantly greater growth rates and significantly higher protein efficiency ratios than did rats fed the standard white bread.

The xanthine oxidase system was significantly more active in livers from rats fed the lysine supplemented bread than in livers from rats fed the standard white bread. Rats fed the lysine supplemented bread had larger livers, which contained a higher per cent of nitrogen and a lower per cent of fat, than did rats fed the standard white bread. The only difference observed between these 2 groups, with respect to carcass composition, was a greater per cent of nitrogen in the carcasses of the rats fed the lysine supplemented bread. This difference was small, but significant.

The lysine supplemented bread provided protein which was superior in both quantity and quality as compared with that of the standard white bread. The blend of wheat flours and the lysine supplement improved the amino acid balance of this protein as compared to that of the standard white bread. The superior nutritional value of the protein in the lysine supplemented bread may be worth the additional cost of .7 cents per pound to individuals who are consuming suboptimum protein. However, it is recognized that the majority of people in the United States do not need improved protein nutrition.

Liver xanthine oxidase activity was an excellent criterion of the nutritional value of the protein in bread. Significant differences in the activity of this enzyme in the livers from young rats were observed after an experimental feeding period of only 14 days, thus, it is a sensitive, fast, and economical method for determining protein nutritional value.

TABLE I

FOOD CONSUMPTION AND GROWTH DATA

GROUP ¹	FOOD INTAKE (g/wk)	WEIGHT GAIN (g/wk)	PROTEIN ² EFFICIENCY (g/wk)
I	46 $\pm$ 2 ³	7 $\pm$ 0 ³	1.31 $\pm$ 0.06 ³
II	46 $\pm$ 2	8 $\pm$ 1	1.12 $\pm$ 0.06
III	75 $\pm$ 1	34 $\pm$ 1	1.99 $\pm$ 0.04

¹Each group contained 11 rats.
 Group I 90 per cent standard white bread.
 Group II 90 per cent bread containing Roman meal.
 Group III 90 per cent lysine supplemented bread.

²Weight gain per gram of protein eaten.

³Standard error of the mean.

TABLE III

LIVER XANTHINE OXIDASE DATA

GROUP ¹	UL O ₂ /HR/10 MG NITROGEN	UL O ₂ /HR/GM LIVER	UL O ₂ /HR/TOTAL LIVER
I	63 ± 3 ²	95 ± 4 ²	237 ± 8 ²
II	73 ± 4	118 ± 4	303 ± 17
III	106 ± 6	214 ± 7	1248 ± 79

¹Group I 9 rats fed 90 per cent standard white bread.

Group II 10 rats fed 90 per cent bread containing Roman meal.

Group III 10 rats fed 90 per cent lysine supplemented bread.

²Standard error of the mean.

TABLE IV

CARCASS ANALYSIS DATA¹

GROUP ²	CARCASS WEIGHT (g dry wt)	NITROGEN (% dry wt)	FAT (% dry wt)	MOISTURE %
I	17.0 $\pm$ 0.3 ³	8.4 $\pm$ 0.1 ³	29.1 $\pm$ 0.9 ³	67 $\pm$ 1 ³
II	16.7 $\pm$ 0.3	8.4 $\pm$ 0.1	27.6 $\pm$ 0.9	69 $\pm$ 1
III	34.2 $\pm$ 1.0	8.9 $\pm$ 0.1	28.9 $\pm$ 1.1	69 $\pm$ 1

¹Data include liver analysis.

²Each group contained 11 rats.
 Group I 90 per cent standard white bread.
 Group II 90 per cent bread containing Roman meal.
 Group III 90 per cent lysine supplemented bread.

³Standard 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 I (diet I)	Bread II (diet II)	Bread III (diet III)
Protein (%)	8.0	10.0	14.5
Fat (%)	3.1	3.1	2.0
Carbohydrate (%)	50.5	47.3	41.0
Moisture (%)	38.0	38.0	38.0
Calories per pound	1227	1172	1035

All breads contained nonfat milk solids and yeast, and were enriched according to government standards with thiamine, riboflavin, niacin, and iron.

The bread used in diet II contained Roman meal, which replaced 1/6 of the white flour. Roman meal is a blend of whole wheat, whole rye, bran, and flaxmeal. The manufacturer stated, "Contains about 25% more protein than do most white breads."

The bread used in diet III was made of a blend of gluten, whole wheat (part bran removed), and unbleached flour. Each pound of bread was supplemented with 1.7 grams of L-lysine monohydrochloride, and 5.4 micrograms of vitamin B₁₂. The manufacturer stated, "53% more protein than the same weight of regular white bread."

TABLE I

GROUP I¹ FOOD CONSUMPTION AND GROWTH DATA
FOR INDIVIDUAL RATS

RAT #	FOOD INTAKE (g/wk)	WEIGHT GAIN (g/wk)	PROTEIN ² EFFICIENCY (g/wk)
I ₁	45	6	1.20
I ₂	61	8	1.30
I ₃	49	7	1.16
I ₄	46	8	1.60
I ₅	47	9	1.80
I ₆	55	7	1.16
I ₇	46	6	1.20
I ₈	40	6	1.20
I ₉	42	7	1.40
I ₁₀	42	7	1.40
I ₁₁	38	4	1.00
AVERAGE	46 $\pm$ 2 ³	7 $\pm$ 0 ³	1.31 $\pm$ 0.06 ³

¹90% standard white bread.

²Weight gain per gram of protein intake.

³Standard error of the mean.

TABLE II

GROUP I¹ LIVER ANALYSIS DATA FOR INDIVIDUAL RATS

RAT #	WEIGHT (g dry wt)	NITROGEN (% dry wt)	FAT (% dry wt)	MOISTURE %
I ₁	0.44	6.2	9.0	75
I ₂	0.62	6.0	15.7	74
I ₃	0.61	6.0	15.5	75
I ₄	0.74	5.7	15.7	76
I ₅	0.63	6.5	10.0	73
I ₆	0.71	5.7	18.3	75
I ₇	0.66	5.8	14.5	73
I ₈	0.59	6.3	11.9	75
I ₉	0.47	5.9	13.4	77
I ₁₀	0.77	5.4	17.7	75
I ₁₁	0.57	5.7	20.6	73
AVERAGE	0.62 ± 0.03^2	5.9 ± 0.1^2	14.8 ± 1.1^2	75 ± 1^2

¹90% standard white bread.

²Standard error of the mean.

TABLE III

GROUP I¹ LIVER XANTHINE OXIDASE DATA FOR INDIVIDUAL RATS

RAT #	UL O ₂ /HR/10 MG NITROGEN	UL O ₂ /HR/G LIVER	UL O ₂ /HR/TOTAL LIVER
I ₁ ²	--	--	--
I ₂	64	102	245
I ₃ ³	--	--	--
I ₄	47	66	206
I ₅	56	96	227
I ₆	64	90	257
I ₇	64	102	245
I ₈	68	108	259
I ₉	86	120	240
I ₁₀	60	84	260
I ₁₁	60	90	194
AVERAGE	63 ± 3 ⁴	95 ± 4 ⁴	237 ± 8 ⁴

¹90% standard white bread.

²Data for this animal recorded for 120 minutes was inconclusive.

³No results were obtained for this animal because of defective experimental procedure.

⁴Standard error of the mean.

TABLE IV

GROUP I¹ CARCASS ANALYSIS DATA FOR INDIVIDUAL RATS

RAT #	WEIGHT (g dry wt)	NITROGEN (% dry wt)	FAT (% dry wt)	MOISTURE %
I ₁	16.3	9.2	24.1	69
I ₂	18.6	8.5	29.1	67
I ₃	16.2	8.4	25.2	69
I ₄	17.5	8.5	30.3	67
I ₅	18.3	7.8	31.3	66
I ₆	17.7	8.4	31.2	67
I ₇	17.2	8.6	24.4	68
I ₈	16.8	8.4	29.4	65
I ₉	16.3	7.8	31.8	67
I ₁₀	16.4	8.1	31.1	67
I ₁₁	15.3	8.3	31.9	66
AVERAGE	17.0 $\pm$ 0.3 ²	8.4 $\pm$ 0.1 ²	29.1 $\pm$ 0.9 ²	67 $\pm$ 1 ²

¹90% standard white bread.

²Standard error of the mean.

TABLE V

GROUP II¹ FOOD CONSUMPTION AND GROWTH DATA
FOR INDIVIDUAL RATS

RAT #	FOOD INTAKE (g/wk)	WEIGHT GAIN (g/wk)	PROTEIN ² EFFICIENCY (g/wk)
II ₁	45	8	1.14
II ₂	42	7	1.16
II ₃	46	8	1.14
II ₄	66	10	1.00
II ₅	40	7	1.16
II ₆	43	7	1.16
II ₇	47	7	1.00
II ₈	48	5	0.71
II ₉	44	11	1.57
II ₁₀	46	9	1.28
II ₁₁	38	6	1.00
AVERAGE	46 $\pm$ 2 ³	8 $\pm$ 1 ³	1.12 $\pm$ 0.06 ³

¹90% bread containing Roman meal.

²Weight gain per gram of protein intake.

³Standard error of the mean.

TABLE V

GROUP II¹ FOOD CONSUMPTION AND GROWTH DATA
FOR INDIVIDUAL RATS

RAT #	FOOD INTAKE (g/wk)	WEIGHT GAIN (g/wk)	PROTEIN ² EFFICIENCY (g/wk)
II ₁	45	8	1.14
II ₂	42	7	1.16
II ₃	46	8	1.14
II ₄	66	10	1.00
II ₅	40	7	1.16
II ₆	43	7	1.16
II ₇	47	7	1.00
II ₈	48	5	0.71
II ₉	44	11	1.57
II ₁₀	46	9	1.28
II ₁₁	38	6	1.00
AVERAGE	46 $\pm$ 2 ³	8 $\pm$ 1 ³	1.12 $\pm$ 0.06 ³

¹90% bread containing Roman meal.

²Weight gain per gram of protein intake.

³Standard error of the mean.

TABLE V

GROUP II¹ FOOD CONSUMPTION AND GROWTH DATA
FOR INDIVIDUAL RATS

RAT #	FOOD INTAKE (g/wk)	WEIGHT GAIN (g/wk)	PROTEIN ² EFFICIENCY (g/wk)
II ₁	45	8	1.14
II ₂	42	7	1.16
II ₃	46	8	1.14
II ₄	66	10	1.00
II ₅	40	7	1.16
II ₆	43	7	1.16
II ₇	47	7	1.00
II ₈	48	5	0.71
II ₉	44	11	1.57
II ₁₀	46	9	1.28
II ₁₁	38	6	1.00
AVERAGE	46 $\pm$ 2 ³	8 $\pm$ 1 ³	1.12 $\pm$ 0.06 ³

¹90% bread containing Roman meal.

²Weight gain per gram of protein intake.

³Standard error of the mean.

TABLE VI

GROUP II¹ LIVER ANALYSIS DATA FOR INDIVIDUAL RATS

RAT #	WEIGHT (g dry wt)	NITROGEN (% dry wt)	FAT (% dry wt)	MOISTURE %
II ₁	0.66	6.4	14.2	74
II ₂	0.77	6.8	11.1	72
II ₃	0.76	6.1	14.5	76
II ₄	0.78	6.5	14.7	75
II ₅	0.74	6.2	14.0	75
II ₆	0.76	6.2	14.1	74
II ₇	0.70	6.6	9.0	74
II ₈	0.66	6.6	12.6	76
II ₉	0.72	6.1	14.8	74
II ₁₀	0.76	6.5	14.1	80
II ₁₁	0.79	6.3	11.7	73
AVERAGE	0.74 ± 0.04^2	6.4 ± 0.1^2	13.2 ± 0.6^2	75 ± 1^2

¹90% bread containing Roman meal.

²Standard error of the mean.

TABLE VII

GROUP II¹ LIVER XANTHINE OXIDASE DATA FOR INDIVIDUAL RATS

RAT #	UL O ₂ /HR/10 MG NITROGEN	UL O ₂ /HR/G LIVER	UL O ₂ /HR/TOTAL LIVER
II ₁	64	103	270
II ₂	66	126	350
II ₃ ²	--	--	--
II ₄	90	144	454
II ₅	64	102	296
II ₆	71	114	331
II ₇	71	120	324
II ₈	71	114	310
II ₉	94	150	420
II ₁₀	78	102	296
II ₁₁	56	96	278
AVERAGE	73 $\pm$ 4 ³	118 $\pm$ 4 ³	303 $\pm$ 17 ³

¹90% bread containing Roman meal.

²No results were obtained for this animal because of defective experimental procedure.

³Standard error of the mean.

TABLE VIII

GROUP II¹ CARCASS ANALYSIS DATA FOR INDIVIDUAL RATS

RAT #	WEIGHT (g dry wt)	NITROGEN (% dry wt)	FAT (% dry wt)	MOISTURE %
II ₁	18.1	8.7	26.7	70
II ₂	16.8	8.4	31.4	68
II ₃	16.5	8.2	26.9	70
II ₄	17.5	8.1	28.9	68
II ₅	16.6	8.4	27.6	69
II ₆	16.3	8.5	27.9	69
II ₇	16.7	8.8	22.4	70
II ₈	15.1	8.1	28.0	68
II ₉	17.5	8.3	24.6	69
II ₁₀	17.2	7.7	33.0	66
II ₁₁	15.0	8.6	26.4	69
AVERAGE	16.7 $\pm$ 0.3 ²	8.4 $\pm$ 0.1 ²	27.6 $\pm$ 0.9 ²	69 $\pm$ 1 ²

¹90% bread containing Roman meal.

²Standard error of the mean.

TABLE IX

GROUP III¹ FOOD CONSUMPTION AND GROWTH DATA FOR
INDIVIDUAL RATS

RAT #	FOOD INTAKE (g/wk)	WEIGHT GAIN (g/wk)	PROTEIN ² EFFICIENCY (g/wk)
III ₁	70	36	2.25
III ₂	73	33	1.9 ⁴
III ₃	66	32	2.13
III ₄	77	39	2.29
III ₅	72	32	2.00
III ₆	75	32	1.88
III ₇	75	32	1.88
III ₈	74	32	1.88
III ₉	79	35	1.9 ⁴
III ₁₀	82	36	1.89
III ₁₁	80	34	1.88
AVERAGE	75 $\pm$ 2 ³	34 $\pm$ 1 ³	1.99 $\pm$ 0.04 ³

¹90% lysine supplemented bread.

²Weight gain per gram of protein intake.

³Standard error of the mean.

TABLE X

GROUP III¹ LIVER ANALYSIS DATA FOR INDIVIDUAL RATS

RAT #	WEIGHT (g dry wt)	NITROGEN (% dry wt)	FAT (% dry wt)	MOISTURE %
III ₁	1.40	7.4	6.2	73
III ₂	1.06	8.7	12.1	73
III ₃	1.38	8.1	8.0	73
III ₄	1.54	7.6	8.6	76
III ₅	1.42	8.2	10.6	72
III ₆	1.67	7.6	8.4	73
III ₇	1.68	7.7	7.8	73
III ₈	1.74	7.0	7.7	75
III ₉	1.78	7.5	10.3	73
III ₁₀	1.43	7.8	12.0	74
III ₁₁	1.76	6.4	9.5	73
AVERAGE	1.53 ± 0.07^2	7.6 ± 0.2^2	9.2 ± 0.6^2	73 ± 1^2

¹90% lysine supplemented bread.

²Standard error of the mean.

TABLE XI

GROUP III¹ LIVER XANTHINE OXIDASE DATA FOR INDIVIDUAL RATS

RAT #	UL O ₂ /HR/10 MG NITROGEN	UL O ₂ /HR/G LIVER	UL O ₂ /HR/TOTAL LIVER
III ₁	96	192	998
III ₂	73	174	679
III ₃	--	--	--
III ₄	120	216	1385
III ₅	112	258	1316
III ₆	111	234	1463
III ₇	109	228	1423
III ₈	109	186	1302
III ₉	80	168	1089
III ₁₀	129	258	1406
III ₁₁	123	222	1421
AVERAGE	106 $\pm$ 6 ³	214 $\pm$ 7 ³	1248 $\pm$ 79 ³

¹90% lysine supplemented bread.

²No results were obtained for this animal because of defective experimental procedure.

³Standard error of the mean.

TABLE XII

GROUP III¹ CARCASS ANALYSIS DATA FOR INDIVIDUAL RATS

RAT #	WEIGHT (g dry wt)	NITROGEN (% dry wt)	FAT (% dry wt)	MOISTURE %
III ₁	31.0	9.3	25.0	70
III ₂	30.9	9.4	27.1	70
III ₃	32.0	8.9	30.1	69
III ₄	34.2	8.8	30.0	68
III ₅	34.6	8.6	32.1	67
III ₆	31.5	9.4	26.1	70
III ₇	35.8	8.4	29.5	68
III ₈	31.1	8.5	21.6	72
III ₉	36.6	9.1	29.6	69
III ₁₀	37.9	8.8	32.8	68
III ₁₁	41.1	8.2	33.4	66
AVERAGE	34.2 $\pm$ 1.0 ²	8.9 $\pm$ 0.1 ²	28.9 $\pm$ 1.1 ²	69 $\pm$ 1 ²

¹90% lysine supplemented bread.

²Standard error of the mean.





TABLE XI

GROUP III¹ LIVER XANTHINE OXIDASE DATA FOR INDIVIDUAL RATS

RAT #	UL O ₂ /HR/10 MG NITROGEN	UL O ₂ /HR/G LIVER	UL O ₂ /HR/TOTAL LIVER
III ₁	96	192	998
III ₂	73	174	679
III ₃	--	--	--
III ₄	120	216	1385
III ₅	112	258	1316
III ₆	111	234	1463
III ₇	109	228	1423
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AVERAGE	106 $\pm$ 6 ³	214 $\pm$ 7 ³	1248 $\pm$ 79 ³

¹90% lysine supplemented bread.

²No results were obtained for this animal because of defective experimental procedure.

³Standard error of the mean.

TABLE XII

GROUP III¹ CARCASS ANALYSIS DATA FOR INDIVIDUAL RATS

RAT #	WEIGHT (g dry wt)	NITROGEN (% dry wt)	FAT (% dry wt)	MOISTURE %
III ₁	31.0	9.3	25.0	70
III ₂	30.9	9.4	27.1	70
III ₃	32.0	8.9	30.1	69
III ₄	34.2	8.8	30.0	68
III ₅	34.6	8.6	32.1	67
III ₆	31.5	9.4	26.1	70
III ₇	35.8	8.4	29.5	68
III ₈	31.1	8.5	21.6	72
III ₉	36.6	9.1	29.6	69
III ₁₀	37.9	8.8	32.8	68
III ₁₁	41.1	8.2	33.4	66
AVERAGE	34.2 ± 1.0^2	8.9 ± 0.1^2	28.9 ± 1.1^2	69 ± 1^2

¹90% lysine supplemented bread.

²Standard error of the mean.

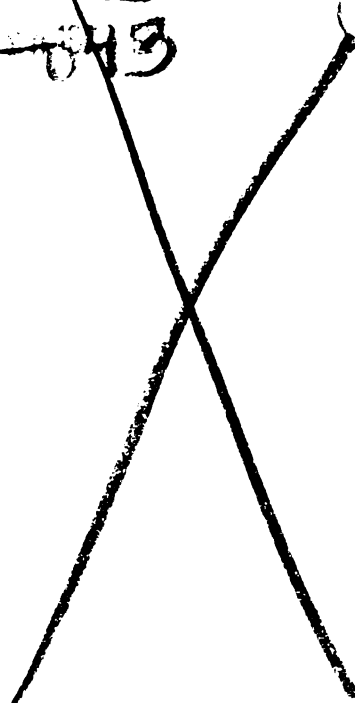
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