PROTEIN QUALITY OF THREE WHEAT MILL STREAMS

by

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B. S., Kansas State University, 1964

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1965

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INTRODUCTION

The world is observing an increasing shortage of foodstuffs, especially in less-developed countries where technological improvements have not kept pace with population growth. The Third World Food Survey by the Food and Agriculture Organization of the United Nations (1) indicated that 10-15% of the world's people were undernourished and that 50% suffered from malnutrition. Undernutrition refers to a lack in quantity of food, that is, calorie intake; malnutrition refers to a lack in quality of food and denotes inadequacy of specific essential nutrients. It was estimated that 60% of the population in less-developed areas was malnourished; that is, their diets were often lacking in high-quality protein, vitamins, minerals and/or fat. Deficiency in protein was named as the foremost problem.

In the FAO survey (1), a relationship was found between calories in the diet and source and intake of protein. Less-developed countries with low calorie diets (2000-2500 cal/day/person) had an average protein intake of 58 g, of which 9 g came from animal sources. Developed countries with high calorie diets (3000 cal/day/person) had an average protein intake of 90 g, of which 44 g came from animal sources.

Scrimshaw (2) stated that the classical form of severe protein malnutrition in children (kwashiorkor), a result of defective feeding of the weaned infant, is a common problem in almost every underdeveloped country and territory in the world. According to Mitchell (3), scientific literature abounds in

studies of kwashiorkor resulting from severe protein deficiency. However, it is often forgotten that there must be an intermediate and large group of children and adults in many countries who receive enough protein to prevent observable deficiency symptoms, but not enough to permit normal growth and maintenance. Although diets in the United States generally are adequate in protein, there is need even in this country for improvement. Watts (4) used the diets of farm families with median income in the North Central and Southern parts of the United States and the diets of homemakers in four cities as the basis of an evaluation of protein in American diets. The diets of the farm families were found to be adequate for maintenance in adults, but inadequate for growth in children. The diets of homemakers in Minneapolis and San Francisco were adequate for both growth and maintenance, but in Birmingham and Buffalo were inadequate for growth.

Mast (5) observed that people in many parts of the world now live on a diet composed largely of cereals, and that one of the most important subjects needing attention is the evaluation of the proteins in a high cereal diet. Wheat is one of the world's major cereals and the principal food cereal of Europe, America and parts of the Middle East. Pyler (6) reported that wheat may contribute approximately 25% of the total calories in the United States diet and from 50-80% of the total calories in the European diet. In the United States, flour and cereal products contribute about 20% of the protein in the diet (7); but in the Middle East, they may contribute 50-75% (1). Shellenberger (8) stated that since 1930, the per capita consumption of wheat in the United

States has dropped from 4.1 to 2.5 bushels; however, total consumption is increasing because of population growth.

Wheat is the second largest field crop in the United States and Kansas is the leading producer of wheat (6). Most of the wheat is milled to flour and during the milling a by-product, millfeed, is produced. Millfeed is a combination of several mill streams which include red dog, bran, shorts and germ. It is sometimes used to feed livestock, but more often is not used adequately for any purpose. In view of the need for more protein foods and the fact that vast quantities of wheat are milled each year, a better way to utilize the by-products of milling needs to be found. Mast (5) emphasized that experiments must be undertaken to determine to what extent common and special wheat foods can supply the protein needs of human beings of all age groups. Before the by-products of milling can be utilized in special foods, it is essential to investigate their properties and nutritive value. The purpose of this experiment was to determine the quality of the protein present in three wheat mill streams as compared to that of flour.

REVIEW OF LITERATURE

Background Information about Wheat

World wheat production. According to Jacob (9), wheat is a relative of the grasses and in its wild form existed in Abyssinia before the dawn of history. The cultivation of wheat began in areas of Asia Minor and Egypt along the fertile crescent, at or

before 6000 B.C. Over a period of centuries, it spread from these regions to Eastern Asia, Europe, the Americas and parts of Africa.

In 1962, the total world production of wheat was approximately 8.67 billion bushels (7). Of this, 1.7 billion came from North America, 1.6 billion from Western Europe and 2.0 billion each from the U.S.S.R. and Asia. The United States, Canada, Argentina, Australia, France, Italy, the U.S.S.R. and Red China are some of the world's major producers of wheat. Of the 1.09 billion bushels of wheat produced in the United States in 1962, approximately 500 million bushels were used for human consumption.

Pyler (6) explained that wheat grown in the United States belongs to three distinct botanical species, but by far the most important of these is Tricitum vulgare or common wheat, which comprises nearly 95% of the total production. There are more than 200 distinct varieties of common wheat grown. These varieties are changing constantly as better ones are developed by plant breeding. They are bred to produce certain characteristics and to be resistant to many adverse conditions.

Winter wheats are planted in the fall in moderate temperature regions and harvested in the late spring. Spring wheats are planted in the spring in colder temperature regions and harvested in the late summer. Hard red winter wheat is grown in 30 states, especially in Kansas, Nebraska, Oklahoma and Colorado. Hard red spring wheat is grown in 23 states, especially in the Dakotas and Minnesota. More than twice as much hard red winter wheat is produced as hard red spring wheat. In 1962, the

percentages of total wheat production were as follows: hard red winter wheat, 49%; hard red spring wheat, 14%; soft red winter wheat, 16%; hard and soft white wheat, 14%; and durum, 7% (10).

Hard red winter and hard red spring wheats are used primarily for bread. Soft red winter wheat is used for cakes, hard and soft white wheat for crackers and pastry, and durum for macaroni, noodle and spaghetti products (6).

Milling of wheat. Pyler (6) stated that the wheat kernel is differentiated into three distinct parts; the branny covering, the germ and the endosperm. The bran is the protective covering of the grain, the germ is the plantlet which upon germination develops into a new plant, and the endosperm constitutes a relatively large reservoir of food for the growing seedling. The bran coat consists of several distinct layers. The outer bran layers constitute the pericarp or dry fruit coat and have a rather tough texture, due to their high content of fiber. This fiber facilitates their removal from the endosperm during milling. The inner bran layers are considered part of the seed proper and contain the red-brown pigments which impart some of the color to red wheats. The aleurone layer, which is next to the endosperm, consists of large, heavy-walled, starch-free cells. Although forming the outer layer of the endosperm, it adheres to the perisperm or inner bran layers and is removed with the bran during milling. The embryo or germ lies at the base of the grain on its rounded side. The scutellum is a layer of epithelial cells around the germ whose function is to secrete enzymes during germination and to absorb and conduct to the growing embryo the

food material from the endosperm. By far the greatest proportion of the wheat kernel is comprised of the endosperm which consists chiefly of starch embedded in a matrix of protein. The purpose of milling wheat is to separate as much as possible of the branny covering and the germ from the endosperm, and to pulverize the endosperm into flour.

Pyler (6) reviewed the various steps in the milling process, including blending, cleaning, tempering and grinding. Because of the variations in quality, wheats of different varieties and from different sources are blended to yield a flour whose baking characteristics are uniform. After the wheat has been blended, it is cleaned and tempered before the actual grinding. Cleaning is necessary to remove any weed seeds, other cereals, dirt or chaff. Tempering is to adjust the moisture content of the kernel's component parts, so that the easiest, most efficient separation of bran and germ from endosperm can be made.

After tempering, the wheat is subjected to a series of grinding operations, of which the first five exert a crushing or shearing action. This is known as breaking and designed to bring about separation of the tough bran from the friable endosperm. The first part of the grinding process is carried out on corrugated iron rolls which revolve in opposite directions at different speeds. The corrugations on the rolls become finer and the setting of the rolls progressively closer as the wheat proceeds toward the fifth break. The first break crushes the wheat into coarse particles and loosens some of the bran. The crushed material passes into a sifter or bolter, and the coarsest

fragments are retained on the top sieves and conveyed to the second break. The medium-sized granular particles (middlings) consisting chiefly of endosperm are separated, and the finest material (break flour) passes through the bottom of the sifter. This process is repeated with less and less endosperm present until after the fifth break, the remaining material consists largely of bran flakes. The middlings are composed of endosperm fragments, with some admixture of bran particles and germ. The middlings next pass into the purifier where by sifting and air aspiration, they are freed as much as possible from the bran. After purification, they are gradually ground into flour between smooth reduction rolls which subject them to a crushing and rubbing action. This produces finer middlings and flour. The germ may be separated from the flour at this point.

These series of breaks and reductions give rise to many mill streams, which differ in degree of refinement. Although the wheat grain is approximately 83% endosperm, with 15% bran and 2% germ, generally only 72-75% of the kernel is extracted as flour. That is, for every 100 lbs. of wheat milled, 72-75 lbs. of flour are produced. Straight grade flour contains all of the 72-75% extraction of the wheat. However, the 72-75% extraction may be divided into three fractions. Patent flour is the whiter, more refined fraction; first and second clear are the darker fractions. Red dog is the residue left on the last flour cloths of the sifter after the final reduction in the milling process. Fine bran usually is obtained from the last of the five breaks during the first phase of milling. Germ stock (scalp) contains the

wheat germ and some endosperm and bran.

Composition of wheat. The average composition of wheat, flour and other mill streams varies widely with environment, variety and fertilizer treatment. Since they are derived from different parts of the kernel, various mill streams from the same wheat differ in chemical composition. Shollenberger et al. (11) reported that the percentage composition of United States wheats might vary as follows:

Protein	6.00-21.00
Moisture	6.00 & up
Fat	0.28- 2.50
Crude Fiber	1.70- 3.72
Ash	1.40- 2.35
Nitrogen Free Extract	66.00-76.00

According to Kent-Jones (12), the following compositions may be found in hard wheat, flour, bran and germ:

	Wheat %	Flour %	Bran %	Germ %
Moisture	9-18	13.0-15.5	10.8	9-13
Protein	8-15	8.0-13.0	11.3	22-32
Fat	1.5-2.0	0.8- 1.5	4.7	6.0-11.0
Fiber	2.0-2.5	0.0- 0.2	13.6	1.8- 2.5
Ash	1.5-2.0	0.3- 0.6	4.8	4.0- 5.0
Sugar	2-3	1.5- 2.0	---	---
Starch	60-68	65-70	---	---

Protein enrichment. In 6 flours with protein contents ranging from 6.7-14.0%, it was found, by McDermott and Pace (13), that as protein content of flours increased, the proportion of lysine in the protein decreased. Yang et al. (14) studied the effect of various levels of lysine supplementation on the nutritional value of wheat flour proteins in rat diets. Growth data and biological values indicated that the nutritional value of the flour proteins was improved by lysine supplementation up to a level of 0.20 or 0.25%, but adverse effects appeared when the diet was supplemented with 1.0% lysine. Clark and Kennedy (15) observed a linear relationship between either the gain in body weight or increase in body nitrogen and the amount of lysine (available) ingested. Heat treatment in baking and roasting was thought to cause some loss of lysine.

From a nutritional standpoint, it is unfortunate that the germ of the wheat kernel must be removed during milling, since it contains 25% protein, 8-11% oil and 15% sugar (16). The nutritional value of flour could be raised considerably if the wheat germ could be retained or added back after milling. Wheat germ in flour has two disadvantages: first, it is likely to develop rancidity because of the fat content and enzymes present; and second, it adversely affects the flour's baking quality due to the reducing substance (glutathione) present. These disadvantages can be overcome by removal of the fat and inactivation of the enzymes and glutathione.

Westerman et al. (17) determined the nutritive effect on rats of adding 2, 4, and 6% levels of wheat germ to enriched and

nonenriched flour. Addition of 2% wheat germ did not increase the growth rate above that obtained with enriched flour alone; but with the addition of 4 and 6% wheat germ, growth was increased. At all three levels, the addition of wheat germ to nonenriched flour produced an increase in growth. Reproduction and lactation showed similar trends to growth. The largest number of litters and the highest survival rate were produced by enriched flour with 4% wheat germ. In a subsequent study, Westerman et al. (18) found that either soya flour or wheat germ was beneficial in promoting growth in rats when added to nonenriched flour, but neither was as good as the stock diet. When either supplement was added to enriched flour, growth was equal to that on the stock diet.

Rand and Collins (19) stated that white flour was inferior in protein content to whole wheat flour, and demonstrated the beneficial effects of supplemental wheat germ on the protein content of white flour. The protein quality of defatted wheat germ was shown to compare favorably with that of high-quality fish meal. Supplementation with 10-15% defatted wheat germ produced striking improvements in the nutritive value of enriched wheat flour. Pomeranz (20) found high and consistent correlations between the quantity of protein supplement added to flour, and the levels of protein and lysine in baked bread. When 10% wheat germ was added, the protein content of the bread increased from an unsupplemented level ranging from 13.8-15.1% to 15.9-17.1%.

Several foreign countries are interested in wheat germ as a source of enrichment. An Italian nutrition program studied by Balliette et al. (21) indicated that the protein content of bread and alimentary pastes was increased by use of white flour (70% extraction) supplemented with 10% wheat germ, and that the flavor of these products was acceptable. However, Parks et al. (22) found that bread containing soy flour and wheat germ was one of the least acceptable from a flavor standpoint, even though it had the most protein per pound of several types of bread baked in their laboratory.

Importance of Protein in the Diet

Growth and maintenance. Proteins are the basis of protoplasm and a component of every body cell (23). Since proteins cannot be synthesized by the animal organism from atmospheric nitrogen or inorganic nitrogenous compounds, protein foods are the only source of the nitrogenous complexes needed to build protoplasm and living tissue. Osborne and Mendel (24) indicated that the first function of protein is maintenance and the second, growth. Protein ingested above the amount needed for the repair of the normal breakdown of the body is used for growth.

After protein in the diet is consumed by an animal, it is broken down to the constituent amino acids and the amino acids are absorbed (23). The absorbed amino acids are the units that form the building blocks with which the animal synthesizes its own protein structures for use in protoplasm.

Labile protein reserves. According to Allison (25), amino acids are organized into cellular proteins by anabolism and cellular proteins are broken down to their component amino acids by catabolism. The body does not store protein in the same way it stores carbohydrate and fat, but holds it in a dynamic state. This dynamic state was characterized by Whipple (26) as a type of "metabolic pool" that amino acids are shifted in and out of, as they are required. Allison (25) remarked that this "metabolic pool" should not be considered as a disorganized fluid component, but as a mechanism for transfer of amino acids from tissue to tissue. These tissue proteins have been called the labile "protein reserves" or "protein stores" (26).

Halac (27, 28) disagreed with the concept of protein stores as it often is used. He found that animals on a high protein diet had less resistance to stress conditions and protein deprivation than those on a medium protein diet. Observations on growing rats indicated that diets containing 15% protein were at the optimum level for resistance to protein deprivation. Diets containing less than 15% protein were in the range of protein undernutrition; diets over 15% protein failed to improve resistance to protein deprivation, since the extra protein was not stored. Halac stated that his studies showed no support for the idea that an increase in protein intake will form protein reserves to protect against future deprivation. It seemed more likely that the body maintained a constant nitrogen composition in the face of elevated protein intake by increasing the rate of protein turnover.

Holt et al. (29) suggested two fallacies in interpreting nitrogen retention as "protein reserves." First, they pointed out that when an individual's protein intake is below his minimum requirement, he lives on his own tissues, which are sacrificed in such an order that the most essential ones are preserved to the last. This could be regarded as a reserve against depletion, but not as compartments which may be filled with stored protein. Secondly, they did not interpret nitrogen losses under stress as the outpouring of protein stores; all the components of tissue are lost, not protein alone. Thus, these are not protein reserves, but tissue reserves.

Liver proteins. A deficiency in quantity or quality of protein affects the liver in two ways: first, a decrease in protein and second, an increase in fat deposition. Kosterlitz (30) reported that liver weights increased in rats on a high protein diet and decreased on a protein deficient diet. Protein content of the livers was reduced as dietary protein decreased, but fat content increased in some instances. Addis et al. (31, 32 and 33) observed that the various organs of the body exhibited different rates of decrease in protein when dietary protein decreased. Livers of rats fasted for 7 days lost 40% of their original protein; whereas the kidneys lost 20%; the heart, 18%; and all other organs, 4%. Each organ also had its own rate and degree of rebuilding of protein. The effect of various dietary proteins on regeneration of depleted liver proteins was studied by Harrison and Long (34). The vegetable proteins, zein and gliadin, produced less regeneration of liver proteins than the

milk proteins, casein and lactalbumin; however, gliadin was better than zein. Allison et al. (35) reiterated that proteins of the liver rise and fall rapidly as dietary protein intake increases and decreases. He observed a rapid loss of liver proteins on a protein deficient diet. Since protein content of the liver correlates with protein in the diet, Henry et al. (36) suggested the use of a liver protein method for the assay of nutritive value of proteins. When their method was tested, the relative values for several types of protein agreed with the values found by nitrogen balance and growth methods.

Kosterlitz (30) found that in the first few days on a protein deficient diet, liver cells contain more fat than normal. A report of Williams (37) might explain why this was observed in the first few days. The ratio of liver weight to body weight increased markedly when rats were placed on a protein deficient diet. The nitrogen concentration per mg of liver decreased in protein deficient rats during the first 30 days, and then slowly began to rise. Perhaps, glycogen and lipids were deposited during the first 30 days, and then the glycogen and lipids were lost from the liver cells and used for energy, which resulted in a relative increase in nitrogen. Winje et al. (38) stated that fat which accumulated in livers of rats fed low protein diets was reduced when dietary protein was increased. They believe that maintenance of normal fat deposition in rats on low protein diets probably depends on the specific ratio of amino acids in the diet, especially lysine, threonine and methionine. The Food and Nutrition Board (39) reported that deficiency of at least three amino

acids, threonine, lysine and tryptophan, resulted in fatty infiltration of the liver with the degree of alteration dependent on the degree of deficiency. Partial restriction produced more alteration than total deprivation. According to Harper et al. (40), accumulation of fat in the liver is one of the signs of kwashiorkor. These investigators think that it is important to study liver fat deposition in rats fed low-protein diets composed of cereals. They found that when rats were fed a low-protein polished rice diet, fat accumulated in the livers up to 8-10%.

Measurement of Quality

The quality of proteins can be measured both by direct analysis of amino acids in the protein and indirectly by analysis of utilization of the protein by the body.

Amino acids. Proteins are made up of more than 20 amino acids, of which 8 are considered essential for human beings and 10 for rats. These essential amino acids cannot be synthesized by the body at the required rate. Thus, the body must obtain them from the diet so that new tissues can be formed.

Reference patterns. The National Research Council (41) stated that the nutritive value of a dietary protein depends on the pattern and quantity of essential amino acids it presents to the body after absorption from the intestine. Assuming that chemical analysis of a dietary protein reveals the pattern of amino acids liberated in and absorbed from the gastrointestinal tract, an estimate of the nutritive value can be made by comparing the amino acid pattern with a reference pattern.

The National Research Council (41) indicated that the reference pattern for a given age, sex and energy expenditure should be the pattern which will allow maintenance of nitrogen balance in the adult or normal growth in a growing subject with a minimum nitrogen intake. Ideally, the pattern should be completely utilized for anabolism; that is, it should possess a net protein utilization and biological value of 100. The reference pattern may be stated as amino acid ratios with tryptophan as unity or in terms of amino acid concentration per unit of nitrogen. Some of the disadvantages of ratios are the difficulty of finding an acceptable amino acid to use as the reference standard, the demonstration that two proteins of dissimilar nutritional value can have similar amino acid ratios and the complications that result if the amino acid that is selected as the reference is adjusted.

Oser (42) stated that although human milk had been suggested and supported by various investigators as the reference protein, he preferred to follow the example of Mitchell and Block (43) and adopt whole egg protein as the reference. Egg is utilized as completely by the rat, the dog and man as any food protein and is not significantly enhanced in biological value by supplementation with any of the amino acids.

The FAO provisional reference pattern was derived from experimental values for the requirements of mature human subjects and infants for individual amino acids, each a part of a mixture of free amino acids (41). However, the experimental values were too few in number and too limited in scope to permit anything but

a very tentative formulation of the reference pattern. There are several advantages and disadvantages to the FAO provisional reference pattern. Some of the advantages are that it can be tested and modified, revised to indicate both minimal and maximal amounts of each amino acid and expressed either as a ratio with one amino acid as base or as mg of each amino acid per g of nitrogen. The disadvantages include: the pattern is provisional; no attention is paid to essential amino acid concentrations greater than those in the pattern; no consideration is given to imbalance and the ratio of essential to nonessential acids is set in the pattern.

According to Mitchell and Block (43), a high correlation was observed between percentage deficits of limiting essential amino acids and the biological value of a protein. They devised a system of "chemical scores" based on the amount of the essential amino acid in greatest deficit in a protein compared to the level present in a reference protein selected for its nutritional excellence. Despite their empirical nature, chemical scores were shown by Mitchell (44) to have a high degree of correlation with published biological values for a series of proteins. However, since it is assumed that the absence of an essential amino acid renders a protein completely unavailable for tissue maintenance (an assumption not entirely accurate), the chemical score is only an index of the value of protein for growth.

Oser (42) defined the Essential Amino Acid index (EAA) as the geometric mean of "the egg ratios," the ratios of the essential amino acids in a protein relative to their respective amounts

in whole egg protein. The EAA index has been calculated for many proteins and good correlations were found with other methods of determining nutritive value. The correlations between the EAA index and the biological value were so consistently high that more reliable estimates of biological value could be made from essential amino acid assay than from biological assay with rats. However, it is necessary to recall that this method of estimation is based on the idea that all the amino acids are available to the animal. Microbiological and chromatographic determinations of amino acids do not take into account the digestibility and absorption of the proteins, or any factors such as insolubility or heat treatment that might impair the degree or rate of digestion and absorption. Sheffner and associates (45) attempted to overcome this difficulty by basing the computation of the index on the essential amino acids in enzymatic digests of proteins rather than on the total essential amino acid content in complete hydrolyzates. They found that in two instances, Labco casein and white flour, the results of the index computation were significantly altered, indicating some impairment of digestion and utilization of these proteins.

Amino acid imbalance. Harper (46) explained the difference between amino acid balance, unbalance and imbalance. A protein that provides amino acids in roughly the proportions needed by the body is termed a balanced protein and has a high biological value. A protein that is low in one or more of the indispensable amino acids is termed an unbalanced protein and has a lower biological value. The term amino acid imbalance has arisen from

studies of adverse effects, beyond the expected fall in the net protein utilization, which have been observed when a protein in the diet has been thrown out of balance by the addition of amino acids or a quantity of an unbalanced protein. Amino acid imbalance is defined as a change in the proportion of the amino acids in a diet that results in an adverse effect which can be prevented by supplementing the diet with a relatively small amount of the most-limiting amino acid or amino acids. This excludes toxicities and antagonisms, which are caused by addition of a large excess of a single amino acid and are not prevented by a relatively small supplement of the most-limiting amino acid. Harper (46) and Kumta and Harper (47) found that amino acid imbalance could consistently be produced by adding a large quantity (3-20%) of a protein or an amino acid mixture with a single amino acid lacking to a diet adequate in amount of protein. Occasionally, a small supplement (0.2-1.0%) of one or two amino acids has been found to cause severe imbalance.

Munaver and Harper (48) obtained evidence that wheat gluten was so poorly balanced in amino acids that the lysine requirement of the rat for maximum growth was increased when this protein was the sole source of lysine in the diet. The digestibility of the proteins in wheat gluten were about 95% and this was unaffected by the level of wheat gluten in the diet over a range from 30-70%. It was concluded that wheat gluten, due to its low level of lysine, was so severely unbalanced that the lysine it contains was not completely utilized. The growth retarding effect of the excess of amino acids from wheat gluten was prevented by the

addition of lysine.

Further studies on the subject of imbalance by Harper and his associates (49, 50, 51, 52, 53, 54) have shown that no adverse effect of poor amino acid balance could be detected if the food intake of the animal on a diet with a poorly balanced pattern of amino acids fell to the point at which the animal consumed only as much protein as it could metabolize efficiently.

Utilization of protein. Mitchell (55) stated that determination of the nutritive value of a protein is a study of the nitrogen economy of animals that are fed that protein. Net protein value can be determined by two methods: first, the ratio of gain in body weight to protein intake of growing rats and second, the gain in nitrogen in the bodies of young rats fed diets complete in everything except protein.

Growth. According to Frost (56), growth or gain in body weight has been the most general and widely used criterion of protein value. However, body weight gain is not entirely a good measure, because it also is produced by other constituents, such as fat and water. In 1919, Osborne et al. (57) introduced the concept of "protein efficiency ratio" as a refinement of the simple growth method. PER (protein efficiency ratio) was defined as the g gained in body weight per g of protein or nitrogen ingested. For each test protein the maximum amount of body weight gain per g of protein consumed was determined in growing rats and generally the better the protein, the lower the level in the diet required to produce the highest PER. PER also varied with the level of protein in the diet. As the protein intake

increased, the PER increased because more of the dietary protein was available for growth. The National Research Council (41) stated that the factors affecting PER assay are age of rat, length of assay period, level of protein and sex of rat. Best conditions of PER assay are a four-week assay period, diets containing 10% protein and providing sufficient amounts of all other essential nutrients, male rats and ad libitum feeding. Using these conditions and specified test diets, Derse (58, 59) reported that reproducible results can be obtained between laboratories.

Bender (60) investigated the relation between PER and NPU (net protein utilization). NPU is the proportion of injected nitrogen that is retained, or the product of biological value and digestibility. PER correlated closely with food intake, but NPU was independent of food intake. Synthetic amino acids cut consumption of the diet, and reduced consumption depressed PER, but not NPU. PER of the proteins correlated highly with NPU. Bender and Doell (61) modified the PER to a PRE (protein retention efficiency) and found an extremely high correlation between PRE and NPU as determined by carcass analysis. PRE was independent of food intake and included maintenance requirement as well as growth. Bender and Doell concluded that body weight was a reasonably accurate index of body protein in young growing rats.

Biological value. Frost (56) stated that in 1909, Thomas proposed a method for determination of the biological value of a protein in terms of percentage of digestible nitrogen retained by an adult from a test food. First, the minimum nitrogen output

occurring during a period of nitrogen starvation was determined; then, a known amount of food containing a known amount of protein was consumed and the increased nitrogen output was measured. Later, Mitchell (62) applied the Thomas method to growing rats and determined the requirements for both growth and maintenance. This method of determining the biological value of protein is still used and is known as the Thomas-Mitchell nitrogen balance method. Fecal nitrogen on a protein-free diet was demonstrated to be directly proportional to the amount of dry food consumed by animals. Hence, fecal nitrogen figures should be corrected by a factor obtained by determination of the quantity of fecal nitrogen excreted per g of dry food consumed during a protein-free diet period. The nitrogen in the feces represents the undigested part of the protein food and some metabolic nitrogen. It is necessary to subtract the metabolic nitrogen because it consists of nitrogen substances that originated in the body, such as residues of the bile, digestive juices, bacterial residues and epithelial cells from the lining of the alimentary canal. Metabolic nitrogen can be determined, according to Mitchell and Bert (63), by measuring the fecal nitrogen from animals fed a low nitrogen or a protein-free diet. A linear relationship was found between the dietary protein and fecal nitrogen. Urinary nitrogen is divided in two parts, similar to fecal nitrogen. Endogenous nitrogen represents nitrogen excreted in the urine when an individual is on a nitrogen-free diet (64). The difference between urinary nitrogen excreted during the protein-test period and during the protein-free period represents nitrogen arising from

the food. Thus, biological value may be calculated as the percentage of the ingested nitrogen that was not eliminated in the urine or the feces.

Two assumptions are involved in the above method of determining biological value (62). First, the metabolic nitrogen of animals is the same, whether they are on a protein-free diet or a protein-test diet. Second, the endogenous nitrogen that occurs in the urine during the nitrogen-free diet period continues at a constant level when a protein diet is fed.

Certain conditions are essential for an accurate measurement of the biological value (62). The diet should contain only the protein or mixture of proteins being tested, and no other protein or nitrogen source. Food intake should be sufficient so that the dietary protein will not be used as an energy source. Forbes and Yohe (65) observed a low biological value for a protein fed to animals on a low intake of energy.

Forbes et al. (66) reported that biological value of proteins, except egg, decreased linearly as the protein level increased above 4% of the diet. Henry and Kon (67) found that rats used protein for growth more efficiently when fed protein levels below 12% of the diet.

The National Research Council (41) emphasized that for certain unheated materials, especially cereals and cereal by-products, lysine is partly unavailable. Mitchell et al. (68) demonstrated that heat treatment of leguminous seeds would increase their biological value and Yang et al. (69) showed that nitrogen retention by rats was greater from cooked than from raw

rice. Rand and Collins (19) showed that mild heat treatments, such as steaming or light toasting, enhanced the protein quality of defatted wheat germ. However, heat may damage the protein of some products and lysine may become less available (41). Mild heat treatment of materials rich in carbohydrate reduced the nutritive value of the protein, but the value was restored by addition of lysine. A decrease in available lysine was observed when protein concentrates containing little or no carbohydrate were subjected to severe heat. Severe heat treatment of protein concentrates was shown to reduce the availability of the sulfur-containing amino acids. Clark and Kennedy (15) also observed that loss in lysine might result from certain types of heat treatment.

EXPERIMENTAL PROCEDURE

Flour and Mill Streams

The enriched flour and 3 mill streams used in the study were milled from a mixture of hard red winter wheat varieties by the Abilene Flour Mill, Abilene, Kansas. The flour was a 67% extraction, medium patent flour. The fine bran represented approximately 10% of the wheat kernel; the red dog, 2% and the scalp (germ), 1%. The red dog was slightly larger than the flour in particle size and a little more granular. The fine bran and scalp were in small flakes.

The proximate composition of the wheat, flour and mill stream as determined by the method of A.O.A.C. (70) is presented in table 1.

TABLE 1

Proximate composition of wheat, flour and 3 mill streams[1]

Nutrient	Wheat	Flour	Red dog	Fine bran	Scalp
	%	%	%	%	%
Protein[2]	10.80	10.83	12.54	14.93	15.68
Fat[3]	1.67	1.28	2.47	3.04	3.97
Crude fiber	2.42	0.33	1.72	8.25	5.92
Moisture	13.77	12.34	10.76	12.94	13.31
Ash	1.53	0.43	1.48	4.85	3.68
Nitrogen-free extract[4]	67.67	73.74	69.82	54.54	55.93
Carbohydrates[5]	70.09	74.07	71.54	62.79	61.85

[1]Analysis by S. N. Rodgers, Chemical Services, Kansas State University.

[2]Factor used for conversion of nitrogen into protein, 5.70.

[3]Ether extract of fat.

[4]Nitrogen-free extract is protein, fat, crude fiber, ash and moisture subtracted from 100.

[5]Carbohydrate is nitrogen-free extract + crude fiber.

The analyses of 17 amino acids and the ammonia in the wheat, flour and mill streams were done on a Spinco (Model 120) Amino Acid Autoanalyzer, which utilizes ion exchange chromatography.

Diets

The percentage composition of the low nitrogen diet and four experimental diets is given in table 2.

Fresh whole eggs were used as the source of protein in the low nitrogen diet. The low nitrogen diet was planned according to the method of the A.O.A.C. (70) to contain protein, 4%; fat, 8%; ash, 5%; fiber, 1%; vitamin mixture, 1%; water, 5% and carbohydrate to make 100%.

The flour and mill streams were used as the sources of protein in the experimental diets formulated for the four groups of animals as follows: I, flour diet; II, red dog diet; III, fine bran diet and IV, scalp diet. The experimental diets were planned as a modification of the method of the A.O.A.C. (70) to contain protein, 10%; fat, 8%; ash, 5%; fiber, 5.5%; vitamin mixture, 1%; water, 11.4% and cornstarch to make 100%. It was necessary to supplement the flour protein with wheat gluten (76.7% protein) to bring the level of dietary protein up to 10%. Because of the high fiber content of the bran and scalp, all of the diets were adjusted to a fiber level of 5.5% rather than the recommended level of 1%. The moisture content of the flour and mill streams made it necessary to adjust the water level to 11.4% rather than the recommended 5%.

TABLE 2

Percentage composition of the low nitrogen diet and 4 experimental diets

Ingredient	Low N diet	Experimental diets			
	Egg	Flour	Red dog	Fine bran	Scalp
	%	%	%	%	%
Protein	31.01	78.48	66.98	79.74	63.77
Gluten[1]	----	1.96	----	----	----
Cottonseed oil	4.43	7.00	5.96	6.03	5.47
U.S.P. XIV salt	4.69	4.66	1.75	3.82	2.65
Vitamin mixture	1.00	1.00	1.00	1.00	1.00
Cellulose[2]	5.52	5.26	----	4.15	1.74
Water	----	1.71	2.73	2.81	2.90
Cornstarch	53.35	----	21.58	2.45	22.47

[1]Wheat gluten was added to the flour diet to bring the protein level to 10%.
[2]Alphacel.

The wheat mill streams were used in the raw state and no additional grinding was done after milling. The vitamins were weighed on a Mettler analytical balance (type B6), combined and added to the diets before mixing. The diets were mixed in a 20-quart Hobart electric mixer (Model A-200-D) for 1 hour at low speed and stored in a household refrigerator. Percentage composition of the experimental diets is presented in table 2. Analysis of the diets for total nitrogen showed that the low protein diet contained 4.38% nitrogen. The flour diet contained 9.61% nitrogen; the red dog diet, 10.30%; the fine bran diet, 10.61% and the scalp diet, 10.39%.

Animals and Their Care

Twenty-four weanling male albino rats of the Sprague-Dawley strain were divided into four groups with similar total weights. The animals were randomly distributed (table 10, Appendix) in individual metabolic cages (8" x 4½" x 2¼") in a room maintained between 24-25°C. Front and back views of the cages (Acme Metal Products, Inc.) are shown in fig. 1. Water bottles and feeders were provided at the back of each cage. The feeders were adjusted to animal size to minimize feed spillage. Food and water were given ad libitum with the feed cups filled every morning and evening. The arrangement of baffle and feces cup in the removable collection funnel provided at the bottom of each cage separated the urine from the feces.

Records were kept of food intake, water consumption and weight gain on the basis of the following periods:

EXPLANATION OF FIGURE 1

Metabolic cages

Top: Front view

Bottom: Back view

Experimental Period I
7-day adjustment
4-day collection

Low Nitrogen Period
3-day adjustment
4-day collection

Experimental Period II
6-day adjustment
4-day collection

Samples

Urine. Urine was collected in 500-ml Erlenmeyer flasks kept under each metabolic cage. Each day of the collection period, the urine in the flasks was transferred to pharmaceutical bottles containing 2 ml of toluene as a preservative. The flasks were rinsed with distilled water and the rinsings added to the bottles. The urine bottles were stored in the refrigerator. At the end of each 4-day collection period, the urine composite of each animal was transferred to a 250-ml volumetric flask. Three ml of concentrated hydrochloric acid was added to each flask as a further preservative, and the composites were diluted to volume with distilled water. The flasks were inverted 50 times to mix the contents and the composites were poured back into the pharmaceutical bottles. The bottles were tightly closed, sealed with paraffin and stored in the refrigerator until analyzed.

Duplicate 50-ml aliquot samples of each urine composite were analyzed for total nitrogen by the macro-Kjeldahl method with boric acid modification (70). The bottles were inverted 50 times before pipetting the first sample and 10 times between the first and second sample.

Feces. Carmine was used as an indicator to mark the beginning and end of each collection period. Fecal material was collected from the feces cups daily, separated from hair and food sticking to it and placed in small wide-mouthed jars covered with porous paper. At the end of each collection period, the feces were dried in an oven at 65°C. for 5 days to constant weight, cooled in a desiccator, weighed and ground to the size of a 40-mesh sieve in a Wiley laboratory mill (intermediate model). Duplicate samples were analyzed by the macro-Kjeldahl method for total nitrogen.

Liver. The animals were without food for one day before being sacrificed, and then were anesthetized with ethyl ether. The entire liver was removed rapidly, placed on a weighed square of aluminum foil and tightly wrapped. The wrapped livers were weighed on a Mettler analytical balance (type B6), frozen and stored in a freezer at -10°C.

Prior to analysis, the solidly frozen liver was removed from the freezer, sliced in approximately 1/16 to 1/32 inch slices as rapidly as possible and placed in a weighing bottle. Duplicate samples were weighed onto the interior surface of opened cotton pads of known weight (1.2-2.9 g). The cotton had previously been ether extracted and dried. The liver samples on the cotton pads were dried in a vacuum oven at 110°C. for 72 hours to constant weight. The dried samples were extracted with ethyl ether for 20 hours on low heat in a Goldfish extractor. The ether was reclaimed after extraction and the beakers were placed in the vacuum oven at 110 C. for 1 hour, then cooled in a desiccator

for 30 minutes and weighed. Weight of fat in the samples was obtained by difference in beaker weights before and after extraction. The percentage of fat was calculated on the wet basis.

The duplicate fat-free liver samples in the cotton were analyzed for total nitrogen by the macro-Kjeldahl method.

Calculations and Statistical Analysis

$$\text{Protein Efficiency Ratio} = \frac{\text{g Body Weight Gain}}{\text{g Protein Ingested}}$$

$$\text{Biological Value} = \frac{\text{N Intake} - (\text{Fecal N-Metabolic N}) - (\text{Urinary N-Endogenous N})}{\text{N Intake} - (\text{Fecal N-Metabolic N})} \times 100$$

$$\text{True Digestibility Coefficient} = \frac{\text{N Intake} - (\text{Fecal N-Metabolic N})}{\text{N Intake}} \times 100$$

$$\text{Net Protein Utilization} = \frac{\text{True Digestibility Coefficient} \times \text{Biological Value}}{100}$$

Analyses of variance were run on all measurements of protein quality to find the differences attributable to diets and periods. Fisher's least significant difference at the 5% level was applied to detect significant differences attributable to specific diets as well as to test periods (71).

RESULTS AND DISCUSSION

The food consumption and body weight gain during each adjustment and collection period for each of the animals are presented

in tables 11 and 12 (Appendix). The protein efficiency ratio, true digestibility coefficient, biological value, net protein utilization and nitrogen balance data of the test diets during each collection period for each of the animals are found in tables 13 and 14 (Appendix). Total liver weight, liver as percent of body weight, and the moisture, fat and nitrogen contents of the liver of each animal are given in table 15 (Appendix). Unless otherwise stated, all figures referred to in the discussion are mean values for each group of animals.

Food Intake

The mean food intakes, F-values and least significant differences at the 5% level for each diet group during each period are given in table 3. During the low nitrogen period, which came between the 2 experimental periods, all 4 groups of animals consumed approximately the same amount of the 4% egg diet. The food intakes of the flour and red dog groups were less in both experimental periods than during the low nitrogen period. The food intakes of the fine bran and scalp groups were less only during experimental period I.

During the experimental periods, food intake varied significantly ($P < 0.001$) among the diet groups. The food intake of the flour group was significantly lower than that of the three other groups during both experimental periods and that of the red dog group was significantly lower than that of the fine bran and scalp groups during experimental period II. The scalp group had

TABLE 3

Mean food intakes of rats during low nitrogen period; and means, F-values and least significant differences for food intakes during two test periods

Group	Low nitrogen[1] period	Exp. period I		Exp. period II
	g	g		g
I, flour	58.3	23.8		24.5
II, red dog	59.3	39.7*	*	47.3*
III, fine bran	60.0	41.3	*	60.0
IV, scalp	59.0	47.7	*	67.0
F values[2]				
Diets			25.70***	
Periods			16.80***	
Diets x Periods			2.55 ns	
Animals: Diets			0.44 ns	
Lsd[3]				
Diets			8.34	
Periods			5.90	

[1]The low nitrogen period came between experimental periods I and II, but was not included in the analysis of variance.

[2]*** significant at 0.001 level, ns-not significant.

[3]Lsd*-least significant difference at 5% level.

the highest food intake during both experimental periods, but this was not significantly higher than that of the fine bran group.

There was a significant ($P < 0.001$) difference in the food intake between the 2 experimental periods. The intakes of the groups on red dog, fine bran and scalp were all significantly higher during experimental period II than experimental period I. No significant interaction was observed for food intake between diets and periods and no significant variation of animals was found within diet groups.

Growth

Weight gain. Figure 2 shows the cumulative mean weight gain for each of the 3 adjustment and collection periods. Throughout the study, the scalp group had the greatest weight gain, followed by the fine bran and red dog groups. The flour group had only a small weight gain during experimental period I, but the rate increased markedly during the low nitrogen period. The weight gains of the other groups during the low nitrogen period were similar to each other, except that the rate of weight gain for the scalp and fine bran groups leveled off somewhat during the adjustment part of the low nitrogen period. The rate of weight gain of the scalp and fine bran groups was higher during experimental period II than during the previous periods, but that of the red dog group was leveling off somewhat and a weight loss occurred in the flour group.

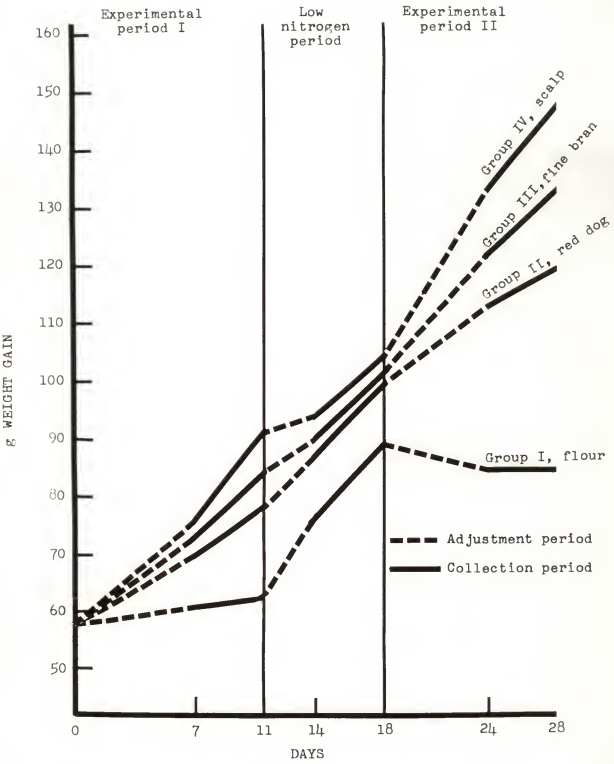

Fig. 2 Cumulative mean weight gain for each diet group during all periods.

The mean percent weight gains, P-values and least significant differences at the 5% level for each diet group during each period are given in table 4. Weight gain varied significantly ($P < 0.001$) among the diet groups. The flour group had a significantly lower weight gain than the other 3 diet groups during both experimental periods. Weight gain of the red dog group was significantly less than that of the scalp group during both experimental periods, and it was significantly less than that of the fine bran group during experimental period II. During experimental period I, the weight gain of the fine bran group was significantly less than that of the scalp group.

There was a significant difference ($P < 0.001$) in weight gain between the 2 experimental periods. Weight gains of the flour, red dog and scalp groups were significantly greater in experimental period I than in experimental period II. No significant interaction was found for weight gain between diets and periods and no significant variation of animals was observed within groups.

Three particularly interesting observations may be made about the weight gain of the animals. First, although the flour group gained the least weight on the experimental diet, their weight gain was similar to the other groups on the low nitrogen diet. The low nitrogen (4% protein, egg) diet produced better weight gain than the flour and red dog diets (10% protein). Second, during experimental period II, some of the animals on the flour diet lost weight, as indicated by the negative weight gain for the group. Third, the weight gains were all lower during

TABLE 4

Means, P-values and least significant differences for percent weight gains and protein efficiency ratios (PER) of rats fed various diets during two collection periods

Group	Weight gain		PER	
	Period I	Period II	Period I	Period II
	%	%		
I, flour	1.89	-0.52	0.49	-0.22
II, red dog	12.32*	6.97*	2.12*	1.60*
III, fine bran	14.71*	12.92*	2.42*	2.46*
IV, scalp	19.27*	13.46*	3.00*	2.57*
F values[1]				
Diets	50.16***		28.23***	
Periods	15.66***		3.35 ns	
Diets x Periods	1.10 ns		0.52 ns	
Animals: Diets	0.77 ns		0.52 ns	
Lsd[2]				
Groups	2.876		0.653	
Periods	2.03		----	

[1] *** significant at 0.001 level, ns-not significant.

[2] Lsd*-least significant difference at 5% level.

experimental period II than experimental period I, despite the fact that the animals were eating more feed.

Protein efficiency ratio. The mean protein efficiency ratios (PER), F-values and least significant differences at the 5% level for each diet group during each period are given in table 4. A significant difference ($P < 0.001$) for PER among diet groups was found. In both experimental periods, the highest PER was obtained for the scalp diet and the lowest for the flour diet. The flour group had a significantly lower PER than the other 3 diet groups during both experimental periods. The red dog group had a significantly lower PER than the fine bran group during experimental period II and the scalp group during both experimental periods. No significant difference was observed for PER between periods. No significant interaction was observed between diets and periods and no significant variation of animals was found within diet groups.

Bender (60) found a mean PER of -0.10 for unfortified bread, 1.15 for bread fortified with lysine, 2.72 for bread fortified with lysine, methionine and threonine and 0.23 for wheat gluten. In the present study, the scalp diet had a mean PER similar to that of the bread fortified with lysine, methionine and threonine; and the red dog diet had a PER somewhat similar to that of the bread with lysine. The flour diet had a low mean PER, similar to the unfortified bread and the wheat gluten.

Nitrogen Balance

Mean nitrogen intake, excretion and retention data for each diet group during two test periods are presented in table 5. The nitrogen intake during the low nitrogen period was about the same for all groups. During experimental periods I and II, the nitrogen intake of the flour group was less than that during the low nitrogen period, but the nitrogen intakes of the 3 other diet groups were higher than during the low nitrogen period. This is another indication that the flour was unsatisfactory as a protein source. It will be recalled that the food intake and rate of weight gain for the flour group were greater during the low nitrogen period than during the experimental periods.

All of the animals during both experimental periods and the low nitrogen period were retaining nitrogen, which would indicate that growth or building of body tissue was taking place. During the low N period, the N retention was similar for all the groups. The retentions of the flour group during both experimental periods were lower than during the low N period. The retentions of the other 3 diet groups were lower during experimental period I than during the low N period, but higher during experimental period II.

Two observations may be made from study of the graph of weight gain (fig. 2) and the values for nitrogen balance (table 5). First, the result of a difference in quantity of protein can be observed in the data for the scalp diet. During experimental period I, when the scalp group was on a 10% protein diet, the nitrogen intake was high and the weight gain was fairly rapid;

TABLE 5

Mean nitrogen intake, excretion and retention of rats on various diets during each collection period

Diet group	Collection period	N intake	N excretion		N retention
			Urinary	Fecal	
		g	g	g	g
I, flour	Exp. I	0.402	0.221	0.054	0.127
	Low N	0.448	0.054	0.056	0.338
	Exp. II	0.413	0.243	0.054	0.116
II, red dog	Exp. I	0.718	0.276	0.114	0.298
	Low N	0.456	0.065	0.059	0.332
	Exp. II	0.856	0.343	0.164	0.349
III, fine bran	Exp. I	0.769	0.279	0.246	0.245
	Low N	0.461	0.073	0.060	0.328
	Exp. II	1.117	0.367	0.284	0.465
IV, scalp	Exp. I	0.869	0.340	0.236	0.293
	Low N	0.453	0.073	0.055	0.324
	Exp. II	1.220	0.389	0.300	0.531

but in the low nitrogen period (4% protein, egg diet), nitrogen intake dropped and weight gain leveled off somewhat. However, when the animals were placed on the 10% protein, scalp diet again, they had a higher nitrogen intake and retention, and gained more weight than in experimental period I. The leveling off of the weight gain during the adjustment part and the increase during the collection part of the low nitrogen period shows that an adjustment to the lowered nitrogen intake was necessary.

Second, the result of a difference in the quality of protein can be observed in the data for the flour diet. During experimental period I, the animals were fed a 10% protein, flour diet, which was known to be poor in quality. The nitrogen intake and retention, and the weight gain were low during this period. However, when placed on the better quality 4% protein, egg diet, the animals had a somewhat higher nitrogen intake, and much higher nitrogen retention and weight gain. When placed on the 10% protein, flour diet again, the nitrogen retention of the animals was less than during experimental period I, and they lost weight. The decrease in nitrogen retention and loss in weight during experimental period II show clearly the heightened effect of a poor quality protein (10% protein, flour) diet, after adjustment to a better quality protein at a lower level (4% protein, egg).

The mean nitrogen retentions, F-values and least significant differences at the 5% level for each diet group during each period are given in table 6. There was a significant difference ($P < 0.001$) in nitrogen retention among diet groups. The flour group had a significantly lower nitrogen retention than the other

TABLE 6

Means, F-values and least significant differences for nitrogen balance and true digestibility coefficients of rats fed various diets during two collection periods

Group	Nitrogen retention		True digest. coeff.	
	Period I	Period II	Period I	Period II
	g	g	%	%
I, flour	0.127	0.116	100.65	100.48
II, red dog	0.298*	0.349*	88.33*	87.52*
III, fine bran	0.245	0.465	76.03	79.79
IV, scalp	0.293	0.531	78.98	79.76
F values[1]				
Diets	61.68***		321.47***	
Periods	60.10***		2.37 ns	
Diets x Periods	14.68***		3.03 ns	
Animals: Diets	2.68*		3.54**	
Lsd[2]				
Diets	0.047		1.71	
Periods	0.034			

[1]*** significant at 0.001 level, ** significant at 0.01 level, * significant at 0.05 level, ns-not significant.

[2]Lsd*-least significant difference at 0.05 level.

3 diet groups during both experimental periods. The retention of the red dog group during experimental period I was significantly higher than that of the fine bran group. However, during experimental period II, the retention of the red dog group was significantly lower than that of the fine bran group and the scalp group. During both experimental periods, the fine bran group had a significantly lower nitrogen retention than the scalp group.

A significant difference ($P < 0.001$) in nitrogen retention was observed between experimental periods. The means for the red dog, fine bran and scalp groups during experimental period II were all significantly higher than during period I. A significant interaction ($P < 0.001$) was found for diets and periods and the variation of animals within diet groups also was significant ($P < 0.05$).

Digestibility coefficient. The mean true digestibility coefficients, F-values and least significant differences at the 5% level for each diet group during each period are given in table 6. In the following discussion, digestibility refers to the true digestibility coefficient unless otherwise stated. A significant difference ($P < 0.001$) was observed among diet groups. During both experimental periods, the digestibility of the flour diet was significantly higher than that of the 3 other diets. The means (100.65, 100.48) for the digestibility of the flour diet indicate that the protein in this diet was completely digested. The digestibility of the red dog diet was significantly higher than that of the fine bran and scalp diets during both experimental periods. During experimental period I, the

digestibility of the fine bran diet was significantly lower than that of the scalp diet, but not during experimental period II. No significant difference was found in the digestibility of the diets between the experimental periods. No significant interaction was found between diets and periods, but the variation of animals within groups was significant at the 1% level.

Chaney (73), quoting E. C. Albritton's Handbook of Biological Data, stated that the coefficient of apparent digestibility of wheat was 79-89% depending on the extraction (70-100%). Dawbarn (74) reported that the apparent digestibility of flour (80-90% extraction) was 80-90%. The true digestibility coefficient should be a little higher than the apparent digestibility coefficient, because it has been corrected to exclude the endogenous nitrogen from the fecal nitrogen excretion. In this study, the true digestibility coefficients of all of the diets except flour were between 76-89%.

Biological value. The means, F-values and least significant differences at the 5% level for biological value are shown in table 7. There was a significant difference ($P < 0.01$) in the biological value among the diets. The biological value of the flour diet was significantly lower than the biological value of the red dog and fine bran diets during both experimental periods, and of the scalp diet during experimental period II. The biological value of the red dog diet was significantly greater than that of the scalp diet in experimental period I, but not in experimental period II. No significant differences in biological value were found between the red dog and fine bran diets or

TABLE 7

Means, F-values and least significant differences for biological value and net protein utilization (NPU) of rats fed various diets during two collection periods

Group	Biological value		NPU	
	Period I	Period II	Period I	Period II
I, flour	58.48	55.06	58.94	55.43
II, red dog	66.84*	63.26*	59.09*	55.40
III, fine bran	64.45*	66.99*	48.97*	53.46
IV, scalp	60.12	67.33*	47.59*	53.79
F values[1]				
Diets	7.05**		6.90**	
Periods	0.20 ns		0.40 ns	
Diets x Periods	2.82 ns		3.58*	
Animals: Diets	2.56*		3.78**	
Lsd[2]				
Diets	4.57		4.07	
Periods	----		----	

[1] ** significant at 0.01 level, * significant at 0.05 level, ns-not significant.

[2] Lsd*-least significant difference at 0.05 level.

between the fine bran and scalp diets for either experimental period. No significant differences were observed in the biological value of the diets between experimental periods I and II. No significant interaction was found between diets and periods, but the variation of animals within diet groups was significant at the 5% level.

The FAO report on protein (72) stated that the biological value of white flour as determined on growing rats was 52. This is comparable with the 58.48 and 55.06 biological values found in this study. The biological value for wheat germ was 75, which is somewhat higher than the biological values (60.12, 67.33) for the scalp diet as analyzed in this study. The scalp or germ stock is the milling fraction from which the wheat germ is taken. It could be expected to have a lower biological value than the purified wheat germ, because of the bran and flour which are present in the fraction.

Net protein utilization. The means, F-values and least significant differences at the 5% level for net protein utilization (NPU) are shown in table 7. A significant difference ($P < 0.01$) in NPU was found between diet groups. The NPU of both the flour and red dog diets was significantly higher than that of the fine bran and scalp diets during experimental period I. However, no significant differences in NPU between any of the diets were observed in experimental period II. No significant differences in NPU of the diets were observed between the experimental periods. The interaction between diets and periods was significant at the 5% level and the variation of animals within

diet groups was significant at the 1% level.

Bender (60) observed that wheat gluten had an NPU of 33; bread, 48; bread with lysine enrichment, 57 and bread with lysine, methionine and threonine enrichment, 78. The NPU in this study varied from 47.59-59.09 during experimental period I and 53.45-55.43 during experimental period II. This would be in the same range with the NPU for bread and bread with lysine enrichment.

Amino Acid Composition

Table 8 shows the amino acid composition of the gluten, wheat, flour and 3 mill streams used in this study. Whole egg is shown as a reference, because it is as nearly completely utilized by the rat and man as any food protein and is not significantly enhanced in biological value by supplementation with any amino acid. Of the 18 amino acids shown, whole egg contained a higher percentage of 12 of them than the wheat products. All of the wheat products were higher than the whole egg in glycine and proline and all of the wheat products, except gluten, were higher in glutamic acid. Flour was higher than whole egg in half cystine and fine bran and scalp were higher in histidine and aspartic acid.

When compared to whole egg, the wheat products contained less of all the essential amino acids. Flour and gluten had the lowest percentages of lysine, and flour was also low in tryptophan and valine. Gluten was lowest in threonine; fine bran, in leucine, isoleucine and methionine; and scalp, in phenylalanine.

TABLE 8

Amino acid composition (% per 16 g N) of gluten,[1] wheat,[2] flour[2] and 3 mill streams,[2] as compared with whole egg[1]

Amino acids	Whole egg	Gluten	Wheat	Flour	Red dog	Fine bran	Scalp
	%	%	%	%	%	%	%
Alanine	6.38	2.24	3.96	3.17	4.35	5.27	5.28
Arginine	6.56	4.35	4.10	3.17	4.56	5.81	5.68
Aspartic acid	7.01	3.61	5.14	4.25	6.09	7.54	7.50
Glutamic acid	12.37	3.67	34.18	39.83	28.91	24.67	23.35
Glycine	3.54	3.67	4.55	3.75	4.64	5.87	5.57
Half cystine	2.34	2.16	2.24	2.42	2.10	2.10	2.04
Histidine	2.40	2.28	2.31	2.17	2.39	2.81	2.61
Isoleucine*	6.64	4.60	3.66	3.75	3.62	3.53	4.09
Leucine*	8.80	7.49	7.39	7.58	7.25	7.06	7.56
Lysine*	6.40	1.91	2.84	2.08	3.40	4.19	4.32
Methionine*	3.13	1.74	1.34	1.25	1.59	1.13	1.19
Phenylalanine*	5.77	5.44	5.07	5.33	4.71	4.43	4.26
Proline	4.24	12.74	10.89	12.67	9.42	7.84	7.73
Serine	8.40	4.74	5.07	5.17	4.86	4.97	4.72
Threonine*	4.99	2.65	3.13	2.83	3.26	3.59	3.58
Tyrosine	4.30	3.24	1.86	1.67	1.67	2.10	2.04
Valine*	7.42	4.74	4.70	4.42	4.71	5.09	5.06
Tryptophan[3]	1.65	1.07	1.09	1.02	----	----	----
Ammonia	----	----	3.58	4.08	3.33	2.75	2.61
Protein (of sample)	72.60	80.00	13.40	12.00	13.80	16.70	17.60

*essential amino acids

[1]From Orr and Watt (75).

[2]Analyzed by Doyle Waggle, Dept. Flour and Feed Milling, Kansas State University.

[3]Tryptophan values for whole egg (75), gluten (75) and wheat and flour (76) gathered from various sources.

In a study by Hepburn et al. (77), the distribution of amino acids in several flour streams, red dog, bran, germ and whole wheat, was similar to that in this investigation.

All the wheat products in this study were very limited in isoleucine, lysine, methionine and threonine. The fine bran and scalp contained a larger proportion of many of the essential amino acids than the red dog and flour, and the red dog was slightly higher than the flour in most of the essential amino acids. This supports the observation that the fine bran and scalp contain higher quality protein than the red dog and flour, as evidenced by weight gain, PER, nitrogen retention and biological value. This also supports the finding that the protein in the red dog fraction may be somewhat better than that in the flour.

Liver

Means, F-values and least significant differences at the 5% level are given in table 9 for percent fat, nitrogen and moisture of each diet group. A significant difference ($P < 0.001$) for fat was found among diet groups. The liver fat content of the red dog group was significantly higher than that of the other 3 diet groups. The scalp group had a slightly higher liver fat content than the fine bran group, although it was not quite statistically significant.

A deficiency in the quality of protein may cause increased liver fat deposition (30). Winje et al. (38) and the Food and Nutrition Board (39) reported that deficiency of lysine, threonine

TABLE 9

Means, F-values and least significant differences for percent fat, nitrogen and moisture in the livers of rats fed various diets during two collection periods

Group	Fat	Nitrogen	Moisture
	%	%	%
I, flour	1.32 *	19.70	77.77
II, red dog	2.62 *	18.09	77.19
III, fine bran	1.26	19.85	77.39
IV, scalp	1.90	19.87	76.91
F values[1]			
Diets	8.88***	2.23 ns	2.19 ns
Lsd[2]			
Diets	0.65	----	----

[1] *** significant at 0.001 level, ns-not significant.

[2] Lsd*-least significant difference at 0.05 level.

and methionine or tryptophan resulted in fatty infiltration of the liver. The amino acid analysis shown in table 9 reveals that the lysine, threonine and methionine in flour and red dog are low. Tryptophan was not determined, but Pyler (76) indicated that tryptophan in wheat, flour and bread was low. The fine bran and scalp contained more lysine and threonine than the flour and red dog. The red dog diet resulted in a significantly greater deposition of liver fat. The flour diet did not produce much liver fat deposition, possibly because of the reduced food intake.

No significant differences were found among groups for percent nitrogen or moisture in the liver.

SUMMARY

The purpose of this study was to investigate the protein quality of three wheat mill streams as compared to that of flour.

Twenty-four male weanling albino rats of the Sprague-Dawley strain were randomly distributed in individual metabolic cages and fed ad libitum. The classical Thomas-Mitchell nitrogen balance method for determination of biological value was used. During 2 experimental periods of 11 and 10 days, respectively, the animals were fed diets containing 10% protein supplied by flour, red dog, fine bran or scalp. A low nitrogen diet (4% protein from egg) was fed to all animals for 7 days between the two experimental periods. During the last 4 days of each period, urine and feces were collected. Weight and food intake of each animal were determined at the end of each period. Nitrogen in

the wheat samples, diets, urine and feces was determined. Percent liver fat, nitrogen and moisture were determined at the end of the experiment.

Weight gain, protein efficiency ratio, true digestibility coefficient, biological value and net protein utilization were calculated. The data were analyzed by analysis of variance and least significant differences were obtained.

Weight gain and nitrogen retention of the flour group were better on the low nitrogen diet than on the flour diet. The flour group had a significantly lower food intake, weight gain, protein efficiency ratio, nitrogen retention and biological value than the other 3 diet groups in every case, with one exception. However, the flour group was significantly higher than the other 3 diet groups in true digestibility coefficient and net protein utilization in several cases. The red dog group often was significantly lower in food intake, weight gain, protein efficiency ratio and nitrogen retention than the fine bran and scalp groups, but was significantly higher in true digestibility coefficient and net protein utilization in most cases. Generally, the red dog group seemed to fall between the flour and the fine bran and scalp groups. In most instances, the scalp group had the highest values for food intake, weight gain, protein efficiency ratio, nitrogen retention and biological value, although the values usually were not significantly greater than those for the fine bran group. However, during both experimental periods, the nitrogen retention of the scalp group was significantly greater than that for the fine bran group. The flour and 3 mill

streams contained less of all the essential amino acids than whole egg. All the wheat products were particularly low in isoleucine, lysine, methionine and threonine. The fine bran and scalp contained a higher proportion of most of the essential amino acids than the red dog, and the red dog a higher proportion than the flour. The red dog diet produced a significantly greater liver fat deposition than the other 3 diets. The fine bran and scalp appeared to be much better sources of protein than the red dog and flour, both in quantity and quality of protein.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Beth Alsup, Major Professor, for her kindness, assistance throughout the experiment and guidance and constructive criticism in the preparation of the manuscript. Appreciation is expressed to Miss Gwendolyn L. Tinklin, Acting Head of the Department of Foods and Nutrition; Dr. Grayce E. Goertz, Professor of Foods and Nutrition; Dr. Thomas H. Lord, Professor of Bacteriology and Dr. John A. Johnson, Professor of Flour and Feed Milling, for being on the advisory committee and reviewing the manuscript.

Recognition is given to Dr. Charles W. Deyoe, Associate Professor of Flour and Feed Milling and to Doyle Waggle, Research Assistant, for the amino acid analysis of the samples; to Dr. Arlin B. Ward, Associate Professor of Flour and Feed Milling, for guidance in planning the experiment and obtaining the wheat samples; to Samuel N. Rogers, Research Assistant in Biochemistry, for proximate analysis of the samples and to Mrs. Vesta J. Kerr, Secretary in Foods and Nutrition, for typing the tentative manuscript.

Appreciation also is extended to Ronald Watson of the Abilene Flour Mills, Abilene, Kansas for the wheat, flour and 3 mill streams used in the experiment.

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APPENDIX

TABLE 10

Random distribution of animals

Rat No.	1	2	3	4	5	6	7	8
Group	IV	III	I	III	IV	II	II	I
Initial weight (g)	62	60	52	58	53	58	54	59
Rat No.	9	10	11	12	13	14	15	16
Group	III	I	II	I	II	III	IV	I
Initial weight (g)	57	61	60	53	58	59	55	67
Rat No.	17	18	19	20	21	22	23	24
Group	II	II	III	IV	I	IV	III	IV
Initial weight (g)	59	57	56	55	56	62	58	60

TABLE 11

Food consumption of rats fed various diets during adjustment and collection periods

Rat No.	Exp. period I		Low-N period		Exp. period II	
	Adjust.	Collect.	Adjust.	Collect.	Adjust.	Collect.
	g	g	g	g	g	g
Group I, flour						
3	36	22	40	47	34	24
8	38	21	48	62	24	22
10	42	28	50	55	39	31
12	39	23	51	65	25	20
16	37	26	46	62	37	26
21	34	23	46	59	31	24
Group II, red dog						
6	54	46	54	56	77	54
7	50	31	45	52	64	45
11	45	31	46	54	52	38
13	59	42	57	61	69	44
17	60	45	57	67	71	50
18	57	43	51	66	74	53
Group III, fine bran						
2	56	42	50	61	84	60
4	48	39	43	53	79	57
9	54	32	43	65	85	59
14	52	43	37	54	84	61
19	58	44	44	60	77	59
23	54	48	54	67	83	64
Group IV, scalp						
1	58	47	52	58	98	70
5	62	48	36	53	92	65
15	62	43	33	49	89	58
20	63	58	50	59	94	70
22	65	51	46	65	98	71
24	62	39	52	70	88	68

TABLE 12

Initial body weights and weights of rats fed various diets at the end of each adjustment and collection period

Rat No.	Init. body wt.	Exp. period I		Low-N period		Exp. period II	
		Adjust.	Collect.	Adjust.	Collect.	Adjust.	Collect.
	g	g	g	g	g	g	g
Group I, flour							
3	52	56	57	67	74	74	74
8	59	65	65	78	93	89	93
10	61	63	64	77	84	85	86
12	53	57	58	77	95	85	82
16	67	66	69	83	96	95	90
21	56	60	61	72	87	83	83
Group II, red dog							
6	58	71	85	95	108	120	129
7	54	65	70	77	86	101	109
11	60	69	78	82	93	106	112
13	58	69	74	85	96	108	115
17	59	76	87	98	115	126	132
18	57	68	76	86	102	116	127
Group III, fine bran							
2	60	75	85	92	102	121	135
4	58	71	82	87	99	116	134
9	57	71	76	84	101	122	135
14	59	71	81	87	95	115	134
19	56	72	87	91	108	124	138
23	58	80	94	102	115	134	150
Group IV, scalp							
1	62	75	89	93	108	131	149
5	53	76	91	90	99	128	144
15	55	73	84	82	94	122	139
20	55	75	90	94	107	131	152
22	62	83	104	106	114	146	164
24	60	77	90	96	110	139	156

TABLE 13

Protein efficiency ratio (PER), digestibility coefficient, biological value and net protein utilization (NPU) of various test diets fed to rats during two periods

Rat No.	Exp. period	PER	Digest. coeff.	Bio. value	NPU
			%		
Group I, flour					
3	I	0.473	99.68	53.46	53.29
	II	0.000	99.95	52.88	52.85
8	I	0.000	101.30	44.58	45.16
	II	1.892	96.36	55.80	53.77
10	I	0.372	98.48	58.04	57.16
	II	0.336	99.54	45.00	44.79
12	I	0.452	102.84	66.21	68.09
	II	-1.561	103.02	55.74	57.42
16	I	1.200	98.81	56.35	55.68
	II	-2.001	100.07	50.65	50.68
21	I	0.452	102.78	72.26	74.27
	II	0.000	103.95	70.31	73.09
Group II, red dog					
6	I	2.955	83.23	69.62	57.94
	II	1.618	88.26	64.64	57.05
7	I	1.566	90.51	74.92	67.81
	II	1.726	88.76	70.18	62.29
11	I	2.819	88.07	60.41	53.20
	II	1.533	83.12	61.84	51.40
13	I	1.184	92.79	76.78	71.24
	II	1.544	88.68	67.07	59.48
17	I	2.373	89.30	52.61	46.98
	II	1.165	89.78	59.80	53.69
18	I	1.806	86.06	66.67	57.38
	II	2.105	86.55	56.01	48.48

TABLE 13

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Rat No.	Exp. period	PER	Digest. coeff.	Bio. value	NPU
			%		
Group III, fine bran					
2	I	2.244	75.58	71.12	53.75
	II	2.199	78.01	65.72	51.27
4	I	2.658	77.76	66.26	51.52
	II	2.976	78.72	70.30	55.34
9	I	1.473	78.98	60.45	47.74
	II	2.077	81.28	71.81	58.37
14	I	2.192	73.93	59.45	43.95
	II	2.936	79.89	60.37	48.23
19	I	3.213	77.42	61.46	47.58
	II	2.236	80.46	66.15	53.22
23	I	2.749	72.53	67.97	49.30
	II	2.356	80.40	67.60	54.35
Group IV, scalp					
1	I	2.867	80.56	66.47	53.55
	II	2.475	77.12	68.84	53.09
5	I	3.008	77.34	59.67	46.15
	II	2.369	80.79	66.82	53.98
15	I	2.462	70.13	55.05	38.61
	II	2.821	73.40	60.75	44.59
20	I	2.489	83.40	71.89	59.96
	II	2.887	82.99	69.73	57.87
22	I	3.963	80.37	62.18	49.97
	II	2.440	81.07	68.61	55.62
24	I	3.208	82.11	45.43	37.30
	II	2.406	83.20	69.22	57.59

TABLE 14

Nitrogen balance of rats fed various test diets during collection periods

Rat No.	Period	Nitrogen intake	Urinary nitrogen	Fecal nitrogen	Retention
		g	g	g	g
Group I, flour					
3	Exp. I	0.371	0.221	0.050	0.100
	Low N	0.361	0.049	0.049	0.263
	Exp. II	0.405	0.240	0.049	0.116
8	Exp. I	0.354	0.251	0.056	0.047
	Low N	0.476	0.053	0.061	0.362
	Exp. II	0.371	0.211	0.074	0.086
10	Exp. I	0.472	0.240	0.062	0.170
	Low N	0.422	0.044	0.055	0.323
	Exp. II	0.523	0.331	0.058	0.134
12	Exp. I	0.388	0.193	0.048	0.147
	Low N	0.499	0.058	0.059	0.382
	Exp. II	0.377	0.212	0.048	0.077
16	Exp. I	0.438	0.253	0.060	0.125
	Low N	0.476	0.064	0.054	0.358
	Exp. II	0.438	0.281	0.054	0.103
21	Exp. I	0.388	0.168	0.045	0.175
	Low N	0.453	0.057	0.056	0.340
	Exp. II	0.405	0.182	0.040	0.183
Group II, red dog					
6	Exp. I	0.832	0.275	0.198	0.359
	Low N	0.430	0.064	0.059	0.307
	Exp. II	0.977	0.369	0.173	0.435
7	Exp. I	0.561	0.181	0.101	0.279
	Low N	0.399	0.053	0.047	0.299
	Exp. II	0.814	0.269	0.139	0.406
11	Exp. I	0.561	0.256	0.125	0.180
	Low N	0.415	0.061	0.059	0.295
	Exp. II	0.687	0.279	0.174	0.234
13	Exp. I	0.760	0.237	0.116	0.407
	Low N	0.468	0.074	0.061	0.333
	Exp. II	0.796	0.306	0.151	0.339

TABLE 14

cont.

Rat No.	Period	Nitrogen intake	Urinary nitrogen	Fecal nitrogen	Retention
		g	g	g	g
17	Exp. I	0.814	0.410	0.153	0.251
	Low N	0.514	0.066	0.066	0.382
	Exp. II	0.904	0.392	0.159	0.353
18	Exp. I	0.778	0.299	0.170	0.309
	Low N	0.507	0.076	0.062	0.369
	Exp. II	0.959	0.441	0.190	0.328
Group III, fine bran					
2	Exp. I	0.782	0.249	0.255	0.278
	Low N	0.468	0.079	0.064	0.325
	Exp. II	1.117	0.377	0.310	0.430
4	Exp. I	0.726	0.258	0.215	0.253
	Low N	0.407	0.068	0.054	0.285
	Exp. II	1.061	0.316	0.280	0.465
9	Exp. I	0.596	0.254	0.188	0.154
	Low N	0.499	0.068	0.062	0.369
	Exp. II	1.098	0.319	0.268	0.511
14	Exp. I	0.800	0.312	0.256	0.232
	Low N	0.415	0.072	0.048	0.295
	Exp. II	1.135	0.431	0.277	0.427
19	Exp. I	0.819	0.310	0.244	0.265
	Low N	0.461	0.066	0.059	0.336
	Exp. II	1.098	0.365	0.274	0.459
23	Exp. I	0.893	0.290	0.317	0.286
	Low N	0.514	0.083	0.072	0.359
	Exp. II	1.191	0.393	0.305	0.493
Group IV, scalp					
1	Exp. I	0.856	0.300	0.226	0.330
	Low N	0.445	0.069	0.059	0.317
	Exp. II	1.275	0.376	0.351	0.548
5	Exp. I	0.875	0.341	0.250	0.284
	Low N	0.407	0.068	0.052	0.287
	Exp. II	1.184	0.385	0.279	0.520

TABLE 14

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Rat No.	Period	Nitrogen intake	Urinary Nitrogen	Fecal nitrogen	Retention
		g	g	g	g
15	Exp. I	0.784	0.312	0.283	0.189
	Low N	0.376	0.065	0.049	0.262
	Exp. II	1.056	0.370	0.330	0.356
20	Exp. I	1.057	0.320	0.230	0.507
	Low N	0.453	0.073	0.055	0.325
	Exp. II	1.275	0.393	0.272	0.610
22	Exp. I	0.929	0.363	0.228	0.338
	Low N	0.499	0.080	0.046	0.373
	Exp. II	1.294	0.409	0.291	0.594
24	Exp. I	0.711	0.402	0.197	0.112
	Low N	0.537	0.084	0.070	0.383
	Exp. II	1.239	0.401	0.278	0.560

TABLE 15

Liver weight, liver weight/body weight, and percent liver fat, nitrogen and moisture of rats fed various test diets

Rat No.	Liver weight	Liver weight/ body weight	Fat	Nitrogen	Moisture
	g	%	%	%	%
Group I, flour					
3	4.41	5.96	1.91	19.00	78.61
8	4.90	5.27	0.61	20.59	78.29
10	4.37	5.08	1.62	18.27	77.84
12	4.28	5.22	0.76	20.42	77.71
16	4.31	4.78	1.92	19.48	76.79
21	3.93	4.73	1.08	20.46	77.40
Group II, red dog					
6	6.35	4.92	2.37	12.87	77.05
7	5.38	4.93	1.19	20.12	77.99
11	5.74	5.12	2.61	19.04	77.33
13	5.43	4.72	2.92	18.44	76.85
17	6.19	4.69	2.95	19.39	77.51
18	6.76	5.32	3.67	18.66	76.43
Group III, fine bran					
2	6.39	4.73	1.15	20.37	77.66
4	6.68	4.98	1.05	19.24	76.83
9	6.48	4.80	1.00	19.63	78.89
14	6.23	4.65	1.57	19.96	77.02
19	6.66	4.83	1.66	19.62	76.64
23	7.32	4.88	1.12	20.30	77.28
Group IV, scalp					
1	7.24	4.86	1.88	19.66	77.01
5	7.66	5.32	1.83	19.48	76.79
15	7.02	5.05	1.56	19.88	77.23
20	7.76	5.11	1.94	20.02	77.04
22	7.59	4.62	1.76	20.20	76.50
24	7.18	4.60	2.41	19.96	76.88

PROTEIN QUALITY OF THREE WHEAT MILL STREAMS

by

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B. S., Kansas State University, 1964

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1965

The purpose of this study was to investigate the protein quality of three wheat mill streams as compared to that of flour.

Twenty-four male weanling albino rats of the Sprague-Dawley strain were randomly distributed in individual metabolic cages and fed ad libitum. The classical Thomas-Mitchell nitrogen balance method for determination of biological value was used. During 2 experimental periods of 11 and 10 days, respectively, the animals were fed diets containing 10% protein supplied by flour, red dog, fine bran or scalp. A low nitrogen diet (4% protein from egg) was fed to all animals for 7 days between the two experimental periods. During the last 4 days of each period, urine and feces were collected. Weight and food intake of each animal were determined at the end of each period. Nitrogen in the wheat samples, diets, urine and feces was determined. Percent liver fat, nitrogen and moisture were determined at the end of the experiment.

Weight gain, protein efficiency ratio, true digestibility coefficient, biological value and net protein utilization were calculated. The data were analyzed by analysis of variance and least significant differences were obtained.

Weight gain and nitrogen retention of the flour group were better on the low nitrogen diet than on the flour diet. The flour group had a significantly lower food intake, weight gain, protein efficiency ratio, nitrogen retention and biological value than the other 3 diet groups in every case, with one exception. However, the flour group was significantly higher than the

other 3 diet groups in true digestibility coefficient and net protein utilization in several cases. The red dog group often was significantly lower in food intake, weight gain, protein efficiency ratio and nitrogen retention than the fine bran and scalp groups, but was significantly higher in true digestibility coefficient and net protein utilization in most cases. Generally, the red dog group seemed to fall between the flour and the fine bran and scalp groups. In most instances, the scalp group had the highest values for food intake, weight gain, protein efficiency ratio, nitrogen retention and biological value, although the values usually were not significantly greater than those for the fine bran group. However, during both experimental periods, the nitrogen retention of the scalp group was significantly greater than that for the fine bran group. The flour and 3 mill streams contained less of all the essential amino acids than whole egg. All the wheat products were particularly low in isoleucine, lysine, methionine and threonine. The fine bran and scalp contained a higher proportion of most of the essential amino acids than the red dog, and the red dog a higher proportion than the flour. The red dog diet produced a significantly greater liver fat deposition than the other 3 diets. The fine bran and scalp appeared to be much better sources of protein than the red dog and flour, both in quantity and quality of protein.