A STUDY OF THE BIOLOGICAL VALUE AND COMPOSITION OF BREADS FROM VARIOUS COUNTRIES

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ABSTRACT

The purpose of this study was to compare the nutritional value of bread baked in Iran and Syrian bread with the American white enriched bread; to determine the nutritional content of these breads; and to evaluate the supplementary action of dried milk solids at the 4% level and lentil powder at the 7.5 and 15% level on Iranian bread.

Two feeding experiments were carried out. Four groups of 10 male weanling rats were fed diets composed of 90% Persian bread, Syrian, American white enriched and Alladin bread. Breads were analyzed for fat, moisture, and nitrogen. Growth and food intake were measured. At the end of 4 weeks the animals were sacrificed, and the following analyses were done on the rats' livers: moisture (by drying to constant weight); nitrogen (by the Kjeldahl technique); fat (ether extraction using the Goldfisch apparatus); and liver xanthine oxidase activity (using the Warburg apparatus).

In the second experiment Persian bread was supplemented with 4% dry milk solids, 7.5% lentils and 15% lentils. Rats were sacrificed at the end of 3 weeks and the same chemical analyses were carried out on the livers of the rats. Growth and food intake were measured. Hemoglobin levels were also determined.

These studies showed the growth promoting effect of Persian bread was the same as that of American white enriched bread. Supplementation of the Iranian bread with 4% dry milk solids or 7.5% or 15% lentils improved its nutritional quality as shown by the increase in weight gain of the rats.

The amino acid intake of rats and humans fed bread diets were calculated. These calculations indicate that man's requirement for amino acids may be met by a diet in which bread serves as the source of 70% of the total calories. These calculations also show that the bread diet does not provide adequate amounts of amino acids necessary for the growth of weanling rats.

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By

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To My Parents

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INTRODUCTION

An adequate food supply is one problem facing the world as its population continues to increase at an ever accelerating rate. Protein is the most difficult nutrient to produce. The less expensive sources of protein including cereals are considered to be poor in nutritional quality. In most areas of the world, bread and cereal make up a large share of the diet. Protein, B vitamins and minerals are deficient in cereals and bread.

More than one half of the world derives over one half of their calories from cereals, chiefly rice and wheat. Cereals are the cheapest source of calories, and throughout the world they are consumed in large proportions by people of low income levels.

Cereals are stated to supply 72.5 percent of the total energy of the diets of people in the Middle East. The estimated total protein intake for these people is 69.2 grams per person per day, of which 83.4 percent is supplied by cereals, mainly wheat, 8.7 percent by nuts and seeds, and 7.9 percent by animal food. Some signs of protein deficiency among young children have been reported from certain areas (Sabry, 1961).

Wheat is the staple food for a large percentage of Iranians. Approximately three-fourths of the usable land in

Iran is given over to the raising of wheat. Most of the wheat is made into bread in local bakeries from 75 percent extraction flour, prepared in government owned mills. The mills in turn buy their supply of wheat from farmers and land owners.

Different varieties of wheat that are raised in different parts of the country are as follows: common wheat, durum wheat, Polish wheat, Polard wheat, shord wheat, Persian wheat and a few others.

Several different kinds of bread are made in Iran. These contain wheat flours, water and salt. The leavening agent is a yeast starter carried over from the previous baking. The bread is baked on hot gravel in sheets about 30x12 inches and 28-40 mm thick. This bread provides 70 percent of the caloric intake of the people of lower economic groups and farmers. The people in this group comprise more than 80 percent of the population. The other foods in the diets of these people do not completely supplement the bread nutritionally. As a consequence, many of the people show mild symptoms of protein and B vitamin deficiency (Browne et al. 1961).

Since bread is the main dietary constituent for the people in the lower and average income groups, it seemed worthwhile to attempt to improve the quality of protein in bread. Any addition of dietary essentials to the wheat flour is likely to be reflected in improvement of the nutritional status of a large proportion of the population.

REVIEW OF LITERATURE

Nutritional studies have shown that the proteins of wheat flour[1], especially gluten, are among the poorer proteins consumed in large amounts. Munaver and Harper (1959) in a study with rats concluded that "wheat gluten due to its very low level of lysine is so severely unbalanced that the lysine it contains is not completely utilized, and lysine requirement of the rat for maximum growth is increased when this protein is the sole source of lysine in the diet". Block et al., (1958) indicate that growth retardation of animals fed wheat flour and bread as the only source of protein, is due to the low percentage of complete protein in wheat flour and bread.

1. Wheat and Wheat Flour Proteins

Protein supplies approximately 13 percent of the total calories of the wheat flour (Block et al., 1958). Howard et al. in 1958 showed that the rate of growth of weanling rats is directly related to the dietary levels of complete protein when wheat flour protein supplies the major part of the diet. Furthermore, addition of proteins which provides non-supplementary essential amino acids as well as excess

1 Wheat flour prepared in the United States is usually 72 percent extraction and white flour is made by further refining of wheat flour to different degrees, depending upon the parts of the country and the type of bread. (The Experimental Study of Foods, Ruth M. Griswold, 1962).

quantities of dietary dispensable amino acids (all amino acids except lysine) do not per se influence the rate of growth.

The studies of Osborne and Mendel on the nutritional values of protein emphasized the poor biological value of the protein of wheat flour, especially gluten. Because of the low levels of lysine in flour protein, animals fed flour as the sole source of protein can utilize less than half the protein to form new tissue (Block et al., 1958).

Work by many investigators indicated that the total protein content of a food is not a good criterion of its growth-promoting action when it is fed to rats. To overcome this difficulty, Howard et al., (1960) suggested the use of the term "complete" protein. They defined this as "that fraction of the total dietary protein which is completely utilized for anabolic purposes". The amino acids required for growth of the young rat were used in calculating the fraction of cereal protein which was "complete". On this basis the "complete" protein should contain 5.3 g lysine, 4.6 g valine, 5.0 g sulfur-containing amino acids (cystine plus methionine), 3.0 g threonine and 1.1 g tryptophan per 16 g nitrogen (Howard et al., 1958; 16 g nitrogen = 100 g protein).

To test the application of this theory, Howard et al., (1960) fed rats wheat flour rations which contained 15% protein. On this basal ration, only 50% of the total protein was complete. Addition of increasing amounts of lysine

improved the growth rate and with that, the fraction of the wheat protein which was "complete". In agreement with the report of Bender (1958) however, Howard et al., (1960) were unable to get growth rates in rats fed the lysine supplemented wheat flour ration which were more than 65 to 70% of that secured when rats were fed a diet containing bread supplemented with lactalbumen, soybean and lysine.

From the results of the amino acid analysis they made of a variety of breads (table 13), Block and Mandl (1958) calculated the percentage of "complete" protein in each of the three commercial breads they studied. These calculations were based on their conclusion that "lysine is the primary amino acid deficiency" in wheat products and that "there is no closely limiting second amino acid". With such assumptions, they calculated that the following values represented the fraction of the total protein which was "complete": wheat flour, 40%; white bread, 52%; whole wheat, 60%; special formula cracked wheat bread, 70% and lactalbumen-soybean milk bread, 80%.

If the assumed estimated amino acid requirement is correct, according to the above figures the second limiting essential amino acid in flour is cystine plus methionine (Howard et al., 1960). However, reports by Bender (1958) and Sure (1957) suggest that threonine is the second limiting amino acid in wheat protein. There is considerable evidence that when an amino acid mixture simulating a good animal

protein is used in the test ration, the threonine requirement is in the order of 3.0 to 3.8 g per 16 g nitrogen. If the requirement for threonine is higher when wheat proteins are used as the source of amino acids the possibility of an amino acid imbalance causing this increased need should be considered.

Rosenberg and Rohdenburg (1954) found that rats raised on a white bread diet supplemented with minerals and vitamins including vitamin B_{12} reached an adult size which was only 75 to 80 percent as great as that of control rats fed a mixed ration in which most of the protein was derived from milk, meat scraps and soybean meals. Flodin (1953) indicates the strong probability that quantity and especially quality of protein in the diet exerts a predominant influence on growth rate and adult size. Racial differences in size are not apparent in the early months of life. However, marked retardation of growth develops as the diet of infants changes from the high quality protein of mother's milk to the low quality protein of vegetables. The adult height and weight of the people in the various countries of the world varies directly with the amount of protein in the diet (Flodin, 1953).

When protein is markedly deficient in the diet, production of plasma proteins declines and the weight of circulating blood proteins per kilogram of body weight diminishes. Prolonged protein deficiency decreases the formation of antibodies and resistance to infections. These defects can be

quickly cured by feeding adequate amounts of high quality protein.

If a pregnant woman consumes less than 75 grams of protein daily during the later part of pregnancy, her infant would tend to be short and light in weight. Mothers produce more milk on high protein diets than on high fat or high carbohydrate diets (Flodin, 1953).

The lysine content of wheat flour limits its protein efficiency ratio (gain in weight per gram of protein eaten) to about half that of the high quality protein. Rats fed wheat gluten had low plasma choline esterase levels while the addition of lysine and methionine elevated the level of enzyme activity[1] (Barrows, 1958). Pearson (1937) has shown that a lysine deficient diet suppresses not only growth but also the estrous cycle.

2. Composition of Wheat and Wheat Flour

J. R. Murlin et al. in 1941 in a study of wheat grain and other cereals showed that the various layers of cereal grains differ not only in their vitamin and minerals, but also in their general protein makeup. The fractions which contain the pericarp and germ and which serve as the distinguishing ingredients of the dietetically superior whole grain flour, possess a much higher nutritive value than

[1] The level of choline esterase activity was considered a means for detection of nutritional status but there are reports that indicate this is not a significant way for measuring nutritional level.

fractions which are composed mainly of endosperm.

Wheat germ has been recognized as having a high nutritive value. It not only has a high thiamine and flavin content, but it also contains 30% protein (Hove et al., 1943).

McDermotte et al. (1960) in studies on amino acid composition of the protein in wheat determined the lysine content of six different varieties of flour. They found that the different varieties of wheat have protein contents ranging from approximately 6.7% to 14%. As the protein content of the flour increases the percent lysine in the protein decreases. The same inverse relationship exists between the content of protein and amount of lysine in the protein of endosperm obtained by microdissection from wheats of different protein content.

Lawrence et al. (1958) confirmed the preceding when they found no significant difference in lysine content of different varieties of wheat. The overall mean lysine content on a unit protein basis was 2.89% for wheat of about 13.5% or more protein. But below this level there was a highly significant inverse relationship between protein content and lysine in protein. The lysine content was not affected by the seasons nor the region in which the wheat was grown.

The availability of lysine in wheat, flour and bread was found to be 75, 72 and 76 percent, respectively, when

wheat gluten was fed as the protein in the basal diet[1]. With an amino acid basal diet the percent availabilities were 78, 80, 83 and 80 for wheat, flour, bread and gluten, respectively (Calhoun et al., 1960).

Kuiken and Lyman (1948) found 92.8% of lysine in the whole wheat to be available to the rats as determined by fecal excretion[2]. Guthneck et al. (1953) reported the lysine of wheat germ to be 65% available as measured by gains in body weight of protein depleted adult male rats. Gupta et al. (1958) reported the lysine of wheat flour to be 70% available as measured by the growth of young rats.

The animal work suggests that the lysine in wheat bread is only about 70% available. No work has been found on the availability of lysine in wheat or wheat products for the human organism. Some of the lysine may be bound in the browning reaction which occurs in the crust of the bread and some might not be absorbed.

3. Amino Acid Loss during Milling and Baking

A. Milling

When converting wheat to flour, the level of the amino acids in flour decreases. Hepburn et al. (1957) reported

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1 A diet containing 20% gluten, 70% starch and 10% salts, corn oil and vitamins plus histidine, methionine, tryptophan, and threonine.

2 This value may have been too high since there is a possibility that the bacteria in the gastro-intestinal tract may have metabolized the lysine.

that in converting wheat to flour the following amino acids were significantly reduced: lysine, 24%; arginine, 19% and aspartic acid, 19%. Hutchinson et al. (1956) indicated that the lysine content of flour is proportional to the extraction rate. The lower the percentage of flour secured from the wheat the lower the lysine content of the flour. Consequently, whole meal bread has a higher lysine content than white bread.

B. Baking

Baking bread from flour produced an 11% decrease in cystine and 19% decrease in lysine (Hepburn et al., 1957). McDermotte and Pace (1957) reported that there are 15 to 16 percent losses of phenylalanine, tyrosine and lysine in the bread; most of the loss is in the crust (browning reaction). Rosenberg and Rohdenburg (1951) showed that considerable loss of lysine (from 9.5 to 23.8%) occurs during the baking process.

C. Toasting and Staling

Toasting further reduces the lysine content from 5 to 10 percent and similar losses occur when bread becomes stale and dry (Rosenberg and Rohdenburg, 1951). Howard et al. (1958) state that browning products formed in the crust during baking are primarily responsible for the loss of lysine in the bread.

4. Lysine Requirement

The minimal daily requirement of l-lysine is the most

variable of all essential amino acids - it appears to have a great interindividual variation. The average lysine requirement of man as found by Clark et al. (1957) is between 500 to 900 mg per day. Rose et al. (1955) in their study found that the minimal daily L-lysine requirement was 0.7 gram. Since some individuals required more than this, Rose et al. tentatively proposed 0.8 g as the minimal daily requirement. They suggest that 1.6 grams of this amino acid is a safe dietary intake for an adult.

Numerous experiments using both human and animal subjects have demonstrated that the protein of whole wheat flour and bread in spite of its low digestibility is superior to that of white flour and white bread (Carlson et al., 1946).

Commercial bread, because of its content of dry milk solids should be decidedly better in protein value than wheat flour (Block et al., 1959). Block et al. rank the different breads they studied in the following order of increasing nutritive value: white bread, whole wheat bread, special formula, cracked wheat and lactablumen-milk-soyabean flour enriched white bread.

5. Bread and Flour Enrichment Program

Before the bread and flour enrichment program started in the United States, the medical literature was filled with reports of pellagra, beriberi, mild anemia and ariboflavinosis. Thiamin was also low in the diets during 1920 and the following decade. The body does not store this vitamin and

it needs to be replenished to prevent the deficiency symptoms associated with a low intake.

The enrichment of bread and flour was one factor that contributed to the decreased death rate for pellagra and beriberi that occurred in the subsequent years. It also contributed to the disappearance of ariboflavinosis.

There have been many studies which demonstrate how the nutritive value of bread, flour and cereal grain can be improved. Milling removes the wheat germ from flour and so reduces very drastically the vitamin, mineral and protein content of the flour. There are a variety of means that have been proposed for overcoming these deficiencies in flour. They are:

A. Fortification of Bread with Milk

Non fat dry milk solids which supply among other things lysine and valine, have been used in white bread rather extensively by the baking industry.

Most commercial white breads contain 2-6 percent dry milk solids. Bakers add the milk solids to bread to increase its softness and stability rather than to improve the biological value of bread protein. The improvement results from the fact that the lysine content of milk protein is 7.5 percent and its valine content is 4.5 percent. Commercial bread has been prepared containing as much non fat dry milk solids as 6 percent of the flour.

Parks et al. (1954) showed that addition of milk improves

the amino acid composition of white bread. The milk also increased the calcium content of the bread. Light et al. (1943) states that addition of 6% dry skim milk powder to the bread formula produces a bread which is equal to whole wheat bread in so far as growth of rats is concerned. Welton et al. (1959) reported that a high nutritive type of white bread containing non fat milk solids at a level as high as 25% of the flour could be made. The addition of this very high level of non fat dry milk solids increased the protein content of the bread about 30 percent, depending on the type of flour used. The biological value of this bread, as shown by rat growth tests, was twice as great as that of commercial bread containing 4 percent non fat dry milk. Thus the nutritional value of the protein (biological value x protein content) is about 2.5 times that of the usual commercial bread.

Jahnke et al. (1957) reported that even when 12 percent non fat dry milk solids were incorporated in white bread, the biological value thereof could be further increased by the addition of lysine. Animals that were fed the bread containing 12% milk solids showed an improved growth rate when 0.25% lysine was added to the bread. They concluded that the rate of growth of rats fed the bread is directly related to the lysine and tryptophan content of the ration.

Kennedy et al. (1954) showed that acceptable bread could be obtained from white flour containing as much as 22% milk solids. The maximum amount of milk solids that could be

added depends to some extent upon the flour. According to L. V. Rogers (1959) when bread containing a high percentage of dry skim milk is made, it is generally necessary to increase the fermentation time due to the increased buffering capacity resulting from the added milk solids.

B. Supplementing Bread with Wheat Gluten and Other High Protein Foods

A number of studies have been carried out to determine how the biological value of bread might be improved by adding a variety of proteins or foods high in protein, other than milk solids. Hutchinson et al. (1962) showed that wheat gluten did not improve the lysine deficiency of white flour. Supplementation of bread with 10-50 percent wheat gluten caused only a small increase in the growth of the experimental animals. For this reason, gluten is not considered a good supplement for white flour.

Wheat germ which is high in protein, iron, threonine, riboflavin and niacin, can be added to bread after the fat has been removed (Westerman et al., 1952). The addition of fat free wheat germ to bread increased the level of the above mentioned nutrients in the diet. When 4 to 6 percent of defatted wheat germ was added to unenriched flour, it increased the growth rate of rats. The only objection to the use of defatted wheat germ is that it is not very palatable.

The protein of soybean flour due to its high lysine and valine content may also be used to increase the nutritional quality of wheat flour protein (Carlson et al., 1946).

Sure (1952) reported that the proteins in whole rye are superior in nutritional value to those in whole wheat. This was true at all levels of flour fed to rats. Prior to this, Kon and Markuze (1931) obtained results indicating the superiority of rye bread with respect to the biological value of the protein.

A new type of bread made with rice and wheat flour has been offered as a substitute for the usual white bread. The use of rice does not change the organoleptic quality of white bread. Alejandro-Mosqueda-Suarez (1958) showed that a suitable flour could be made by replacing 30 percent of white flour with rice flour. The protein efficiency of rice-wheat bread compared with wheat bread was increased by 9.1 percent. They suggested that the improvement in protein efficiency probably resulted from the changed amino acid pattern produced by the rice. This bread requires 25 percent more yeast and also needs a longer fermentation time.

Algae have also been used for supplementing white flour. Hundley et al. (1956) reported that chlorella is a better source of thiamine than purified soy protein. When chlorella was added to white flour on an isonitrogenous basis, it was equal to several animal proteins of high biological value. They also suggested that algae might serve as a source of protein for the supplementation of various flours and cereals.

Sure (1946, 1947, 1948) in an extensive series of studies on the supplementation of cereal grains observed that

rats fed enriched white flour supplemented with 1-5 percent nonviable dried yeast exhibited large increases in weight gain and food utilization as compared to those fed unsupplemented enriched white flour. These increases were not entirely accounted for by the lysine content of the yeast. The diet used by Sure contained adequate amounts of 8 crystalline B vitamins. Seeley et al. (1950) stated that "Although dried yeast contains 3.5 percent lysine and is therefore an excellent source of this substance, the amount of lysine and valine which it (the yeast at a 3% level) provides at the low level employed evidently is not enough to produce a large increase in the biological value of the bread protein". The increase in the weight of the rats fed the yeast-supplemented bread depended on the protein, vitamins and minerals in the yeast as well as the lysine.

Parks et al. (1954) showed that the level of thiamine was increased in bread made with brewer's yeast.

C. Supplementation of Wheat Bread and Flour with Lysine

Many studies have been carried out to determine the effect of lysine in improving the protein of white flour and bread. In 1943, Light and Ferry reported that the protein of white and whole wheat bread can be further improved by supplementing the flour with lysine and valine. Rosenberg and Rohdenburg in 1952 reported that if sufficient lysine was added to the bread diet to bring its total lysine content to 0.8% or more, growth similar to that obtained with

the stock diet was observed[1]. Sure (1953) found that the addition of lysine and valine increased the protein efficiency ratio of the proteins in wheat flour from 0.68 to 3.11. Lysine and threonine were found to play a "significant specific" role in food utilization. In 1954, Sure carried out rat growth studies with a whole wheat cereal breakfast food and with whole wheat. Feeding these cereals at an 8 percent protein level, he observed an increase in protein efficiency when lysine alone was added. However, he obtained a further increase by adding valine and threonine to the lysine supplemented cereal.

When lysine supplemented white bread was fed at a 12.5% protein level no supplementary growth effect was obtained from the addition of valine and threonine. He also reported that vitamin B_{12} in the presence of lysine and threonine increased the protein efficiency of the processed wheat. The vitamin B_{12} also increased food intake. The addition of lysine alone to wheat gluten has been shown to raise its protein efficiency ratio to a value equal to those for animal proteins. This was shown to be true for both infants and adults (Flodin, 1956).

Hutchinson et al. (1956) found that the difference in

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[1] Composition of the basal bread diet was: dried bread, 90%; salt mix no. 351, 3%; crude soybean oil, 4.5%, cod liver oil, 0.5%; biotin or starch (15 ug/g), 1.0% and vitamin concentrate, 1%. Bread used in this experiment was commercial loaf bread baked according to the standard formula. It contained 3% non fat milk solids and 2 parts of yeast per 100 parts of flour.

lysine content was responsible for the increased growth rate of male weanling rats fed rations containing protein from whole wheat meal as compared with that of white flour. They showed that the optimum response in growth rate occurred with a lysine addition of between 0.2 and 0.3 percent to white bread. Addition of more than 0.3 percent lysine depresses growth rate and also food intake of rats. The latter is probably due to an amino acid imbalance.

Culik et al. (1958) showed that lysine supplementation of bread improved reproduction and lactation performance of rats uniformly in each of 7 litters of the same generation and in successive generations. Rations containing, as the sole source of protein, flour supplemented with 0.25% L-lysine HCl produced normal or nearly normal reproduction and lactation of rats.

Rosenberg and Rohdenburg (1958) showed that the protein efficiency ratio of bread protein can be increased from 1.01 to between 1.89 and 2.12 by fortification with lysine. Brown et al. (1959) concluded that "for protein efficiency ratios in the high quality range of 2 or higher it appears necessary that the bread protein contain at least 200 mg of L-lysine per gram of nitrogen".[1]

Clark et al. (1961) reports that supplementation of

[1] In my experiment the protein efficiency ratio was found to be 1.34 for American standard white bread with 2% dried milk solids added (table 3).

flour diets with lysine up to the level of 0.5 percent significantly increased the food intake and weight gain. Maximal weight gains were noted with 0.2 percent of added lysine. The biological value of flour protein (the percentage of absorbed protein retained by the experimental animals) was increased in a stepwise fashion by lysine supplementation up to the 0.25 percent level. Higher levels did not further increase the biological value of the protein.

The nitrogen efficiency value reached a maximum when lysine supplementation was at a level of 0.20 or 0.25 percent.

From the above observations, it is well established that wheat bread and flour are poor sources of complete protein. If they supply the major part of the diet, growth retardation in animals results. Various methods of bread and flour enrichment have been studied and several supplements were used such as dry milk solids, soybean flour, rye flour, cultured and brewer's yeast, rice, algae, pure amino acids, etc.

At present in Iran it is economically unfeasible to consider the fortification of flour or bread with pure amino acids. However, skim milk powder is produced by a number of plants throughout Iran. This product has not found a very extensive market largely because the people are not accustomed to this food. For this reason it should be possible to mix skim milk powder with the flour prepared in the government mills. It may also be possible to use legumes that

are indigenous to Iran for supplementing the protein in the flour.

6. Measures of Nutritional Status

Liver xanthine oxidase activity is a method used by investigators to study the levels of protein in the diet. W. Westerfield et al. (1951, 1956) showed that this enzyme is very sensitive to levels of protein intake. Low protein diets cause a loss or decreased activity in liver xanthine oxidase. Measuring the activity of this enzyme was used in this experiment to compare the protein content of the 4 types of bread used in Experiment I.

Choline esterase activity was used by Barrows (1958) for measuring the quality of wheat protein. McCance et al. (1948) reported that mean serum choline esterase levels are related to the protein and food intake, but Saunders et al. (1952) showed there is no relation between the level of this enzyme and protein intake. In view of the controversial nature of this method, it was not used in this experiment.

Another factor that is very sensitive to the level of dietary protein is hemoglobin levels. In starvation studies, Keys et al. (1950) showed that both the level of hemoglobin in the blood and total circulating hemoglobin reflect the protein losses more accurately than any other factor. The adequacy of the protein in the 4 diets in Experiment II was assessed by the hemoglobin levels in the rats at the end of the study.

GENERAL EXPERIMENTAL PROCEDURE

This study consisted of two experiments. Each experiment will be discussed separately. The first experiment is a comparison of the growth produced by rations in which the primary ingredient was either Persian, Syrian, Alladin or U. S. white enriched bread (see p. 23 for description of breads).

The second experiment was designed to study the improvement of Persian bread by supplementing the bread with dry milk solids and/or lentils.

Male weanling rats of the Sprague-Dawley strain, weighing 45-55 grams were used in both experiments. The animals were divided into groups of ten on the basis of weight. The mean weight of all groups in the same experiment did not vary by more than 0.5 gram. The animals were housed individually in wide mesh, wire bottom elevated cages. Food and water were given ad libitum. All animals were weighed weekly during the experimental period. Food intake was measured and recorded.

The composition of the basal diet was:

Dried ground bread	90%
Corn oil[1]	5%
Mineral mixture[2]	4%
Vitamin mixture	0.25%
Choline chloride	0.15%
Sucrose	0.60%

1 Containing 7.5 mg alpha tocopherol per 5 g.
2 Salt mixture W, obtained from Nutritional Biochemicals Corp.

The vitamin mixture provided in each 100 g of diet: 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 B_{12}, 0.01 mg biotin, 1.0 mg p-aminobenzoic acid, 10 mg vitamin A, 0.18 mg vitamin D, and 0.38 mg menadione.

EXPERIMENT I

EXPERIMENTAL PROCEDURE

Four groups of 10 rats each were fed rations in which the dried ground bread was: Group I, American white enriched bread which was obtained on the open market; Group II, Syrian-Lebanese bread, baked in Detroit and having a label which indicated the bread was made of flour, water, salt, yeast and preservatives; Group III, Persian bread which was bought, dried and ground in Iran and shipped here; Group IV, Alladin bread which was ordered from New York. The label on the latter bread indicated that the same ingredients were used in this bread as those in the Syrian bread.

All four types of bread, including the dried bread from Iran, were dried in a vacuum oven at 50°C for a period of 5 hours then ground to a fine powder.

The rats were fed these diets for a period of four weeks. Food intake and body weight were recorded each week. From these values, protein efficiency ratio (gain in body weight per gram of protein consumed) and gain per gram of food consumed were calculated. At the end of the 4th experimental week, 5 rats from each group were sacrificed with a sharp blow on the head and decapitated. The livers were removed, homogenized with water in a Potter-Elvehjem apparatus and then dried to constant weight in an oven maintained at 100°C (requiring 12 hours). On the 29th experimental day,

one rat from each group was sacrificed. One gram of its liver was immediately homogenized and a fraction of it added to a Warburg flask for the determination of xanthine oxidase activity. On each of the next 4 days, another rat from each group was sacrificed and one gram of its liver used for the determination of xanthine oxidase activity. This was done for all of the rats killed during the 5 days after the experiment proper was terminated. The liver remaining after the removal of the 1 gram sample was homogenized. The moisture in the liver was estimated by drying to constant weight at 100°C. After drying, the livers were ground to a fine powder in a small Wiley mill. The fat from one gram of the dried, ground liver was extracted by ether on a Goldfisch apparatus. Nitrogen was determined by the Kjeldahl technique. For liver xanthine oxidase activity, the procedure of Axelrod and Elvehjem (J. Biol. Chem., 140:725, 1941) for the Warburg apparatus was used.

The breads in the four diets were analyzed for nitrogen and fat and the protein contents of the breads were calculated using 5.7 as the conversion factor (Assoc. Off. Agric. Chem., Official Methods of Analysis, p. 215.).

RESULTS

Table 1 presents the results of the bread analyses. This table shows that the nitrogen content of Persian bread is almost equal to that of American standard bread (with 2 percent milk solids). Alladin bread has the highest nitrogen content. The level of fat in American bread is 1.36 due to the addition of 5 percent tri- and diglycerides (mostly as stearate) while the other 3 breads are much lower in fat since they have none added.

The growth per week of rats in Groups III and IV were similar to those of the control group (American white bread, table 2). Group II gained significantly less weight than the control animals ($P<0.05$, table 3). The protein efficiency ratio of Group I is significantly greater than that for Group II and Group IV and that of Group III is significantly greater than Groups IV and II ($P<0.01$).

The liver nitrogen of animals in Group I is significantly less than that of Groups III and IV ($P<0.01$, tables 4 and 5) and the animals in Group II have significantly less nitrogen in their livers than Group IV ($P<0.05$).

Table 1. Composition of breads used in the four experimental diets. All values are expressed as percent of the dried breads or diets prepared for the rats.

Group	Bread	Nitrogen in Bread	Fat in Bread	Moisture	Protein in Bread	Protein in Diet
I	American	2.17	1.36	1.29	12.37	11.13
II	Syrian	2.47	0.13	4.29	14.08	12.67
III	Persian	2.00	0.39	9.86	11.40	10.26
IV	Alladin	2.78	0.31	4.37	15.85	14.26

Table 2. Mean food intake per week, weight gain, protein efficiency ratio and gain per gram of food eaten.

Group	Food Intake g/week	Growth g/week	Protein Intake	Gain/g Protein	Gain/g Food
I	63.0±2.6	9.45±0.55	7.13	1.34±0.05	0.15±0.006
II	66.8±2.8	8.80±0.42	8.46	1.04±0.03	0.13±0.004
III	76.6±3.3	10.40±0.48	7.86	1.32±0.04	0.14±0.004
IV	63.4±2.7	9.93±0.70	9.04	1.08±0.04	0.16±0.005

Table 3. Level of statistical significance of differences between groups (see table 2).

Groups Compared	Food Intake	Growth	Gain/g Protein	Gain/g Food
I vs II	none	none	1%	5%
I vs III	2%	none	none	none
I vs IV	none	none	1%	none
II vs III	5%	5%	1%	none
II vs IV	none	none	none	1%
III vs IV	2%	none	1%	1%

Table 4. Liver fat, nitrogen, moisture and xanthine oxidase activity. All values are expressed as percent of the fresh livers.

Group	Moisture	Fat	Nitrogen	Xanthine Oxidase ul O_2/hour/g liver
I	72.5±0.6	4.36±0.3	2.15±0.05	175±26
II	72.8±0.8	4.15±0.1	2.28±0.09	196±38
III	73.0±0.7	3.88±0.1	2.45±0.08	214±40
IV	70.8±1.1	4.08±0.1	2.59±0.09	196±44

Table 5. Level of statistical significance of differences between groups (see table 4).

Groups Compared	Moisture	Fat	Nitrogen	Xanthine Oxidase
I vs II	none	none	none	none
I vs III	none	none	1%	none
I vs IV	none	none	1%	none
II vs III	none	none	none	none
II vs IV	none	none	5%	none
III vs IV	none	none	none	none

DISCUSSION

Body weight gain. During the 4 weeks of the study, the animals in Group III fed the Persian bread diet had an average weight gain which was significantly greater ($P<0.05$) than that for the rats in Group II, fed the Syrian-Lebanese bread (10.4 g vs 8.8 g). In the other groups the differences in weight gain were not significant.

Food intake. The amount of food consumed by the rats was measured by recording the differences in the weights of the feed cups when empty and when filled with food. The amount of spilled food was recovered and weighed to obtain more accurate food intake values.

The average food intake of the animals in Group III was significantly greater than Groups I, II, and IV; these groups consumed the same weight of food.

These differences can very well be the result of food spillage. It was observed that in Group III, the animals fed the Persian bread diet spilled their feed more than the rats in the other groups. However, special attention was given to make sure that this spilled food was accounted for.

Liver analyses. There were no significant differences in liver moisture and fat among the 4 groups of animals. The nitrogen concentration in the livers of the rats in Group I was significantly less than in Groups III and IV ($P<0.01$) and

that in Group II was significantly less than in Group IV ($P<0.05$). This could be due to the better protein efficiency of the bread fed to the animals in Groups III and IV. The nitrogen content of the bread used for the diet of the animals in Group IV was higher than that fed the controls (table 1).

The primary purpose of this part of the study was to see whether the biological values of the bread proteins were the same. On the basis of the above results, there are no significant differences between the growth promoting effects of the American commercial white bread, Persian bread and Alladin bread. But that of Syrian-Lebanese bread is significantly less than that of the Persian bread. In other words, the growth promoting effect of Persian bread is equal to American standard white bread which is made with 2.7 percent yeast, 2% milk solids and vitamin and mineral enriched flour. This could be due to several factors. First, Iranian bread is baked in the oven for only 5 minutes or less, at a high temperature. But on the other hand, this bread being baked in a sheet has more surface area exposed to the heat and consequently the browning reaction is greater. Theoretically, there should be more lysine lost in this bread than American bread baked in loaves. According to Rosenberg and Rohdenburg (1951) and McDermotte and Pace (1957), the amino acids destroyed due to the browning reaction in the crust of bread amounts to between 9.5 and 23%. Thus, the time and heat

involved in baking the bread does not explain the similarity between the nutritive values of Iranian bread and white American bread. Another factor may be the lower extraction of flour in Iran. They discard a smaller portion of the wheat in the milling process and thus more of the protein is conserved in the flour than is conserved in the American white flour (the usual extraction of flour in Iran is between 75 and 80%). The type of wheat may be another factor. According to McDermotte et al. (1960) different varieties of wheat have a protein content of from 6 to 14 percent. However, Lawrence et al. (1958) indicated that there were no significant differences in the lysine content of different varieties of wheat.

For a variety of reasons, the different varieties of wheat in Iran are bought and pooled at the mills. Usually the wheat flour that is used for bread is prepared from a mixture of different varieties of wheat, depending on yield, price and season and other factors as well. If the pattern of amino acids in different varieties of wheat differs, they may balance each other and as a result the mixture may have a better protein content.

The length of the fermentation can be another factor. The dough which is thin (almost like a batter) is left overnight to rise, the raised dough is not as elastic as the dough used for American bread. According to Clifton (1957)

the number of yeast cells increases with time. The rate of cellular division remains constant, but the actual number of bacteria or amount of bacterial protoplasm formed per unit of time, during this phase increases with time since growth is proportional to the number of cells present at any given time. Huffman and coworkers (1941) found that in standard dough containing 1.67% of yeast fermented at 80°F there was practically no increase in the number of yeast cells during the first 2 hours. The greatest increase of about 26% took place between the second and fourth hour. The rate of increase dropped to about 9% between the 4th and 6th hour of fermentation. The long period of fermentation of Iranian bread may help increase the total amount of yeast cell protoplasm thus increasing its protein content.

The factors that may be important in bringing the biological value of the Iranian bread up to that of American white bread containing 2% skim milk powder are: 1) short baking period of Iranian bread; 2) lower extraction rate of Iranian flour; 3) the variety of wheat used in making Iranian bread; and 4) the long period of fermentation.

EXPERIMENT II

EXPERIMENTAL PROCEDURE

Forty male weanling rats were divided into 4 groups of 10 each and fed the following diets for a period of 3 weeks: Diet I, basal diet which was the same as that listed on page 23 and made with Persian bread; Diet II, the same as Diet I except that 4% skim milk powder (Carnation instant) replaced an equal amount of bread; Diet III, same as Diet I except that 7.5% ground lentils (Lens esculenta) replaced an equal weight of bread; Diet IV, same as Diet I except that 15% ground lentils replaced the same amount of bread.

The measurements for food intake and body gain in weight were taken as previously described. The chemical analyses were also the same as previously described.

Hemoglobin levels in the blood of these rats were determined in all animals in place of liver xanthine oxidase activity determinations.

Blood samples were taken from the rats' tails prior to sacrificing. The blood was transferred into spot plates which contained crystalline potassium oxalate to prevent blood clotting. Then 0.02 ml of blood was transferred to tubes containing Drabkin's solution. Drabkin's solution was made from 1.0 g $NaHCO_3$, 50 mg KCN and 200 mg $K_3FE(CN)_6$ and brought up to 1 liter volume. The tubes were read in a Bausch and Lomb colorimeter and percent hemoglobin was read

from the standard curve prepared using known concentrations of cyanomethemoglobin standard (Drabkin, 1932).

The nitrogen content of diets was determined and protein content was calculated using 5.7 as the conversion factor. The average protein intake of animals was also calculated (table 7) as previously described.

RESULTS

In table 7 the average group food intake per week is shown for the 3 week period. Average growth per week, gain per gram protein eaten and gain per gram food intake were calculated therefrom.

The food intake, body weight gain, protein efficiency ratio and grams of body weight gain per gram of food intake in the rats in the 4 groups were significantly different ($P < 0.01$, table 8).

The moisture content of the livers of the rats in Group III was significantly less than that of rats in Groups I, II and IV ($P<0.01$, 0.02 and 0.01, respectively; tables 9 and 10). The fat content of the livers of the animals in different groups were not significantly different but the nitrogen content of the livers of the rats in Group III was significantly greater than that of animals in the other groups (table 9 and 10).

There was a significant difference ($P<0.05$) in the hemoglobin levels of the rats in Groups I and III, Group III being greater. Hemoglobin levels in the blood of animals in other groups were not significantly different (tables 11 and 12).

Table 6. Diet composition and percent protein in the diet.

Group	Composition	% N in Diet	% Protein in Diet
I	Persian (9.86% moisture)	1.91	10.89
II	Persian + 4% skim milk	1.98	11.29
III	Persian + 7.5% lentils	2.01	11.46
IV	Persian + 15% lentils	2.16	12.31

Table 7. Mean food intake per week, weight gain, protein efficiency ratio and gain per gram of food eaten.

Group	Growth g/week	Food Intake g/week	Gain/g Food	Protein Intake	Gain/g Protein
I	10.8±0.5	63.4±2.1	0.17±0.005	6.92	1.57±0.05
II	18.1±0.4	75.1±3.3	0.24±0.005	8.48	2.13±0.04
III	21.3±0.4	86.0±1.3	0.25±0.004	9.86	2.17±0.03
IV	31.6±1.0	105.6±3.4	0.30±0.005	12.99	2.43±0.04

Table 8. Level of statistical significance of differences between groups (see table 7).

Groups Compared	Growth	Food Intake	Gain/g Food	Gain/g Protein
I vs II	1%	1%	1%	1%
I vs III	1%	1%	1%	1%
I vs IV	1%	1%	1%	1%
II vs III	1%	1%	none	none
II vs IV	1%	1%	1%	1%
III vs IV	1%	1%	1%	1%

Table 9. Fat, moisture and nitrogen content of the livers. All values expressed as percent of the fresh liver.

Group	Moisture	Fat	Nitrogen
I	71.56±0.33	3.84±0.15	2.17±0.07
II	71.22±0.26	3.84±0.13	2.27±0.05
III	71.51±0.19	3.81±0.14	2.41±0.03
IV	70.40±0.15	4.18±0.15	2.23±0.06

Table 10. Level of statistical significance of differences between groups (see table 10).

Groups Compared	Moisture	Fat	Nitrogen
I vs II	none	none	none
I vs III	none	none	1%
I vs IV	1%	none	none
II vs III	none	none	5%
II vs IV	2%	none	none
III vs IV	1%	none	5%

Table 11. Hemoglobin levels of blood of rats. Sample secured at 18th day of experiment. The experiment was of 3 weeks' duration.

Group	Bread	Hemoglobin Level
I	Persian	11.55±0.16
II	Persian + 4% milk solids	11.65±0.24
III	Persian + 7.5% lentils	12.05±0.15
IV	Persian + 15% lentils	11.95±0.28

Table 12. Level of statistical significance of differences between groups (see table 11).

Groups Compared	Hemoglobin Level
I vs II	none
I vs III	5%
I vs IV	none
II vs III	none
II vs IV	none
III vs IV	none

DISCUSSION

The bread used in the rations for all groups was Persian bread which was used at a level of 90% in the control diet. For the other groups, the bread was supplemented with 4% dried milk solids, 7.5% and 15% ground lentil powder, respectively. The amount of bread used in Groups II, III and IV was 86%, 82.5% and 75%.

Food intake and growth. There was a significant difference ($P<0.01$) for all four groups of animals in so far as body weight gain and food intake were concerned. This indicates that the supplements used in this study enhanced the rate of body weight gain. For the lentils, the higher the level of supplementation, the better was the food intake and growth. The addition of 4% dried milk solids increased the food intake of the animals during the 3 week study. Lentil powder (7.5% and 15%) further increased the food intake and growth which were proportional to the percent of supplementation.

Table 7 shows that protein efficiency ratio of the diet increased as the level of supplementation increased.

Liver analyses. The moisture content of the livers in Groups I, II and III were significantly greater than those in Group IV ($P<0.01$, 0.02 and 0.01, respectively). There was no significant difference between any of the groups in the percent fat in the livers.

Liver nitrogen in Group III was significantly greater than that in Groups I and II ($P<0.01$ and 0.05, respectively). The nitrogen in Group III was significantly greater ($P<0.05$) than that in Group IV.

The hemoglobin level in the blood of all animals was determined and the statistical analyses show that Group III had significantly greater ($P<0.05$) than that of the control group. There were no significant differences between the level of hemoglobin in the blood of animals in the other groups.

The level of supplementation of the Iranian bread with dried skim milk solids was only 2% above that of the American white bread. In the first experiment it was found that unsupplemented Iranian bread produced the same growth in rats as the standard American white bread, containing 2% milk solids. In the second experiment, rats fed Iranian bread which was supplemented with 4% dry skim milk solids, gained almost twice as much weight as the rats fed the standard American white bread in the first experiment (18.1 g vs 9.45 g).

Supplementation of the bread with lentils at the levels of 7.5% and 15% respectively in the diets of Groups III and IV in Experiment II further increased the weight gain in proportion to the level of supplementation. However, the liver nitrogen in Group IV was significantly less than the liver nitrogen in Group III. This might be due to the larger size

of the livers in Group IV (an average of 5.45 g vs 4.51 g).

The lower moisture content and nitrogen content of the livers in Group IV could be due to the toxicity of lentils (legumes) at high levels. Ponseti et al. (1952) reported that higher levels of legumes in human diets causes lathyrism. Geiger, Steenbock and Parsons in 1933 noted scoliosis and hernias in white rats fed a diet containing 50 percent sweet pea seeds. Those authors found that the water extracts of peas fed to the rats were toxic while the extracted peas fed at the 50 percent level were not toxic. Lewis et al. (1948) noted spinal curvature in rats fed various species of legumes. When peas are eaten as a smaller proportion of the diet, they are said to be harmless.

Stockman (1929) believed that the toxic principle was an alkaloid which he obtained from the peas and used to produce paralysis in frogs. Acton and Chopra (1922) reported paralysis in the hind leg of monkeys, rats, etc., produced by a pure hydrochloride of an amine obtained from peas. Young (as reported by Stockman, 1929) suggested that the disease was due to a vitamin A deficiency but sources of vitamin A did not protect animals against the disease. Normal growth was obtained in 20 weeks in rats fed 12.5% and 5% sweet peas in the diet.

Lentils, being a legume, might cause the same problem as peas do when used to supplement the diet at high levels. If so, this would be a limiting factor in trying to supplement

the bread with higher levels of lentils. Another factor would be the taste of the bread. This should be tested and the acceptability of the lentil supplemented bread should be proved before any attempt to actually supplement bread with lentils is begun.

EPILOGUE

This study was started with the purpose of improving the nutritional quality of the protein of Iranian bread. Many experiments conducted by various investigators over long periods of time have shown that bread is a poor source of protein as evidenced by feeding experiments. These studies further showed that the quality of the protein in the bread could be improved by adding different proteins or amino acids to it. Most of these experiments were done using the rat as the experimental animal. The results of the 2 feeding experiments with the rat that are reported here are in agreement with other experiments done before by others. They show that the Persian bread can be improved nutritionally by supplementing it with 4% dried milk solids, or with 7.5 and 15% lentils.

After the experiments were completed, it seemed desirable to find out how much of the amino acid requirement of man was met by a diet consisting of 70 percent unsupplemented bread. This would be comparable to the diet of the Iranian farmers and people in the lower classes. Surprisingly, the results of our calculations showed that the above mentioned diet would meet all the amimo acid requirements of human beings. Further research of the literature and calculations showed that the same bread is definitely deficient in amino acids as far as the growth of the rat is concerned (table 13).

Table 13. Comparison of the extent to which bread provides the amino acids required by adult human beings and growing rats.

	In Bread		For Man (g/day)		For Rat (g/day)	
Amino Acid	g/16 g N[1]	per 100 g[2]	Needed[3]	Intake[4]	Needed[5]	Intake
Arginine	4.3	0.41		2.59	0.045	0.033
Histidine	2.5	0.24		1.51	0.080	0.019
Lysine	2.8	0.27	0.8	1.69	0.138	0.022
Tyrosine	3.3	0.31	1.1	1.99		0.254
Tryptophan	0.8	0.08	0.25	0.48	0.045	0.062
Phenylalanine	4.6	0.44	0.30	2.77	0.138	0.035
Cystine	3.0	0.29	0.81	1.81		0.023
Methionine	1.9	0.18	0.2	1.15	0.090	0.015
Threonine	2.4	0.23	0.5	1.45	0.080	0.018
Leucine	4.8	0.46	1.1	2.89	0.138	0.037
Valine	3.7	0.35	0.8	2.23	0.115	0.029
Glutamic Acid	29.0	2.76		17.47		0.223
Glycine	3.1	0.30		1.87		0.024
Alanine	3.0	0.29		1.81		0.023
Isoleucine	3.9	0.37	0.7	2.35	0.090	0.030

1. Table I, Block and Mandl, 1958.
2. The values for g amino acid per 100 g of bread were secured by multiplying the values in column 1 by the factor 9.5/16x6.25. The 9.5 is the value for the protein content of fresh bread (pooled commercial white bread secured from a number of cities in the U.S. See Block and Mandl, 1958).
3. Taken from H. H. Williams. New York State of Med., 59:4008 (1959).
4. The calculated amino acid intakes were based on a diet consisting of 70% bread and supplying 2500 calories. The amino acid intakes as listed represent only that which is secured from bread.
5. Taken from Albritton's "Standard Values in Nutrition and Metabolism", McGregor and Werner, Inc., Dayton, Ohio, p. 23 (1955). These figures represent the amino acid need of a 50 gram rat for growth.

This leads to the question: Are the results obtained from studies with rats directly applicable to man especially in so far as the adequacy of the amino acid composition of the diet is concerned?

Assuming the average caloric intake of Iranians to be 2500 calories and Iranian bread to have the same lysine content as American white enriched bread (containing 2% dry milk solids), lysine intake from bread alone, of a diet composed of 70% bread would be 1.6 grams. This was calculated using the equation:

$$\frac{2500 \times .70 \times .252^{1}}{276^{2}} = 1.6$$

According to W. C. Rose (as reported by H. H. Williams, 1947) the minimal daily lysine requirement of man is 0.8 g. The safety requirement for lysine brings this value to 1.6 g. The above mentioned Iranian diet seems to meet the safety lysine requirement of the adult man, even with bread proteins alone. On the other hand, this study shows that supplementing the bread with 4% dry milk solids and 7.5 and 15% lentils caused weanling rats to gain 2 and 3 times as much, respectively, as control rats. If the human lysine requirement is met by the above diet and if lysine is the most limiting amino acid, the requirement for other amino acids will also

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1 0.252 g lysine/100 g bread (table 13)
2 276 calories are obtained from each 100 g of bread (U.S.D.A. Handbook no. 8)

be met. The values give in table 13 prove this point.

Mitchell (1958) indicates that the amount of absorbed nitrogen required for nitrogen equilibrium in human beings is 4.61 mg/basal calorie and for the rat, 3.32 mg/basal cal. when wheat flour is fed. However, when the total caloric requirement is considered for both rat and man, the ratio for man decreases to almost one-half, while that of the rat remains almost the same. This is based on the fact that the total energy requirement for man is about twice his basal needs. If the individuals do as much physical work as the Iranian laborers and farmers do, the basal energy required may have to be multiplied by a factor greater than two. Under such circumstances, the reduction in the above ratio for man becomes even greater. For the rat, there is probably little difference between its basal and total energy requirement. The rat confined to the small cage in which these studies were carried out is restricted in his total activity. Consequently the energy above basal needs to provide for spontaneous activity is very small.

A possible explanation for the differences in the results secured with rat feeding trials and the extent to which bread appears to meet the amino acid requirement of man may be the difference in the ratio of calories to amino acid requirement. The weanling rat receiving the bread diet containing the Iranian bread consumed 9 grams of diet which contained 90% bread with 9.86% moisture. Their bread intake

was 7.3 grams per day. The rat requires 138 mg lysine per day, therefore the animals obtained each mg of their lysine intake from .223 calories. Man requires 800 mg lysine per day. If he receives 1750 calories from the same bread, each mg of lysine is obtained from 2.2 calories. This ratio of caloric and lysine requirements differs by a factor of 10 from that of the rat.

From the amount of amino acid available to rats eating a diet consisting primarily of bread, it can be shown that this diet does not meet the amino acid requirement of the rat (table 13). The rats in Experiment I, Group I, that were fed the diet consisting of 90% American white enriched bread, and the rats in Experiment II, Group I that were fed a diet consisting of 90% Iranian bread had an average food intake of 63 g and 63.5 g per week, respectively. Since the amino acid content of American bread is known, the amino acid intake of rats on this diet can be calculated. The rats had an average intake of 9 grams each day of the diet that had 90% bread, or 8.1 grams of bread each day. Using the amino acid content of bread (table 13) the intake of each individual amino acid was calculated. The result of this calculation shows that the amino acid requirement of the rat is not met by a bread diet.

J. C. Winter (1947) in a feeding experiment with rats fed the animals freshly prepared human diets to determine the effect of bread enrichment. She concludes "these adverse

factors (sterility of females, failure of survival of young and many still-births and the young beingeaten by their mothers) rendered statistical comparison of the effects of enriched versus unenriched diets impossible. No significant effect of enrichment could be obtained. Such human diets appear highly defective for nutrition of rats during the reproductive cycle".

She concluded therefore, that the human diet under study was not satisfactory for the rat. There is a possibility of food spoilage as a result of enzymatic changes that may have occurred in the fresh moist human food left in the rat cages for an indefinite period of time. If she had used a dried sample of human food this problem of changes, resulting from enzymatic or microbial activity in the food would have been minimized. Nevertheless, Winters made no attempt to evaluate the diet she tested in terms of man's nutrient requirements.

In view of the above calculations we can conclude that the rat is not a satisfactory animal to use for studies of the extent or degree to which human diets may meet man's requirement for amino acids. The studies of McCance and Widdowsen confirm this statement. They studied children in German orphanages who drew most of their caloric intake from bread. They concluded that diets consisting largely of breads and vegetables provided the children with double the amount of all essential amino acids (except methionine) for nitrogen equilibrium.

Improvement of Iranian bread may be possible as far as minerals and vitamins are concerned. Since no calcium or vitamins are added to the bread, such supplementations are important since the Iranians are not accustomed and do not have access to milk and milk products. The possibility of calcium and vitamin deficiency occurring among the Iranians is great. Supplementation of bread with calcium and needed vitamins, especially B vitamins, would be an excellent way to incorporate these daily essentials into the diets of the middle and lower economic classes of people in Iran.

GENERAL SUMMARY AND CONCLUSIONS

Bread provides about 70% of the calories for a large proportion of the people in Iran. The bread used by the Iranian people is made with a flour that is comparable to the 72% extraction flour used in the United States. Since studies by many other investigators showed that the nutritional value of the protein in wheat and bread is poor, it appeared desirable to determine how the Iranian bread might best be improved. To be adopted by the people of Iran, any proposed improvement would have to utilize other foods that are readily available in Iran.

To this end, two feeding studies were carried out with rats. They were done to compare the nutritional value of bread baked in Iran with American white enriched bread and a Syrian-Lebanese bread secured from local grocery stores. In the second experiment, a study was made of skim milk powder and lentils as supplements for improving the nutritional value of Iranian bread.

Four groups of 10 male weanling rats were used in each experiment. In the first experiment, the nutritional values of breads from Iran, Syrian-Lebanese and American white enriched bread were compared. All animals were sacrificed at the end of 4 weeks. Body weight gain and food intake were measured and recorded. The livers of the animals were used for the following analyses: liver xanthine oxidase activity

was determined on a part of the livers from 5 rats in each group. The remaining liver from these animals and the livers from the rest of the animals were dried to a constant weight (for moisture content) and ground to a fine powder.

In the second experiment, the same number of rats was used as in Experiment I. This experiment was designed to evaluate the effectiveness of skim milk and lentils as supplements to the Iranian bread. For these studies, the control group of rats was fed a ration containing 90% of Iranian bread plus adequate amounts of minerals and vitamins. Other groups of rats received similar rations in which a part of the bread was replaced with 4% skim milk powder, or lentils at either a 7.5 or 15% level. The changes in body weight and food consumption were measured for a 3 week period. Chemical analyses similar to those described for the first experiment were carried out with this group. In addition thereto, hemoglobin levels were determined on blood samples taken from all rats prior to sacrifice.

The results of the first experiment showed that Persian bread, despite having no nutrients other than flour in significant amounts, has the same nutritional value as American white bread containing 2% skim milk powder. This was shown by the rat growth studies. Syrian bread was inferior as compared with Iranian and American bread. In the second experiment, it was shown that the nutritional quality of protein in Iranian bread can be improved by supplementing it

with 4% dried skim milk powder or 7.5 and 15% lentil powder as far as the growth of rats is concerned. The growth promoting effects of the breads increased with the level of supplementation when lentils were used.

After the biological studies were completed, the contribution of Iranian bread to the amino acid requirement of adults was calculated. This calculation was based on the assumption that the amino acid pattern of Iranian bread is similar to that of American enriched white bread. Furthermore, it was assumed that 70% of a 2500 calorie intake would come from bread. Under such circumstances, it could be shown that an adult would receive all essential amino acids in adequate amounts from the bread. The amounts of amino acids provided by the bread according to these calculations equalled the "safe" levels suggested by W. C. Rose for adults.

On the basis of these calculations, it appears desirable to evaluate the adequacy of bread as a source of amino acids for human subjects. An extension of this problem should be directed that way.

This study emphasizes the fact that although rations may be inadequate for supporting normal growth of rats, such results may not be applicable to man himself.

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