THE EFFECT OF WHEAT PROTEIN CONCENTRATE SUPPLEMENTATION UPON SORGHUM DIETS AS MEASURED BY RAT PERFORMANCE

by

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INTRODUCTION

Problems of nutrition are of world-wide interest because of their social and economic implications. Malnutrition is one of the most serious of disasters that affects humanity today. This is especially true in the developing countries, where malnutrition takes a huge toll in terms of human incapacity and inefficiency, lowered production, loss of energy, and susceptibility to disease (1).

In the etiology and pathogenesis of malnutrition, inadequate intake of essential nutrients is basic to the problem. The causes of malnutrition are numerous, but the greatest one is lack of sufficient food because of underproduction (2). Social and economic factors deterring adequate food production and nurturing wrong food habits vary widely in developing countries, but fall into familiar patterns. Unchanging landholding systems, inadequate farming and stock raising techniques, unorganized marketing, lack of storage and transport facilities, almost non-existent credit systems, demagogy on the question of prices, age-old traditions, vague superstition and mysticism are some of the manifold social patterns built upon ignorance, thus perpetuating poverty and in turn, malnutrition (2, 3).

The most formidable barrier to social and economic progress is the low level of education among people in these areas (2). Progress in raising a developing society to acceptable nutritional status depends on education and on a sufficient food supply. However, studies made in education, science and technology will be of no avail if the present rate of population growth does not decline. Concerted efforts by all nations have resulted in an awareness of the overpopulation problem among the

people, and positively reduced family size in some cases. Until such time as the population rate is adequately lowered, and food production remains sufficient to feed world populations, the problem of malnutrition will be of primary concern to many nations and individuals (4, 5).

The most efficient use of available foods is the vital concern of all countries. The investigation of the feasibility of using wheat protein concentrate as a supplement in diets for human consumption would be a significant contribution in alleviating the problem of protein deficiency. Animal studies are necessary prior to the consideration of adding protein concentrates into human diets. Therefore, the study using albino rats, was designed to evaluate the nutritional adequacy of wheat protein concentrate from mill-feed and to investigate the possibility of supplementing a sorghum diet with wheat protein concentrate to improve its nutritional performance.

REVIEW OF LITERATURE

The Problem of Protein Malnutrition

The justification of singling out protein malnutrition over lack of other nutrients is its world-wide prevalence (6). A lack of protein dominates the food problems of the world (7, 8, 9, 10). While the dire need for food supplying calories is quite clear, the requirement for protein is not widely understood in developing countries (11), where agriculture is diverted to producing hunger-satisfying cereals and tubers, having a high yield of calories per acre. This sets the scene for protein malnutrition (6), which results from too great a dependence on starchy foodstuffs at the expense of protein-rich foodstuffs. The Third Joint

FAO/WHO Expert Committee on Nutrition (1953) defined protein malnutrition thus: "the concept includes the effects of a deficiency in the quantity of protein consumed, of imbalance of amino acids, and deficiency of factors such as Vitamin B_{12}, commonly found in foods associated with animal protein and concerned with protein metabolism" (12). The basic problem as put forth in this definition is a lack of adequate quantity of protein, particularly of animal origin.

In the Food and Agriculture Organization statistics of food consumed in thirty-two countries Dole (13) observed that dietary protein supplied 10% to 13% of the calories throughout the world. He suggested a possible correlation between protein and calories which did not necessarily indicate food consumption patterns or availability, but indicated a relationship between protein requirements and total calories. Results of later surveys by the U.S. Department of Agriculture, cited by Jansen and Howe (14) compare with Doles' observations. In these studies the per capita protein intakes, in all countries surveyed other than the United States and Canada were supplying at least 8% of the calories, an amount apparently fulfilling protein requirement.

Protein deficiency can be either of total dietary protein or of one or more essential amino acids. Vegetable proteins, which are major sources of protein in developing countries, are known to be low in essential amino acid content (15), and as a consequence are limiting in one or more essential amino acids. Lysine, sulphur amino acids, threonine and tryptophan are the amino acids most likely to be limiting in diets based on cereals, millets and pulses (14, 16).

It was suggested by Holt (17) and supported by Waterlow (18) that the

factor limiting growth in underdeveloped areas of the world is total dietary nitrogen rather than any of the essential amino acids. His observation is based on 2 studies (19, 20). Jansen and Howe (14) however contradicted these suggestions, since it appears extremely unlikely that total nitrogen can be the limiting factor on a cereal diet.

The deficiency in protein in much of the world today is primarily qualitative rather than quantitative. The major reason for the qualitative difference is the small quantity of animal protein consumed in developing countries where the present production of animal proteins is far below minimum protein needs (10, 11), and the exorbitant prices almost prohibitive (21). With existing calorie deficits and growing populations, it seems most unlikely that intakes of animal proteins can be significantly raised in these areas (14, 21). Increasing animal protein production presents the greatest scientific, technical and economic challenge of our times (21), and until this is met, the maximum use of available vegetable sources needs to be exploited.

Protein Requirements

Proteins are unquestionably the most important of all substances in the organic kingdom. They are normal constituents of the cells and fluids of plants and animals; they are important in controlling the balance between cells and intercellular fluid, between tissues and blood, and in maintaining fluid balance; they are constituents of enzymes and hormones and are involved in immunological and allergic substances (26).

Of the three basic food substances--fat, carbohydrates and proteins, it is only the proteins that supply nitrogen without which growth,

development and replacement of tissue is impossible and death is inevitable (27).

The number of different proteins found in nature is nearly infinite, because of variations in the amount and arrangement of some twenty-one or twenty-two amino acids (28). The early work of Osborne and Mendel (29, 30, 31) demonstrated that protein of different composition might vary in nutritive value. Those workers developed quantitative studies utilizing rat growth to estimate the efficiency with which various proteins would meet body needs.

Proteins for growth and maintenance. Dietary intake of protein beyond the amount needed for the repair of the normal breakdown of body tissues, is used for growth (29). The primary function of protein is, therefore, maintenance and its secondary function that of growth.

In an early view largely defined by Folin (32) synthesis of new proteins was believed to cease at adulthood, and it was considered that nitrogen entering the cells was required only to replace constant endogenous metabolism. The first studies using isotopically labeled amino acids made it clear that cellular proteins constantly liberate amino acids that are mixed with those from the diet and other tissues and used for resynthesis of proteins (26).

The ability of a dietary protein to promote tissue synthesis depends on its amino acid content, which in turn determines the total nitrogen content (28). Information on amino acid composition of specific proteins and foods has been compiled (33, 34). Literature abounds in findings demonstrating the superior performance of animal proteins as compared to vegetable proteins in promoting growth in animals. Vegetable proteins

contain a smaller proportion of the indispensable amino acids (28, 35, 36). A survey of the literature (35), emphasizes the importance of an intake of the essential amino acids in approximately the proportions and quantities found in milk and eggs for optimum growth of tissue proteins. Essential amino acid patterns required for growth and maintenance in rats and man were studied and compiled by Allison (35, 36). The Food and Agriculture Organization (FAO) Committee on Proteins has developed a reference pattern using data from experiments where amino acid mixtures had been used to determine the requirements for maintenance and growth in human beings (37).

Emphasis has been placed upon the importance of the proportions and quantity of the essential amino acids. The essentiality of the non-essential amino acids should not be overlooked. Non-specific dietary sources of nitrogen (19, 38, 39, 40) particularly the non-essential amino acids (40) are more effective in meeting the non-specific nitrogen requirements than the essential amino acids. The ability, therefore, of a dietary protein to adequately perform in maintenance and growth is limited by the nitrogen content, and the essential amino acid pattern presented in the diet.

Protein reserves. Of the two variables limiting utilization of dietary protein mentioned in the preceding section, nitrogen intake is considered important, because the synthesis and maintenance of tissue proteins is expressed simply as a nitrogen balance (41).

When an intake of protein in the adult exceeds the minimum requirement, some excess nitrogen is stored within the body (35). This retention could be accounted for by what Mitchell (42) called "adult growth." The

retention was in excess of these minimum requirements (43), and was reflected in increased body weight. That retention of nitrogen in healthy adults is not always accompanied by gain in weight has been demonstrated by several investigators (44, 45). The lack of a satisfactory understanding of this peculiar phenomenon raises pertinent questions about protein reserves in the body.

The "metabolic pool" of amino acids first described by Whipple (46) is an essential component of modern concepts of cellular protein synthesis (26). This "pool" is a mechanism of transfer of amino acids between cellular proteins providing for a balance between anabolism and catabolism of amino acids (36). Those tissue proteins that can be depleted to contribute amino acids to the metabolic pool are called protein reserves (47). The contribution that different tissues make to this loss have been determined in experimental animals (48, 49, 50, 51, 52). The proteins of the brain are resistant to depletion, those of the heart less so, the kidney still less, while cellular proteins of the liver are depleted easily (52). The estimated losses in terms of original tissue protein were reported as follows: liver, 40%; prostate and seminal vesicles, 29%; alimentary tract, pancreas and spleen, 28%; kidney, 20%; drawn blood, 20%; heart, 18%; muscle, skin and skeleton, 8% (48, 49, 50). The rate at which depletion occurs is directly related to the protein reserves. Urinary nitrogen excretion is great when protein stores are high (53, 54). Some of the enzyme proteins in tissues may be considered to represent labile protein. According to Kosterlitz and Campbell (51) it is the cytoplasmic protein including ribonucleoprotein that is readily lost from certain tissues in starvation and when on a protein-free diet. Flavo proteins are concerned

in enzyme action; therefore, depletion in protein reserves results in reduction of activity of enzyme systems (27). The presence or absence of tissue proteins is reflected in plasma proteins with albumin contributing most to the changes (55). Thus it is likely that labile protein reserves may have a varying composition in different tissues and may also be lost under stress at different rates (52, 56).

Labile proteins. Liver proteins. When animals are fed a diet containing insufficient quantities of an essential amino acid, either by using a protein of poor quality or a purified diet devoid of one amino acid, growth is retarded, and abnormal tissue development occurs. Extensive studies have established the considerable sensitivity of liver protein content to dietary changes (48, 49, 50). Direct measurements of liver proteins and enzymes have shown problems of changes associated with severe protein malnutrition (56). The special lability of liver enzymes has been reported (52). The functional activity of the liver suffered during gross protein deficiency (27). The quantity of dietary protein resulted in concommitant rise and fall in liver proteins (57, 58).

Kosterlitz (57) observed that liver weights of rats increased on a high protein diet and decreased on a diet deficient in protein. However, in some cases, an increase of fat was observed in the first few days on a protein deficient diet. Williams (59) found that the ratio of liver weight to body weight increased on a protein deficient diet. The nitrogen concentration per mg of liver decreased in those rats in the first thirty days, and then slowly increased. He suggested that initial deposition of lipids and glycogen were lost later from the liver and used for energy, which resulted in a relative increase in nitrogen. Increasing the protein

in the diet reduced the fat content of livers (60). Winje et al. (60) believed the specific ratio of amino acids in the diet, especially lysine, threonine and methionine, affected normal fat deposition. It has been shown that fatty infiltration of livers resulted from deficiency of lysine, threonine and tryptophan (61), and was a characteristic sign of kwashiorkor (62).

Vegetable proteins produced less regeneration in depleted liver proteins than milk proteins (63). Zein and gliadin produced lesser quantities of liver nitrogen than the milk proteins, casein and lactalbumin. However, gliadin was better than zein. The addition of cystine or methionine improved the liver protein regeneration.

The effect of milder deficiencies on the liver is not known, since liver biopsy in man obviously cannot be done routinely to detect the degree of malnutrition. Animal liver biopsy, therefore, remains the best research tool in laboratory investigations.

Blood proteins. In field studies investigating protein malnutrition it is simple to obtain blood samples to appraise the protein status of a population. "In spite of all the advances in this field of nutritional biochemistry, there is no new test available which can be used in field surveys as an indicator of protein malnutrition" (64). The most widely used laboratory values on blood as an appraisal of protein status are the hemoglobin content, the total protein content and serum-protein spectrum, and the hematocrit (65).

Whipple (66) concluded that there were reserves of protein in tissues that contributed to the maintenance of plasma protein concentration in fasting or dietary protein deficiency. Those plasma proteins may exceed

in quantity the dietary protein in conditions of protein restriction. The efficiency with which dietary proteins are used for the formation of blood proteins has been studied widely (67, 68, 69, 70, 71), and used extensively as a measure of nutritive value. Whipple (72, 73) demonstrated that foodstuffs vary in their ability to regenerate hemoglobin and plasma proteins. Liver was used efficiently to build both, whereas, egg white, casein, soybean meal and salmon bread were used more efficiently for plasma proteins.

The lowering of serum amino acid levels was cited as a sign of deteriorating nutritional status (74). Forty-eight hours after fasting, valine and leucine were observed to increase as a result of tissue protein breakdown (75), while values for threonine, lysine, methionine, arginine and tryptophan dropped. Cystine appears to be the most important amino acid in building of plasma protein regeneration (76). Albanese et al. (77) found that a cystine deficient diet did not affect blood protein levels in rats, but if deficient in methionine and cystine, hypoprotinemia and anemia resulted.

The quantity of hemoglobin is a useful index for establishing malnutrition in the widest sense, even if anemia is unspecific (65). According to Goldsmith (78), in protein deficiency there is always macrocytic anemia because of a deficiency of Vitamin B_{12}. Hemoglobin anabolism was affected by inadequate protein intake (79), and chronic anemia was produced in rats by feeding them diets low in protein (80). Dietary deficiency of methionine and tryptophan produced marked anemia (77). Using protein-depleted rats, Sebrell and McDaniel (81) observed that histidine values and leucine deficiencies had a marked effect on the recovery of red blood cells, whereas arginine, methionine and tryptophan had little effect

on hemoglobin regeneration. Isoleucine and lysine did not affect hemoglobin regeneration to the extent as the other amino acids.

Protein and amino acid requirements of man. The accepted technique to determine any nutritional requirement is to establish the minimum requirement and add an allowance of safety to arrive at the recommended figure. The determination of minimal protein requirements is based on two premises: (a) that the maintenance requirement is proportional to the basal metabolic rate, and (b) that a protein with a biological value of 100 is completely utilized for maintenance and growth (27).

The first premise is a biological constant due to catabolism under basal conditions, represented by nitrogenous losses in the urine. Extensive work by Terroine and Sorg-Matter (82), Smuts (83) and Brody (84) indicated that in several animals studied, including man, the minimum level of urinary nitrogen excretion is proportional to the basal metabolic rate and approximates 2 mg of nitrogen per basal calorie. This value is not minimal for man (85). The urinary loss of nitrogen has been expressed mathematically in terms of body weight as $N = 146\ W^{0.72}$.

Hegsted et al. (86) indicated that the nitrogen requirement of an individual is more closely related to the body surface than body weight.

A measure of minimal excretion of nitrogen based on endogenous nitrogen alone is inaccurate without considering fecal and dermal losses. The origin of fecal nitrogen is not known with certainty. It may be mostly endogenous in origin (87, 88), and is influenced by the bulk of food consumed (89). Variability of the diet affect both composition and amount of fecal nitrogen. The loss it represents is covered by allowing a 10% intake above the estimated requirement based on urinary nitrogen excretion.

Hegsted et al. (86) found that total fecal losses were 0.8 to 1.1 gm per day on diets supplying 4.5 gm of nitrogen.

Losses through skin and hair are difficult to measure and have rarely been considered in nitrogen balance studies. A recent review of the limited evidence available was presented by Mitchell and Edman (90). Conflicting data, and the technical difficulties in accurate assessment make it difficult to estimate an allowance for that loss.

The second factor is associated with the biological value of the protein in the diet and the catabolism of such amino acids as are used in daily metabolism. Such requirements represent an ideal distribution of particular amino acids. Proteins of animal sources usually are considered better than plant sources. Hegsted et al. (86) showed that when one-third of an all-vegetable diet was replaced by meat, the protein requirement of adults was 17% less than on the vegetable diet. They recommend that half the dietary protein should be of animal origin. When protein intake exceeds the ability of the organism to utilize it the biological value falls (15).

Another factor to be considered in mixed diets is the indigestibility of some proteins and their incomplete digestion. On an average, the loss from that factor is estimated at 8% (91).

The relationship between growth patterns and calorie-protein intakes resulted in the expression of protein requirement in terms of calories (92). An adequate figure of 15% of the total calorie intake from protein was suggested by Johnston (93). Hegsted (94) proposed a theoretical approach to estimating protein requirement, based on the basal metabolism of children and the biological values of various proteins. This method

takes into consideration both the basic premises for evaluating minimum protein requirements.

In the light of present knowledge on amino acids the term "protein requirement" may be considered vague and outmoded. Living organisms do not need protein as such, but they require nitrogen, which is available from the amino acids in the protein.

The primary data on man were reported by Rose (95, 96) for adult men; by Leverton (97), Swendsied (98), Swendsied et al. (99), and Jones et al. (100) for adult women. The criteria for adequacy have been uniformly measured by nitrogen balance. Rose defined the minimum requirement as "the smallest amount capable of inducing a distinctly positive nitrogen balance," and gave the value for the highest requirement of any individual in the group. Leverton denoted it as the amount that "will keep a subject in a zone of nitrogen equilibrium, where the difference between intake and excretion is within $\pm$ 5 per cent."

Rose et al. (101) concluded that caloric requirements to maintain nitrogen equilibrium were considerably higher on an amino acid-containing diet than on a whole protein diet. Later studies by Metta et al. (102) failed to demonstrate a relationship between caloric need and amino acid intake.

Evidence on the nutritional requirements of man are derived from three sources: (a) nutrition surveys, or epidemiologic data on appropriate large groups in relation to nutrient intake, (b) controlled specific feeding experiments on a limited number of individuals and (c) critical metabolic and comparative studies on other species.

It generally is agreed that studies with laboratory animals are the

most exact, but cannot be applied directly to man until the proper baseline or method of conversion of the data to man has been demonstrated by studies on man himself (41). Because of the difficulties associated with obtaining specimens, limited information is available on man relative to the morphological and histological changes in various tissues and organs associated with malnutrition. Information on changes in structure and function of vital organs during fasting and feeding experiments has come mainly from animals.

Protein and amino acid requirements of the rat. In spite of the inadequacy of information for all the physiological processes, there are still sufficient data to permit a fairly accurate estimate of the nutrient needs of the rat, for growth, maintenance and reproduction (103). A deficiency of protein in the growing animal results in reduction of growth and body nitrogen (104) and a chronic deficiency leads to edema (105). Low protein diets result, also, in reduced food intake (106) that is reflected in growth rates.

The adequacy of a protein is related to three factors (a) energy concentration of the diet, (b) amino acid composition of the protein, and (c) digestibility of the protein (103). The Committee on Animal Nutrition (103) suggested an optimum ratio of net protein per gross kcal for weanling rats of 29 mg per kcal. If ingredients are selected to provide adequate amounts of essential amino acids, levels of 15 to 20% of protein are sufficient (107).

The amino acid requirements of rats are based on the studies of Rose et al. (108, 109) who used a 3% fat diet with adequate B vitamins. Rama Rao et al. (110) reported the essential amino acid requirements of the rat

using a diet containing 5% casein. The utilization of non-specific nitrogen sources in the synthesis of non-essential amino acids was studied by Rechcigl et al. (111) and Birnbaum et al. (112).

The rat in nutrition investigations. The rat is an excellent laboratory tool and possesses characteristics suitable for research intended for possible application to man (113). The value of the rat in experimental work is related to a number of factors such as small size, low cost of feeding, small space requirements, tractability, omnivorous dietary, short time span of reproduction, large litters and its conduciveness to standardization (113, 114).

Nutritional research has made valuable use of the rat in the last four decades (114). One of the earliest recorded use of rats for nutrition studies is that of Savory (115), who, more than 100 years ago, used them in nitrogen balance experiments, and showed that non-nitrogenous diets did not maintain life. Osborne and Mendel (29, 30, 31) made extensive use of the rat to determine protein quality by rat growth. Nutritional investigations using the rat allow for observation of gross changes and post-mortem pathologic investigation. Entire organs instead of fragments of organs can be studied because of their small size. The universal availability and use of the rat in nutrition research around the world makes it possible to adequately compare results of various investigators (116).

Assessment of Protein

Measurement of protein content. The most frequently used method of measuring the amount of protein in a given material is by means of the

nitrogen assay. The assumption that the nitrogen value consists essentially of protein is not strictly correct (27). The presence of large proportions of non-protein nitrogen, some of which are not nutritionally available (117, 118, 119) may exaggerate the true protein content of the food. This is especially true for many plant foods (118, 119), particularly roots and tubers. However, the assumption that dietary nitrogen occurs essentially as protein is reasonable in the case of mixed diets (27).

Protein content commonly is calculated by multiplying the nitrogen content by a factor, 6.25, since most proteins do not deviate markedly from a 16% nitrogen content. The use of this average theoretical value may be misleading for certain foods in which the percentage of nitrogen in the total protein is above 16% (120).

Automatic amino acid analytical techniques using column chromatography, and microbiological assay of amino acids have been used widely, and have their own merits. Information on amino acid content of foods has been assembled (34). Hughes (121) demonstrated that the amino acid content of mixed diets can be accurately predicted from published data of the amino acid composition of foods, but he found the application of such information to a vegetarian diet less successful.

Measurement of protein quality. In the determination of protein quality, one is concerned with the qualitative and quantitative adequacy of at least nine amino acids, the quantities supplied per unit of protein or nitrogen, and their balance and utilization (28). Tests designed to evaluate protein quality are of two kinds. First the quality of a dietary protein can be tested in relation to some function of the body such as

growth or nitrogen balance. Such tests do not demand any knowledge of the amino acid composition of the protein. Secondly, the pattern of amino acids present in the protein can be compared with the pattern required by the recipient. Such methods of evaluation of protein quality require precise information about the amino acid composition of the protein and also about the needs of the subject for individual amino acids. The different assay methods have different parameters of measurement and different end-points, and include biological, chemical and clinical evaluation.

Animal growth and protein efficiency ratios (PER). It generally is accepted that the rate of growth of weanling rats, under standardized conditions, estimates gain in body nitrogen, and is a reliable measure of dietary protein (122). Osborne et al. (31) first used growth of rats as a measure of nutritive value of protein and introduced the concept of "Protein Efficiency Ratio" (PER). This ratio is defined as the gain in body weight per gram of protein or nitrogen consumed.

The data from the study of Osborne et al. (31) showed that the better the quality of the protein, the lower the level in the diet required to produce a high PER. Other experiments indicated an increasing PER with increasing protein intake (123, 35). Where a single intake is used, a 10% level of protein gives the best comparison between various protein efficiency ratios.

Although gain in body weight generally correlates well with gain in body protein in the rat (124, 125), this is not always reliable. Gain in body weight may not be constant in composition for different proteins, and may be a result of addition of constituents other than protein, such as fat and water (15, 126).

Another fundamental criticism of PER is that this ratio makes no allowances for maintenance, but assumes that nitrogen intake is only available for growth. Attempts to avoid this criticism have been made by Bender and Doell (127) by their "Net Protein Ratio" and Allison (36) by the "Nitrogen Growth Indexes."

The above disadvantages of the PER method do not constitute a serious criticism of the method when carried out under standardized conditions (126). Those disadvantages do not apply to the assessment of protein quality by the nitrogen balance method, which can be used on adults as well as growing subjects and is applicable to studies on man.

Nitrogen balance and biological value. The intake of nitrogen, its retention in the body, and its excretion; and the interrelationship of growth and maintenance can be summarized in a limited way through the concept of nitrogen balance (128). Nitrogen balance is a direct measurement of nitrogen retention and is the difference between dietary nitrogen intake and nitrogen excreted in the urine and feces plus dermal losses (128). The accepted practice is to compute nitrogen balance from output of nitrogen in the urine and feces (28) as the values for dermal losses in dogs and man are believed to be negligible (15).

The validity of nitrogen balance studies is questioned by some research workers. Costa (129) observed nitrogen retentions over a long period of time, without a corresponding gain in weight, and Costa concluded that loss of dietary nitrogen may have been via an unknown route. Wallace (130) pointed out earlier that the errors of nitrogen balance determination, namely, overestimation of nitrogen intake as a result of diet unconsumed, and underestimation of nitrogen output from excreta lost,

favor a positive nitrogen balance, and the resulting cumulative error can be considerable.

There is evidence of concordance between weight changes and nitrogen balance data in adult rats (131) and in growing chicks (132). In the latter instance a close agreement was found between the nitrogen retention of chicks by nitrogen balance and carcass analysis.

The most useful measurement of nitrogen balance is the determination of Biological Value. Thomas (133) defined the Biological Value of dietary protein as the fraction of absorbed nitrogen retained in the body for maintenance and growth. He published the now classical procedure for measuring biological values of proteins by nitrogen balance determinations. Later this method was used by Mitchell (134) with growing rats to determine the requirements for both growth and maintenance. The method of determining biological value, in practice today, is known as the Thomas-Mitchell nitrogen balance method.

The calculation of the biological value requires an estimation of the amount of nitrogen absorbed into the body, and the amount of absorbed nitrogen retained. From the discussion, earlier, on the dynamic state of nitrogen metabolism, it is clear that both diet and the body contribute to the metabolic pool, a portion of which is excreted continually. Allison and Anderson (135) demonstrated a linear relationship between nitrogen balance and nitrogen absorbed in dogs in negative or low positive balance. Bricker et al. (85) demonstrated a linear relationship of those parameters in man, and in rats (136). This linear correlation makes the calculation of biological value possible if it is assumed that endogenous excretion of urinary and fecal nitrogen is constant and independent of nitrogen intake

(85, 135, 136). The original determinations corrected for fecal and urinary losses for endogenous nitrogen, which was measured by a nitrogen-free diet, with the protein test diet being a low protein level and isocaloric to the nitrogen free diet. Today suboptimal levels are used to measure endogenous nitrogen (126).

There is evidence that feeding nitrogen may conserve body nitrogen, thus giving unusually high biological values (137, 138, 139, 140). Results of those studies were interpreted to mean that excretion of body nitrogen was lesser during nitrogen feeding than during a protein-free diet. High biological values in protein-depleted animals were reported by Allison (141).

The biological value of a protein depends on the level of protein in the diet. A high biological value on low levels of protein were reported by Chick et al. (142). On levels higher than 4%, utilization of nitrogen fell progressively (142). Henry and Kon (143) indicated efficient utilization of proteins at levels below 12% of the diet. In consequence, determinations of biological value to assess protein quality are carried out at suboptimal levels of protein intake (144) and represent relative merits of different proteins in partly protein-depleted rats, assuming the maintenance of high levels of labile protein stores on these protein values (27). Therefore, studies of nitrogen balance are supplemented by examination of dietary protein quality on the protein content of individual tissues, which are especially sensitive to protein level of the diet. Henry et al. (145, 146) reported fair agreement between the ranking of relative values of various dietary proteins in the deposition of labile liver proteins, and the determination of biological value by the nitrogen

balance method. For the measurement of the effects of amino acid supplementation or imbalance, and for comparing the protein value of diets at the protein level commonly consumed, intakes may approximate requirements (126).

Nitrogen balance methods have the disadvantage of being expensive and involve time consuming analyses. Compared to growth methods, however they have the advantage of being relatively rapid. Balance studies on each experimental diet are required for three successive 3-day balance periods, following a suitable period of initial adaptation, to obtain valid results in evaluating protein quality (126). Nitrogen-balance methods are useful, reliable and reproducible, providing certain precautions and standard procedures are observed (27, 126, 144).

Amino acid patterns and protein quality. The ability of dietary protein to meet nitrogen requirements varies, a variable that is an expression of nutritive value (141). The biological value of egg proteins is close to 100, interpreted to mean that all the absorbed nitrogen is retained in the body, or more correctly that the amino acids supplied by whole egg are in the proper proportion to provide maximum efficiency of utilization (144). Thus biological value estimates how closely the essential amino acid composition of a protein conforms to the amino acids utilized most efficiently by the animal for protein synthesis. Attempts to demonstrate this are available in the literature (147, 148, 149).

Mitchell and Block (148) have compared the amino acid content of many foods with whole egg and proposed that the biological value of a protein may be estimated approximately from the maximum deficit of the most limiting amino acid relative to egg protein. This they called a "chemical

score." The use of Chemical Scoring in the assessment of protein quality of human diets compares favorably with biological values (150). The concept is valid in considering amino acid patterns for subgroups and smaller populations (126).

The concept of chemical scoring assumes that the nutritive value of a dietary protein is determined by its gross amino acid content. Availability of amino acids can be reduced by incomplete digestion and absorption (27), presence of inhibitors of digestive enzymes in legumes (151), and damage to food proteins and amino acids from heat treatment (152, 153).

A calculation of nutritive value from a consideration of amino acid patterns often is called a "Protein Score" (15). Oser (154) showed that the most limiting single amino acid may not determine biological value, but that all deficits contribute. He therefore proposed an "Essential Amino Acid Index." Recent studies emphasize the value of the "Chemical Scoring" method of Block and Mitchell (155) for its reliability and reproducibility.

Amino acid analyses of proteins can be an inadequate guide to their nutritive value, and need to be supplemented by biological methods, even though biological value and chemical score generally correlate well (28). The amounts of amino acids that escape absorption are measured by "true digestibility" (27). Platt et al. (157) introduced the term "Net Protein Utilization" to express in a single index both digestibility of the protein and the biological value of the amino acid mixture absorbed, i.e., it represents the amount of food nitrogen retained. The determination of net protein utilization by carcass analysis was described by Bender and Miller (158).

Improvement in patterns of amino acids supplied by diet is arrived at by supplementation. That requires reference patterns for estimating nutritive value of the protein sources, that are best for maintenance and growth. Besides egg proteins, discussed earlier, the essential amino acids of human milk for man have been proposed as a reference pattern by Gyorgy (159) and that of rat carcass analysis by William et al. (160).

The FAO Committee on Protein Needs decided to seek a provisional reference pattern based on available data on amino acid requirements of man for growth and maintenance (37). This pattern is calculated by assigning a unity to tryptophan or to threonine. An extensive evaluation of these reference patterns is available (126). Possibly the best reference pattern for estimating the nutritive value of dietary proteins will be developed from studies on amino acid requirements for maintenance, growth and metabolic needs (56).

This discussion of nutritive values of dietary protein is incomplete, without emphasizing the importance of balance between all constituents of the diet. Water, inorganic elements, amino acids, vitamins and fats and carbohydrates in adequate amounts and proportions are essential to the development and well being of each tissue through nitrogen metabolism (161, 162, 163, 164, 165, 166, 167).

Foods Consumed in India

General Dietary in India. In spite of the economic development through three 5-year plans (22) growth of population, low income, rising costs of living and inadequate supply of protective foods have kept the dietary pattern in India almost static during the last decade.

Simpson (23) stated that poverty, ignorance, traditional beliefs and customs are the main causes of undernutrition and malnutrition in the rural populations of India.

Diet and nutrition surveys (22, 24, 25) conducted in India reveal that the diets consumed by a vast majority of the people, regardless of socioeconomic status, are characterized by disproportionately high amounts of cereals and negligible amounts of protective and protein-rich foods. The mean intake of protein, fat, carbohydrates and calories during the period, 1953-1963, was 70g, 30g, 440g, 2325 calories per day, respectively. Of these, cereals contributed 70% of the protein, 35% fat and 80% total calories. Protein, though quantitatively adequate in these diets was qualitatively of low biological value, being largely of plant origin.

No significant change in dietary patterns were observed from 1953 to 1963. However, trends to decrease the use of cereals, pulses and vegetables, and increase milk, milk products, flesh foods and fats were discernible (22). Other nutrients deficient in diets of middle-class families were calcium, vitamin A, thiamine, and riboflavin (25).

Cereal consumption in India. Cereals such as rice and wheat, and millets such as sorghum (jowar) and ragi occupy a predominant position in the Indian diet (168). A study of diet survey records (24, 168) revealed that a combination of rice, wheat and millet are consumed in most parts of the country, and over the years a trend to include more than one cereal in the diet is noticed (24).

In Andhra Pradesh rice, wheat, sorghum and ragi are consumed. The production of principal crops in that state compared to the all India production, for the years 1963 and 1964 are presented in Table 1. The

percentage of area sown for each crop to the total area sown in Andhra Pradesh for 1963 and 1964 is given in Table 2. Andhra Pradesh produces a substantial amount of the country's sorghum, and devotes vast acreage, second only to rice, to its cultivation.

Sorghum as a staple food in India. The consumption level of sorghum in India is difficult to estimate, as it differs from State to State and District to District. However, considering the annual output, the per capita consumption would be 1.77 oz/day, with the Andhra Pradesh figure at 3.32 oz/day.

Protein levels of sorghum grain vary considerably (169, 170). The protein content of sorghum grown in Kansas ranged from 5.7% to 12.6%. Gopalan (171) analyzed sorghum varieties at Mysore and obtained values ranging from 7.17% to 12.14%. The amino acids limiting in sorghum grain are lysine, arginine, threonine, glycine and methionine. Waggle and Deyoe (170) demonstrated, that the higher the protein content, the greater the amounts of amino acids, with an increase in concentration of glutamic acid, proline, alanine, isoleucine, leucine, and phenylalanine, and a decrease of amino acids lysine, histidine, arginine, threonine, and glycine. The amino acids whose concentration was unaffected by protein levels were aspartic acid, serine, valine, and methionine. Waggle et al. (169) performed rat growth studies and demonstrated that sorghum grain of low protein values promoted growth better than sorghum grain of high protein levels.

The biological value and digestibility of the proteins of sorghum fed at the 5% level were 83% and 91% respectively, by Swaminathan (172). Later he reported the PER values at a 5% level to be 0.78 (173). At a

TABLE 1

The position of Andhra Pradesh as compared to all India acreage and production of principal crops for 1963-64*

S. no.	Name of crop	Estimated area in 1000 acres				Estimated area in 1000 acres			
		All India	Andhra Pradesh	State % age	State rank India	All India	Andhra Pradesh	State % age	State Rank
1	Rice	87,650	8,298	9.5	6	35,913	4,274	11.9	4
2	Wheat	32,878	51	0.2	13	9,555	5	—	13
3	Jowar	44,910	6,472	14.4	3	9,081	1,377	15.2	2
4	Bajra	26,713	1,492	5.6	6	3,677	328	8.9	5
5	Ragi	5,841	844	14.4	3	1,823	284	15.6	3
6	Maize	11,234	500	4.5	8	4,456	161	3.6	9
7	Small millets	11,402	2,355	20.7	2	1,999	398	19.9	1
8	Total millets	100,000	11,663	11.7	-	21,036	2,552	12.1	-
9	Total millets and cereals	220,637	20,012	9.1	-	66,504	6,831	10.2	

*Personal communication Bureau of Economics and Statistics Hyderabad, A. P., India.

TABLE 2

The percentage of area sown under each of the principal crops to the total area sown under all crops in Andhra Pradesh for the years 1963-64*

	Crop	Current	Previous	Normal
1.	Paddy	26.3	27.1	26.1
2.	Wheat	0.2	0.2	0.2
3.	Jowar	20.5	20.7	21.8
4.	Bajra	4.7	4.8	5.1
5.	Ragi	2.7	2.6	2.8
6.	Maize	1.6	1.6	1.6
7.	Korra	4.1	4.5	4.8
8.	Variga	1.8	1.7	2.1
9.	Samai	0.6	0.8	0.8
10.	Other millets	1.0	1.1	1.0
11.	Total small millets	7.5	8.1	8.7
12.	Total millets	37.0	37.7	40.0
13.	Total cereal and millets	63.5	65.0	66.3

*Personal communication Bureau of Economics and Statistics Hyderabad, A. P., India.

ten per cent level, PER values of 1.61 have been obtained by Phansalkar and Ramachandran, and a PER of 1.2 at the 8% level (174).

The study of the nutritive value of a poor vegetarian diet based on sorghum, indicated its superiority in promoting growth in rats, over a similar diet based on rice. Diets containing sorghum as the only source of protein, supplemented with B vitamins and minerals, were inferior to similar diets based on wheat (171).

The effect of partial (25 or 50%) or complete replacement of rice by sorghum in poor vegetarian diets did not affect the over-all nutritive value of the diet as judged by growth of rats, and there was no significant difference in the hemoglobin and red blood cell count or average fat content of livers (171). The metabolism of nitrogen, calcium and phosphorous in children fed a poor diet based on jowar was studied by Kurien et al. (175). Positive balance for nitrogen, calcium and phosphorous was reported. The apparent digestibility was 55.4%. Replacement of sorghum in a poor rice diet resulted in a decrease of retention of nitrogen and calcium and increase retention of phosphorous in these children.

Wheat as a staple food in India. The consumption pattern of cereals and millets (24) in Andhra Pradesh indicate that wheat is consumed to a lesser degree than sorghum. Wheat proteins vary greatly in protein content. Watson et al. (176) investigated quality potential of wheat from several developing nations and report variations of 13.8% to 19.8% in 1965. Percentage composition of United States wheats vary from 6.00% to 22% in protein content (177, 178). Wheat proteins are limiting in lysine (179).

Protein Supplementation and New Protein Rich Foods

Dietary protein supplementation. The most promising vegetable protein-rich raw materials available in developing countries are legume seeds, oil seed meals and cakes, and palm kernels (11, 180). Legumes represent that vast group of vegetable foods available to draw upon for any real attack on the protein problem (11). Their quality as single proteins is not better than cereals; however they are good sources of lysine, and therefore can supplement cereals. The peanut (arachis hypogaea), soybean (glycine max), common bean (phaseolus vulgaris), and chick pea (cices avientinum) are the legumes most investigated. Of the oilseed meals and cakes, cottonseed, soybean, and peanuts have been considerably investigated because of their availability in large quantities. Other possible oilseed sources are sesame, safflower, rape, mustard, poppy, linseed and hemp. Coconut alone of the palm kernels has received attention.

Among various solutions to overcome the problem of malnutrition, Jeliffe (181) suggested that supplementary feeding programs appear to be promising and likely to be most effective. In recent years numerous countries have made efforts to develop protein-rich foods of vegetable origin, suitable for supplementing diets low in protein quality (11, 180, 182, 183, 186). Without using any animal products, adequate protein nutrition is possible with suitable mixtures of cereals, legumes and oil seed products (11). INCAP Vegetable Mixture 8, made up of lime treated maize flour, sesame flour, cottonseed flour, Torula yeast and Kikuyu leaf meal closely approximates the quality of milk protein as judged by growth experiments in rats, chicks and metabolic balance studies in children recovering from protein malnutrition (180, 184). INCAP Vegetable

Mixture 9B (Incaparina) contains whole ground maize, whole ground sorghum, cottonseed flour, Torula yeast, with Calcium and Vitamin A, and is highly acceptable as a gruel in Central America. Several formulas of Indian multipurpose food (MPF) have been developed (185) two of which, Mysore Food A and B, have undergone extensive biological trials in both animals and man. The former is based on bengal gram and peanut meal, and the latter is based on bengal gram, peanut meal and sesame flour. CSM is a new food for infants and children based on corn, soy and milk formulated by the USDA's Agriculture Research Service, and was successfully tested for acceptability in Southeast Asia and Latin America (186).

From a practical point of view, the elaboration of protein-rich vegetable mixtures involves the supplementation of cereal grains which form the staple food in most developing countries. Several cereal-legume mixtures have been successfully used in Ceylon, Africa, South America and India (187, 188, 189). The supplementation of cereal diets with vegetable mixtures (190, 191), with cooked leaf meal (192) and improvement of the nutritive value of cereal proteins by fortification with limiting amino acids (193), also, were reported.

In the search for new vegetable protein-rich foods, interest of late has been displayed in the upgrading and conversion of wheat millfeeds into products of greater stature, value and market stability (194, 195). Millfeeds have been shown to contain substantial amounts of good quality protein (196).

Supplementation of cereal proteins. The biological value of cereal grains is improved by adding the amount necessary to bring the level of the first limiting amino acid in such a way as to correct and improve the

balance or proportion among other essential amino acids (197). Bressani et al. (198, 199) made extensive studies on the amino acid supplementation of wheat. The addition of lysine, which was the most limiting factor, gave a nitrogen retention similar to milk or addition of all limiting amino acids. Relative amounts of the essential amino acids required for maximal response increased with the percentage of protein in the diet. The effect of supplementing a sorghum protein at the 8% level with lysine improved the PER values from 0.95 to 2.44, and the addition of threonine and lysine gave a PER of 2.92% (200). The PER of a poor sorghum diet at the 10% level was improved from 1.99 to a PER of 2.71 on supplementation with both amino acids.

The essential supplementation of dietary proteins has been emphasized as a means of meeting and overcoming protein shortage in developing countries (201). Supplementary value of a blend of groundnut flour, Bengal gram flour and coconut meal, to poor Indian diets based on cereals and millets was reported by Tasker et al. (202). Similar studies using full-fat soya flour, groundnut flour, fish flour, and cotton seed flour, in various combinations to supplement wheat flour, rice diets, and maize-tapioca diets are available (203, 204, 205, 206). The possibilities of better protein nutrition with balanced protein mixtures of cereal and legume blends have been reported (207, 208). Though quality of the proteins was significantly inferior to casein it was comparable to Incaparina.

The success of the use of Indian Multipurpose Food in India (209) and Incaparina in Latin America (210) in school lunch programs is indication of the benefits of supplementation. Protein foods based on groundnut protein isolate and skim milk powder to supplement diets of weaned infants

and preschool children are being investigated (211). Besides increasing standard sources of protein, international organizations have been considering new sources of protein to reduce the problem of malnutrition.

New protein rich foods. The pressing need for protein rich foods has urged several enlightened nutrition workers to try and fulfill the protein requirement by investigating various foods not utilized previously. Biological trials with lyophilized algae, fed at 50 to 100 g levels per day for 23 days had no apparent long-term ill effects on human beings (212). The possibility of mushroom mycelium to stave protein shortage has been explored by Litchfield (213) and Falanghe (214) and its future potential is promising. The amino acid composition of a protein hydrolysate from lichens was shown to compare with that of casein hydrolysate by Subramanian (215). Giok et al. (216) investigated the nutritional value of rubber-seed protein and suggested its possible use to supplement a maize diet. The enzymatic modification of the extractability of protein from coconut flour improves protein quality and decreases crude fiber (217), and its usefulness for infant feeding is recommended.

Agriculturists have been striving to improve the nutritional value of cereal grains by the use of gene mutations and selective breeding (218). Initial studies by Mertz and his associates (218) on rats with opaque-2 maize demonstrated more gain in body weight than with normal maize. The endosperms of this mutant are higher in lysine content.

Wheat protein concentrate. Recent interest in millfeeds as a potential human food has been gaining the attention of several investigators. Millfeeds have been shown to contain good quality protein (196). The milling operation concentrates essential amino acids in millfeeds, thus

on the basis of amino acid content, mill products have higher nutritional value than the wheat from which they are milled (219).

A complete knowledge of millfeeds is lacking. A review by Johnston (195) has been made. Mennell (221) has recommended that millfeeds be considered as a product rather than a by-product.

Fellers et al. (220) found that milling and sifting wheat millfeeds gave flours high in protein, low in fiber and suitable for use in food products. Coarse bean, fine bean and shorts at various moisture levels (3 to 17%) were milled. Flour yields were highest from shorts and lowest from coarse bean. Flour fractions were richer in protein and starch, slightly high in total sugars and fats, but lower in fiber, pentosan and ash, compared to millfeeds. Similar results are reported by Farrell et al. (222). It was observed by Fellers et al., that the millfeed flours could be further fractionated by a sifting process. These fractions differed considerably in composition, indicating a flexibility in the ability to obtain millfeed flours of desired composition.

At the present time millfeeds are used for animal feeding. Today, all countries are vitally concerned with the most efficient and economical use of wheat crops, that are a staple cereal in many parts of the world. The Millers National Federation are supporting investigations on millfeeds obtained with small-scale and pilot-plant experiments in various laboratories (219, 220, 221). Sullivan (223) reviewed the present availability, economics and use of wheat protein concentrates. Blends of 70% wheat and 30% wheat protein concentrate from shorts were evaluated in Cairo, Egypt, India and Pakistan, and are superior in nutritional quality to local wheat flour.

Grains are valuable sources of protein in developing countries. Sorghum, next to wheat, is a staple cereal consumed by large number of people in Eastern countries, and especially in India. Data on nutritive value of sorghum grain in comparison to wheat are limited. Lamb et al. (224) compared the nutritive value of three sorghum grains to wheat and recommended supplementation of sorghum grains before they are used to substitute for wheat in a diet.

EXPERIMENTAL PROCEDURES

Materials

The wheat protein concentrate (18.9% protein) used was a product obtained from shorts, from a mixture of hard red winter wheat varieties, supplied by the Dixie Portland Flour Mills, Arkansas City, Kansas. Wheat was a hard red winter variety 64-183 Concho containing 11% protein and the sorghum was C 18107 variety containing 12.2% protein. Both grains were obtained from the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas. The proximate analysis and amino acid content of the three products are presented in Tables 3 and 4.

Animals and Their Care

Thirty weanling male albino rats, of the Sprague-Dawley strain, ranging in weight from 38 to 52 g were used. The animals were distributed to individual metabolic cages ($8\frac{1}{2}$" x $4\frac{1}{2}$" x $2\frac{1}{4}$") to which the experimental diets were assigned randomly, so that each experimental diet had a homogenous group of rats in relation to body weight (Table 11, Appendix). The front and rear views of the metabolic-cage unit (Acme Metal Products Inc.)

TABLE 3

Proximate analysis of experimental food stuffs[1]

Food stuff	Protein Nx6.25	Ether extract	Crude fiber	Moisture	Ash	Nitrogen free extract	Carbohydrates
	%	%	%	%	%	%	%
Wheat	13.25	1.74	2.75	9.26	1.56	71.44	74.19
Wheat protein concentrate	22.13	6.16	4.19	7.01	5.74	54.77	58.96
Sorghum	12.06	3.42	2.12	9.04	1.89	71.47	73.59

[1]Analyzed by chemical laboratories, KSU.

TABLE 4

Amino acid analysis of wheat, wheat protein concentrate and sorghum[1]

Amino acid	% of Sample			% of Protein		
	Wheat	Wheat protein concentrate	Sorghum	Wheat	Wheat protein concentrate	Sorghum
Lysine	.351	.808	.238	2.953	4.278	2.057
Histidine	.280	.535	.270	2.356	2.832	2.333
Ammonia	.413	.590	.373	3.471	3.126	3.218
Arginine	.582	1.362	.429	4.896	7.206	3.700
Aspartic Acid	.584	1.326	.828	4.908	7.020	7.140
Threonine	.362	.670	.382	3.042	3.546	3.296
Serine	.581	.937	.532	4.888	4.161	4.591
Glutamic Acid	3.931	4.753	2.651	33.033	25.149	22.855
Proline	1.340	1.462	.980	11.260	7.742	8.451
Glycine	.510	.988	.347	4.292	5.230	2.993
Alanine	.436	.912	1.163	3.666	4.828	10.033
Half Cystine	.433	.426	.252	3.646	4.254	2.175
Valine	.488	.928	.604	4.105	4.915	5.210
Methionine	.136	.316	.139	1.149	1.672	1.203
Isoleucine	.440	.812	.495	3.705	4.295	4.271
Leucine	.808	1.374	1.696	6.793	7.274	14.620
Tyrosine	.370	.622	.489	3.109	3.294	4.217
Phenylalanine	.576	.874	.648	4.844	4.627	5.594

[1]Department of Grain Science and Industry, KSU, Kansas.

are shown in Fig. 1. Water bottles and feed cups were provided in the rear of the unit. The arrangement of baffle and feces cup in the removable collection funnel provided at the bottom of each cage allowed for separation of urine and feces. The urine was collected in Erlenmeyer flasks kept below the funnel. Since the metabolic cage unit consisted of only twenty-four cages, six animals were housed in circular individual metabolic cages, slightly different in construction.

Food and water were given ad libitum. Initially feed cups were filled once, and later twice, a day in the morning and evening. Water bottles were washed and refilled at the end of each collection period.

Urine and feces were collected daily, the baffle and funnel washed, dried and replaced, and the Erlenmeyer flask changed.

At the beginning of the experiment and at the end of each experimental period the animals were weighed to the nearest gram on a Toledo balance. The food intake of individual animals was recorded for each period, whereas an approximate measurement of water consumption was made during the last experimental period.

Experimental Periods

The experiment lasted 30 days, divided into six periods of 5 days each. The periods comprised of an initial adjustment period and five collection periods. Determinations of nitrogen balance and biological value were made for the initial four collection periods; growth and protein efficiency ratio (PER) studies for all five periods.

EXPLANATION OF FIGURE 1

Top — Front view of metabolic cages

Below — Rear view of metabolic cages

Experimental Diets

The experimental diets were comprised of a low nitrogen diet and five test diets. Table 5 describes the content and percentage composition respectively. The low nitrogen diet contained approximately 3% egg protein, and the test diets contained approximately 9% protein, from sources as indicated in the table. In the sorghum plus wheat protein concentrate diet 6.75% protein was from sorghum and 2.25% protein was from wheat protein concentrate. Lysine was added at the 0.2% level in the sorghum plus lysine diet. The diets were planned according to the method of the Association of Official Agricultural Chemists (228) to contain protein, 3% in the case of the low nitrogen diet, and 9% in test diets, with fat 8%, ash 5%, fiber 1% and the remainder, carbohydrate and moisture in all diets. Adjustment was made for fiber and moisture content to make diets comparable.

The wheat, hard red winter variety, and Concho sorghum grain was ground to pass through 70x mesh sieve. The wheat protein concentrate obtained from shorts was received already ground. All the ingredients, except vitamins, were weighed on a Tension balance. The vitamins were weighed on a Mettler analytical balance (type B6) and added to the diets prior to mixing. Vitamin E was dissolved in the oil. The diets were mixed in a 20 quart Hobart electric mixer (Model A-200-D) for one hour at medium speed, and stored in jars in a household refrigerator. The proximate analysis of the diets is presented in Table 6.

TABLE 5

Percentage composition of experimental diets

Ingredients	Diets					
	I LN	II W	III WPC	IV S	V S+WPC	VI S+L
Protein source	23.33	67.92	40.58	74.62	66.10	74.62
Cottonseed oil	8.00	8.00	8.00	8.00	8.00	8.00
U.S.P. XVI salt	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin mixture	1.00	1.00	1.00	1.00	1.00	1.00
Cellulose	1.86	1.86	1.86	1.86	1.86	1.86
Water	17.04	6.54	6.54	6.54	6.54	6.54
Cornstarch	43.77	9.68	37.02	2.98	11.50	2.98

Note: L. N. ——— Low nitrogen
W. ——— Wheat
WPC ——— Wheat protein concentrate
S ——— Sorghum
S+WPC ——— Sorghum + wheat protein concentrate
S+L ——— Sorghum + lysine

TABLE 6

Proximate analyses of the experimental diets[1]

Diet	Protein Nx6.25	Ether extract	Crude fiber	Moisture	Ash	Nitrogen free extract	Carbohydrates
	%	%	%	%	%	%	%
Wheat	10.44	8.95	1.85	9.20	4.10	65.48	67.33
Wheat protein concentrate	10.50	9.16	2.01	9.84	4.68	63.81	65.82
Sorghum	10.56	8.39	1.73	8.51	4.31	66.50	68.23
Sorghum + wheat protein concentrate	10.75	8.68	1.82	8.67	4.43	65.65	67.47
Sorghum + lysine	10.81	8.27	1.74	8.97	4.20	66.01	67.75
Sorghum, lysine + methionine	11.00	8.33	1.76	8.71	4.23	65.97	67.73

[1]Analyzed by chemical laboratories, KSU.

Collection, Preservation and Analysis of Samples

Urine. Each day of the collection period, the urine collected in individual flasks, was transferred to pharmaceutical bottles containing approximately 2 ml of toluene as a preservative. Flasks were rinsed with distilled water and rinsings added to the urine. Bottles were then stored in the refrigerator. At the end of each collection period, urine composites were made up to a volume of 250 ml with distilled water after addition of 3 ml concentrated hydrochloric acid, and composites were poured back into the pharmaceutical bottles. The composites were stored in the refrigerator until analyzed, and prior to analysis they were brought to room temperature. Bottles were inverted 50 times before obtaining the first aliquot and 25 times before the second aliquot for analysis. Duplicate samples of 50 ml aliquots each were analyzed for total nitrogen by the macro-Kjeldahl method with boric acid modifications (225). The procedure is given on page 83 of the Appendix.

Feces. Daily fecal material was collected from the fecal cup, separated from food and hair, placed in small wide-mouthed jars and covered with a cloth. At the end of each collection period the fecal composites for the period were dried in an oven at 65°C, cooled in a desiccator, weighed and ground to the size of a 40 mesh sieve in a Wiley laboratory mill. Samples of 1.5 g to 2.0 g were analyzed in duplicate by the macro-Kjeldahl method for total nitrogen.

Blood. At the end of the 30-day experimental period, samples of blood from the heart were drawn for hemoglobin and hematocrit determinations. Hemoglobin was determined by the cyanmethemoglobin method (225) with a Coleman Junior Spectrophotometer. The light transmission values

obtained were converted to milligrams of hemoglobin per 100 ml blood, using a standard curve. The capillary tubes with blood samples were centrifuged in a microhematocrit for 3 minutes. The volume of the packed blood cells was read as percentage of the whole blood using a Crito-cap microhematocrit tube reader.

Liver. At the end of the 30 day experimental period, the animals were fasted for 16 hours, anesthetized with ether and sacrificed. The whole liver was excised immediately, washed in distilled water to remove adhering blood, gently pressed in folds of filter paper to remove water, and weighed on a Mettler analytical balance. Each liver was wrapped in aluminum foil, frozen and stored in a freezer. Prior to analysis, frozen livers were defrosted overnight at refrigerator temperature. Each liver was sliced in approximately 1/16 to 1/32 inch slices as rapidly as possible and placed in weighing bottles. Duplicate samples were weighed into cotton pads of known weight. These cotton pads previously had been extracted with ether and dried. The liver samples on the cotton pads were dried in a vacuum oven at 110°C for 72 hr to constant weight. The dried samples were extracted with ethyl ether for 20 hr in a Goldfisch extractor. The method is given on page 86 of the Appendix. The percentage of fat was calculated from the original weights of frozen livers. The duplicate fat-free liver samples were analyzed for total nitrogen by the macro-Kjeldahl method.

Calculations. The following measurements of protein quality were calculated from the data collected.

1. Protein efficiency ratio (PER)

$$= \frac{\text{g gain in body wt.}}{\text{g protein ingested}}$$

2. Apparent digestibility coefficient

$$= \frac{\text{N intake} - \text{Fecal N}}{\text{N intake}} \times 100$$

3. True digestibility coefficient

$$= \frac{\text{N intake} - (\text{Fecal N} - \text{Metabolic N})}{\text{N intake}} \times 100$$

4. Biological value (BV)

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

5. Net protein utilization (NPU)

$$= \frac{\text{True digestibility} \times \text{Biological Value}}{100}$$

6. Feed efficiency ratio (FER)

$$= \frac{\text{g food ingested}}{\text{g gain in body wt.}}$$

Statistical analyses. Analyses of variance were computed for the test diets for both collection periods and total period for protein efficiency ratio, apparent and true digestibility coefficient, biological value, net protein utilization, and feed efficiency ratio. Correlation coefficient ratios were calculated for effect between the experimental diets.

RESULTS

Food Intake and Feed Efficiency Ratio—FER

The food intake of rats on various test diets for each experimental period is given in Table 12 of the appendix. The intake for all groups by period appeared to be constant within experimental limitations except on the group consuming the sorghum plus lysine diet. Analysis of variance of the feed efficiency ratio showed significant differences among diets, ranking from high to low in the following order: sorghum plus lysine, sorghum, sorghum plus wheat protein concentrate, wheat, and wheat protein concentrate. There were significant differences ($P<0.05$) between wheat protein concentrate, and the sorghum plus lysine, and sorghum diets; and again between wheat, and wheat protein concentrate and sorghum diets.

Growth

The average percentage cumulative weight gain of rats fed various test diets is shown in Fig. 2. Detailed data are in Table 13 in the Appendix. Animals fed the wheat protein concentrate diet had significantly ($P < 0.05$) higher weight gains than those on the other diets. There was no significant difference in growth performance between the sorghum plus wheat protein concentrate, and wheat. The wheat diet was significantly ($P < 0.05$) different from the sorghum and from the sorghum plus lysine diet. Analysis of variance for the individual periods of growth indicated a similar pattern with more marked variations for the sorghum plus lysine diet in the first period (Table 7).

EXPLANATION OF FIGURE 2

Per cent average cumulative weight gain of rats fed various test diets.

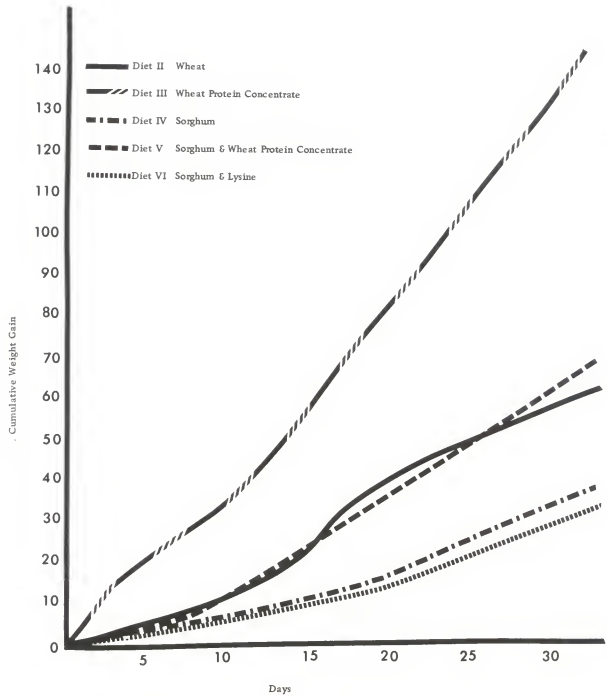


Fig. 2 Per cent average cumulative weight gain of rats fed various test diets.

TABLE 7

Initial body weights and weight gain of rats fed various diets at the end of adjustment and collection periods

Rat no.	Initial weight (g)	Adjustment period (g)	Period 1 (g)	Period 2 (g)	Period 3 (g)	Period 4 (g)	Period 5 (g)
Diet I. Low nitrogen							
3	42	41	44				
7	48	49	51				
24	46	47	44				
27	49	53	56				
28	47	50	54				
Diet II. Wheat							
1	52	50	54	63	67	72	77
4	48	50	51	57	64	70	74
11	45	47	53	59	64	70	75
21	46	47	52	60	64	70	73
23	39	40	43	49	52	58	65
Diet III. Wheat protein concentrate							
5	41	47	52	63	74	82	96
9	47	53	63	79	94	105	116
13	50	57	62	75	86	98	112
15	48	54	62	78	90	104	122
26	45	50	63	72	77	87	100
Diet IV. Sorghum							
2	46	45	45	49	51	55	58
16	45	47	48	52	55	57	61
17	49	49	51	56	62	65	68
20	52	51	54	58	59	64	69
22	41	44	45	49	52	54	58
Diet V. Sorghum and wheat protein concentrate							
8	49	49	54	63	68	76	81
14	44	46	50	58	63	67	75
19	46	46	49	55	60	66	73
29	48	52	57	61	66	71	76
30	47	48	53	60	66	71	76
Diet VI. Sorghum and lysine							
6	38	39	45	44	45	48	52
10	48	47	47	49	54	54	59
12	46	46	47	50	51	54	55
18	52	50	51	54	57	60	65
25	46	48	49	54	59	65	71

Protein Efficiency Ratio and Digestibility

The animals fed the wheat protein concentrate diet had the greater protein efficiency ratio, g gain/g body weight, followed by sorghum plus wheat protein concentrate, wheat, sorghum, and lastly sorghum plus lysine (Table 14 in Appendix). There was a significant difference ($P < 0.05$) between the sorghum plus lysine diet and the wheat protein concentrate, sorghum plus wheat protein concentrate; and between the wheat and wheat protein concentrate diets. The effect of the experimental periods was not a significant factor. A high negative correlation (-0.9098) was observed between the sorghum plus lysine and wheat protein concentrate diets. There was no significant difference in the apparent and true digestibility among test diets attributed to experimental period.

Biological Value

During the first period, for biological value there were significant differences ($P < 0.05$) between the wheat protein concentrate diet, and each of the following diets: sorghum, sorghum plus wheat protein concentrate, and sorghum plus lysine. In the fifth period there was a significant ($P < 0.05$) difference between sorghum plus lysine and all the other test diets. The sorghum diet was significantly ($P \leq 0.05$) different from the wheat protein concentrate diet and from the sorghum plus lysine diets, but not from the wheat and the sorghum diets. Sorghum and wheat diets were significantly ($P < 0.05$) different from the wheat protein concentrate and sorghum plus lysine diet.

Net Protein Utilization

In net protein utilization the sorghum plus wheat protein concentrate diet and the sorghum diet were significantly ($P < 0.05$) different from the wheat protein concentrate diet. A significant negative correlation ($r = -0.9098$) was observed between the wheat and the sorghum plus wheat protein concentrate diets. The effect of experimental periods was not significant (Table 8).

Liver

The data on protein, fat and total moisture on rat livers were not statistically analyzed because values were similar in all test diets (Table 15 in Appendix). Data in liver weight, liver weight/body weight ratio, protein, and moisture content are given in Table 9. There was a noticeable difference between the average fat content of livers of rats on the different diets placing them from high to low in the following order: sorghum plus wheat protein concentrate, sorghum plus lysine, sorghum, wheat, and wheat protein concentrate. The protein content of the livers varied inversely with fat content.

Blood

The average values for hemoglobin and hematocrit of rats on various test diets varied slightly, Table 9. However, the test diets could be ranked in the following manner in their ability to support hemoglobin levels: sorghum plus wheat protein concentrate, 11.28%; wheat protein concentrate, 10.86%; sorghum plus lysine, 9.98%; wheat, 9.95%; and sorghum, 7.96%. Hematocrit values were as follows: sorghum plus wheat

TABLE 8

Average and total values for the biological parameters of protein quality, based on experimental periods, for rats fed various test diets

Parameters of assay	Test diets	Experimental periods				Average for total experimental period
		1	2	3	4	
Apparent digestibility coefficient	II	81.53	73.48	72.40	46.84	68.49
	III	81.20	72.98	75.99	62.42	73.15
	IV	80.75	78.95	81.79	50.15	72.91
	V	79.28	80.66	82.60	59.00	75.39
	VI	79.83	81.91	80.50	49.81	73.01
True digestibility coefficient	II	91.12	82.34	79.55	58.90	77.98
	III	88.19	66.71	81.23	67.21	75.84
	IV	90.96	88.90	90.55	58.97	82.35
	V	88.41	88.19	89.69	66.00	83.07
	VI	76.87	92.12	90.04	59.18	79.55
Biological value	II	62.88	59.05	48.20	51.27	55.35
	III	78.92	55.30	69.13	62.66	66.50
	IV	54.35	52.14	59.47	33.98	49.99
	V	55.19	48.53	53.28	39.07	49.02
	VI	99.93	68.69	61.57	44.79	63.75
Net protein utilization	II	57.23	48.92	37.04	30.49	43.42
	III	71.61	43.10	56.65	42.27	53.41
	IV	49.36	46.56	53.93	20.66	42.63
	V	49.35	42.80	47.80	26.51	41.61
	VI	52.79	63.25	54.60	29.10	49.54
Feed efficiency ratio	II	8.86	6.00	7.22	9.26	7.84
	III	6.07	5.22	5.29	4.78	5.34
	IV	8.14	15.05	10.93	14.36	12.12
	V	7.37	7.83	8.10	8.22	7.88
	VI	15.20	4.40	20.00	14.01	13.40
Protein efficiency ratio	II	1.27	2.19	1.20	1.22	1.47
	III	1.95	2.74	2.18	1.88	2.19
	IV	0.50	1.49	0.92	0.98	0.97
	V	1.44	1.80	1.32	1.39	1.49
	VI	0.77	0.80	0.96	0.93	0.87

TABLE 9

Average values for liver and blood analyses for rats on various test diets

	Liver weight (g)	Liver wt/ body wt (g)	Fat %	Pro. %	Moisture %	Hemoglobin g/100 ml.	Hematocrit
Diet II Wheat	2.41127	0.03311	0.95	22.93	76.06	9.95	35.0
Diet III Wheat protein concentrate	3.32193	0.03041	0.88	24.24	73.61	10.86	38.3
Diet IV Sorghum	2.24502	0.03595	2.62	20.50	76.33	7.96	28.6
Diet V Sorghum plus wheat protein concentrate	2.33708	0.03067	1.21	22.03	75.06	11.28	42.6
Diet VI Sorghum plus lysine	2.20595	0.03660	1.39	21.64	75.66	9.98	21.8

protein concentrate, 42.6; wheat protein concentrate, 38.3; wheat 35.0, sorghum 28.6; and sorghum plus lysine, 21.8.

DISCUSSION

The superior ability of the wheat protein concentrate diet to promote growth in rats as compared to the other test diets indicated that the amino acids were available to the animal in proportions needed for growth. The anabolic activity of the body proteins for both essential and nonessential amino acids and reserve proteins exceeded the catabolic activity thus resulting in a net gain in body nitrogen, reflected in growth and PER values. Supplementing sorghum with the wheat protein concentrate brought the growth promoting level up to that of wheat, indicating that the amino acids in wheat protein concentrate did improve the sorghum amino acid patterns. The supplementation of sorghum with lysine did not result in an increase in growth. The lack of growth spurt upon supplementation of sorghum with lysine perhaps may be attributed to the imbalance of the amino acid pattern of the resulting mixture (27). This finding is important since the fortification of a main staple cereal grain with lysine resulted in a decrease in growth rate in rats rather than the expected increase. At present, in certain sections of India, sorghum is used solely as the main source of nutrients in the diet. On the basis of the findings in this study where lysine plus sorghum affected growth of rats adversely, the fortification of Indian sorghum diets with lysine should not be recommended without additional research.

The enrichment of one amino acid to the exclusion of others can cause an increased need for another nutrient, especially in the growing

animal (15). This factor was observed by comparison of the growth curve for animals on the sorghum, and sorghum plus lysine diets. The two parallel each other for a while, but as the growth of the animal increases the demand for nutrients, the sorghum plus lysine diet no longer was sufficient and growth declined.

The depressed growth rate induced by a diet of sorghum plus lysine may be attributed to (a) amino acid imbalance, (b) amino acid toxicity, (c) amino acid antagonist. Salmon (224) suggested that the depressed growth rate was a result of the biochemical response to the amino acid imbalance. The excess of amino acid causes an amino acid imbalance, which stimulates amino acid catabolism and excretion. This results in some loss of amino acid that is already limiting or marginal for growth. The growth depression from amino acid imbalance can be prevented only by increasing the level of the limiting amino acid in the diet. Another possible contributing factor could be competition among amino acids leading to delayed absorption and transportation. This has not been widely investigated, but the work of Rosenberg et al. (227) showed the addition of lysine to a rice diet caused an imbalance corrected by threonine and addition of threonine caused an imbalance corrected by lysine.

Sauberlich (228) studied the toxic effect of the excessive intake of amino acids. Inclusion of 5% l-lysine resulted in a decrease in the growth rate. The growth depression caused by an excess of an individual amino acid is more severe when the diet is low in protein, indicating that the organism can tolerate an excess of one better, when it has an adequate supply of all others. In this study there was a significant difference (P 0.05) between sorghum plus lysine and wheat protein concentrate on

growth and net protein utilization. The sorghum plus wheat protein concentrate diets were significantly different from sorghum plus lysine ($P < 0.05$) on biological value. The greater toxicity of amino acids on low protein diets suggests enzyme limitation especially in diets low in pyrodoxine, riboflavin and cobalamine.

Jones (229) demonstrated the lysine-arginine antagonism. Excess lysine increased the arginine requirement for the rat.

The basis for the adverse effects attributed to the amino acid imbalances, antagonism and toxicities have not been established (27). The most consistent and general finding is that the amino acid pattern of blood and body fluids is more frequently out of balance than the pattern of the diet producing the adverse effect.

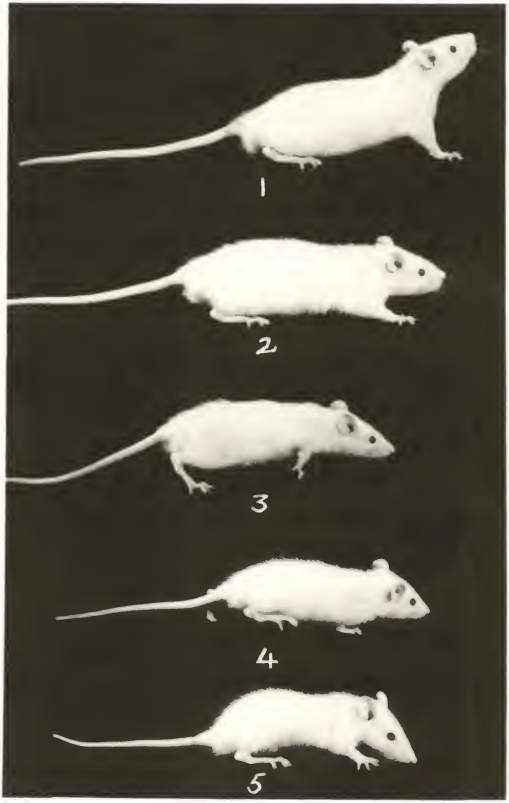
Rats receiving wheat protein concentrate diets appeared the best (Fig. 3), and those on the sorghum plus lysine diet were in poor condition with poor body form and scanty hair. Sorghum plus wheat protein concentrate was next to wheat protein concentrate and superior to wheat and sorghum diets.

The average weights of livers of rats on each test diet were proportional to the protein content of the livers. Wheat protein concentrate was utilized the best in terms of reserve protein in the liver. It is known that excess above sufficient protein in the diet for growth and maintenance are channeled to depot stores. Though the rats on sorghum plus wheat protein concentrate diets stored less protein in the liver, blood proteins were high. This is perhaps a result of the maintenance of labile blood proteins by liver protein reserves on an adequate protein diet. A deficiency in the quality of protein causes increased liver fat

EXPLANATION OF FIGURE 3

Photographs of representative rats from each group of test diets in the following order.

1. Diet III Wheat Protein Concentrate
2. Diet V Sorghum plus Wheat Protein Concentrate
3. Diet II Wheat
4. Diet IV Sorghum
5. Diet VI Sorghum plus lysine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deposition (57). The wheat protein concentrate diet produced least fat deposition in rat livers. An inverse relationship between fat and protein content indicates the utilization of amino acids on diets of adequate protein quality going to growth and maintenance, and on poor quality protein diets, the amino acids are utilized for energy purposes.

The normal average standard values for hemoglobin and hematocrit for rats are 14.8 g/100 ml Hb, and 46% hematocrit (230) with a range of 12-17.5 g/100 ml for Hb, and 39-53% hematocrit. The average values obtained for various test diets (Table 9) indicate that for Hb levels, sorghum plus wheat protein concentrate, and wheat protein concentrate are closer to these standards than the other diets. Values for hematocrit for rats on the sorghum plus wheat protein concentrate diet fall within the range for normal hematocrit values. The hemoglobin level is maintained at the expense of other labile proteins. The sorghum plus wheat protein concentrate diet had low protein levels for livers compared to wheat, and wheat protein concentrate. Apparently the amount of amino acids in the sorghum plus wheat protein concentrate diet was more conducive to maximum blood protein maintenance than those from the sorghum only diet.

The poor response of the rats on the sorghum plus lysine diet indicates that lysine did not improve the pattern of amino acids in sorghum for the building of blood. Yoshimura (231) has suggested that protein deficiency affects hemoglobin levels by affecting enzyme systems, thus depressing the metabolic activity of erythrocytes, which in time reduces resistance of the erythrocyte membrane of the animal.

The digestibility among all the diets was not significantly different, hence the biological values and net protein utilization are comparable so

far as this parameter is concerned. The ranking of sorghum plus lysine second for biological value and net protein utilization and last on the growth producing basis appears inconsistent. The average values as presented in Table 9, show that the sorghum plus wheat protein concentrate diet was inferior to the wheat diet. These values were considered somewhat inaccurate because of the problems encountered with one rat in the group (Table 16 in the Appendix). Rat no. 25 excreted such small quantities of urine that the collection was difficult to make. If the data for Rat 25 were excluded, the corrected biological values and net protein utilization would result in the sorghum plus wheat protein concentrate diet being ranked second to the best diet—wheat protein concentrate (Table 10). Bressani et al. (232) stated that a good amino acid supplement tends to produce a sustained response, while a poor or less satisfactory supplement causes a negative balance or a transient increase in nitrogen retention caused by a temporary benefit from filling N needs with incomplete protein which do not require the same amino acid proportions as overall growth and maintenance. A decrease in nitrogen retention following an initial use caused by an amino acid change suggests the amino acid supplementation is inadequate. It is, therefore, necessary to consider the effect of excesses of lysine on a sorghum diet. The relative participation of growth and maintenance in making up the biological value varies markedly depending upon the quantity and the nutritive quality of protein that is ingested (233). When a diet is deficient in protein, a greater amount of nitrogen resulting from catabolic processes, may not be excreted but be cycled into anabolic pathways. Urinary and fecal nitrogen are therefore less in animals on a diet of low protein, than those on adequate

TABLE 10

Diets as classified from greatest to least effect for each parameter

	Growth	PER	BV	NPU	Liver			Corrected		Blood	
					Protein	Fat	Weight	BV	NPU	Hb	Hematocrit
D	WPC	WPC	WPC	WPC	WPC	S+WPC	WPC	WPC		S+WPC	S+WPC
I	S+WPC	S+WPC	S+L	S+L	W	S+L	W	S+WPC		WPC	WPC
E	W	W	W	W	S	S	S	S+L		S+L	W
T	S	S	S+WPC	S+WPC	S+WPC	W	S+WPC	W		W	S
S	S+L	S+L	S	S	S+L	WPC	S+L	S		S	S+L

Legend:

Diets

WPC . . . Wheat Protein Concentrate
S+WPC . . Sorghum plus Wheat Protein Concentrate
W . . . . Wheat
S . . . . Sorghum
S+L . . . Sorghum plus Lysine

Parameters

PER . . . Protein Efficiency Ratio
BV . . . Biological Value
NPU . . . Net Protein Utilization
Hb . . . Hemoglobin

protein intake. In the experiment, the sorghum diet had the lowest biological value and net protein utilization which was significantly increased by the addition of wheat protein concentrate. The sorghum supplemented with wheat protein concentrate was of such improved protein quality as to promote growth, and to maintain protein reserves. The amount digested, retained and released compared positively (Table 16 in the Appendix).

Merely increasing the intake of a poor quality protein can compensate for part of its qualitative deficiency and result in greater absolute nitrogen retention (234). Yoshimura (231), after a series of studies, concluded that consumption of larger amounts of low quality proteins is better than low amounts of high quality protein. Physiological effects of protein deficiency are more serious with lower intake of animal protein, than those with vegetable protein.

The developing countries can benefit from this observation by raising poor quality proteins in the diets to a higher level of protein quality by supplementation even though the resulting level is below that of animal protein. The improvement of sorghum proteins by the addition of wheat protein concentrate in this initial study using rats as the experimental animal, suggests a great potential use of this concentrate, even though it is now used primarily as an animal feed. The sorghum grains in the United States, too, are used only as animal feeds, but in many other countries of the world, the sorghums are used by the people as major source of their food.

Sullivan (223) suggested several uses of wheat products and explored the possible use of wheat protein concentrate in developing countries,

particularly those that receive large wheat supplies now from the U.S.A. The expanded market possibilities for wheat mill feeds on the basis of their chemical constituents has been considered (235) but biological trials are lacking. The present study provides some evidence on the basis of the important biological trials using rats as the experimental animal. This evidence would indicate that the use of wheat protein concentrate as a supplement for improved diets for people in developing countries is promising. The wheat protein concentrate is now available even though it is used in animal feed in the U.S.A. Use of the concentrate as a supplement in diets for people in countries such as India could be initiated rapidly and thereby make a much needed contribution to the solution of serious malnutrition problems in the world.

SUMMARY

The effect of supplementing a sorghum diet with wheat protein concentrate (obtained from millfeed from wheat shorts) and with lysine was investigated, using a wheat diet as a standard. Thirty weanling rats of the Sprague-Dawley strain were randomly divided into 6 groups and assigned to one of 6 diets, namely a low nitrogen diet based on 3% protein from whole egg, and 9% protein diets based on wheat, wheat protein concentrate, sorghum, sorghum plus wheat protein concentrate, and sorghum plus lysine. The first 5 days of the 30-day experimental period comprised the adjustment period, followed by 5 collection periods of 5 days each. Growth and protein efficiency ratios, nitrogen balance, biological value, net protein utilization, and estimation of liver and blood proteins were the parameters used to evaluate protein quality.

Rats on the wheat protein concentrate diet had significantly ($P < 0.05$) higher weight gains over those on the other test diets. Growth of rats on wheat and sorghum plus wheat protein concentrate diets did not differ significantly; however, the wheat diet was significantly different ($P < 0.05$) from the sorghum and sorghum plus lysine diets. The protein efficiency ratio, biological value, and net protein utilization were highest for the wheat protein concentrate diet, followed by the sorghum plus wheat protein concentrate diet, wheat diet, sorghum diet, and sorghum plus lysine diet. The protein content of liver from rats on diets ranked according to diet, highest to lowest, in the following order: wheat protein concentrate, wheat, sorghum, sorghum plus wheat protein concentrate, and sorghum plus lysine. There was an inverse relationship between protein and fat in rat liver and the protein quality of the diet. The higher the protein quality in the diet, the less liver fat; the lower the protein quality, the higher the liver fat value. Hemoglobin and hematocrit values were highest for sorghum and wheat protein concentrate diet followed by wheat protein concentrate, wheat, sorghum, and sorghum plus lysine diets.

Addition of lysine to the sorghum diet resulted in decreased growth rate and raises a question concerning lysine supplementation of this grain. The addition of wheat protein concentrate to sorghum markedly improved the response of test animals for all variables over those for sorghum alone.

On the basis of this study the supplementation of sorghum with wheat protein concentrate to improve protein quality is promising. The potential use of wheat protein concentrate in human diets merits investigation.

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APPENDIX

TABLE 11

Random distribution of animals and diets to cages

Cage and rat no.	Diet group	Initial weight, g
1	II	52
2	IV	46
3	I	42
4	II	48
5	III	41
6	VI	38
7	I	48
8	V	49
9	III	47
10	VI	48
11	II	45
12	VI	46
13	III	50
14	V	44
15	III	48
16	IV	45
17	IV	49
18	VI	52
19	V	46
20	IV	52
21	II	46
22	IV	41
23	III	39
24	I	46
25	VI	46
26	III	45
27	I	49
28	I	47
29	V	48
30	V	47

Nitrogen Determination by the Macro-Kjeldahl Method

Reagents and solutions

Sulphuric acid:	93-98% H_2SO_4; nitrogen-free
Mercuric oxide:	red; reagent grade; nitrogen-free
Potassium sulphate:	reagent grade; nitrogen-grade
Potassium sulphide solution:	dissolve 40 gm of commercial K_2S in 1 litre of water
Sodium hydroxide:	flakes; nitrogen-free; dissolve approximately 450 gm solid NaOH in water and dilute to 1 litre (specific gravity of the solution should be 1.36 or higher)
Zinc granules:	reagent grade
Mixed indicator:	dissolve 0.125 gm methyl red and 0.083 gm methylene blue in 100 ml of 95% alcohol
Sulphuric acid standard solution:	0.1 N; dilute 28.4 ml H_2SO_4 (approximately 94% H_2SO_4) to 10 litres
Boric acid:	4% solution

Standardization of the acid. Accurately weigh enough Na_2CO_3 (about 0.2 gm) to titrate approximately 40 ml of acid and transfer to a 300 ml flask. Add 40 ml of CO_2 free water and stopped flask. Swirl gently until the sample dissolves. Add 4 drops of methyl red and titrate with the solution that is to be standardized. Normality of the acid is given by:

$$\frac{\text{gm } Na_2CO_3 \times 1000}{\text{ml acid} \times 52.997}$$

Procedure

I. Sampling:

a. Urine: Invert and revolve sample bottle 50 times for the first sample aliquot. Repeat another 25 times for duplicate sample.

Pipette 50 ml vito 500 ml Kjeldahl flask.

b. Feces: Accurately weigh duplicate samples of ground feces (between 1-2 gm). Secure in nitrogen-free filter paper in a packet. Place in 500 ml Kjeldahl flask.

II. Digestion:

Add to the sample approximately:

0.7 gm	HgO
15 gm	K_2SO_4 (powdered)
25 ml	H_2SO_4

Shake flask to insure that the contents are well mixed. Place in the digestion rack and heat until the mixture has turned light green, a very pale straw color or completely clear. (Start digestion slowly, increasing heat gradually. Undigested portions adhering to the sides of the flask may be digested by gentle rotation. After foaming and bumping heat strongly for rapid digestion. Wash down particles in the neck of the flask with distilled water, after cooling. If material becomes solid, cool flask, and add approximately 5 ml of sulphuric acid and redigest.) Continue heating for one-half hour after contents clear to insure complete digestion. Cool flask, add 200 ml distilled water and cool again.

III. Distillation:

Add 3 drops of mixed indicator and 50 ml of 4% boric acid into a 500 ml Erlenmeyer flask, and place it at the receiving end in the distillation rack, so that the lip of the glass tube is just below the surface of the acid. Turn on the cold water through the condensers. To the Kjeldahl flask add:

25 ml of potassium sulphide solution and mix to precipitate mercury, and a few zinc granules to prevent bumping.

Tilt flask and add layer of NaOH, enough to make the contents strongly alkaline, without agitation. Immediately connect flask to distilling bulb on condenser, shake flask until contents are thoroughly mixed, and apply heat immediately. Distill until distillate reaches the 225 ml mark. Lower the receiving flask so that the glass lip of the condenser is above the distillate. Remove the Erlenmeyer flasks and turn off the heat. Stopper if not titrated immediately.

IV. Titration:

Titrate with standard 0.1 N H_2SO_4. Compare the end point with a reference solution of 50 ml of 4% boric acid, 175 ml of distilled water and 3 drops of mixed indicator. This solution should be violet, with a pH 5.0.

Goldfisch's Fat Determination

Reagent: Ether

Procedure:

Weigh into a rectangular piece of filter paper, the weight of which is known approximately an accurate amount of the sample--about 2 gm. Record weight of filter paper and sample. Package the sample by means of an apothecary's packet.

Dry the sample in the vacuum oven at 110°C till a constant weight is obtained, about 24 hours. Deposit the weighed dried sample in the ceramic (alundum) thimble for extraction, such that it could be removed easily on completion. Place the thimble in the glass tube with the bulb at the end, and set it in the extractor. Measure 30-40 ml of anhydrous ether in the dried, weighed beakers. Place the beaker in the ring, attach to the extractor. Turn on the water for cooling. Turn on the heat, low. Allow extraction for 20-24 hours.

After extraction, remove tube and sample and attach reclaiming cup in its place. Slip safety cover over heating surface during the operation. Allow the ether to collect in the reclaiming cup. The fat in the beaker should not be heated to dryness, and thus burn fat.

Remove beakers containing the fat and set in a tray. Dry the beakers and contents in a vacuum oven for one hour at 110°C. Cool in the desiccator for one-half hour. Weigh the beaker and contents. Avoid fingerprints which will alter the weights.

TABLE 12

Food consumption of rats fed various diets during the collection periods

Rat no.	Period 1 g	Period 2 g	Period 3 g	Period 4 g	Period 5 g
Diet I. Low nitrogen					
3	48				
7	41				
24	38				
27	60				
28	53				
Diet II. Wheat					
1	38	40	51	45	43
4	26	33	38	44	47
11	35	35	43	37	43
21	33	37	44	37	48
23	28	31	43	41	45
Diet III. Wheat protein concentrate					
5	39	50	56	65	67
9	47	59	69	73	71
13	39	50	55	62	65
15	44	60	66	69	76
26	57	42	53	57	62
Diet IV. Sorghum					
2	29	26	37	34	36
16	32	29	36	32	32
17	30	34	41	39	39
20	32	35	36	42	40
22	29	34	29	31	36
Diet V. Sorghum and wheat protein concentrate					
8	40	46	46	46	44
14	42	43	41	39	43
19	32	37	45	47	48
29	24	38	45	45	44
30	39	43	42	45	43
Diet VI. Sorghum and lysine					
6	24	28	29	30	20
10	24	24	33	31	17
12	26	29	28	28	27
18	27	35	35	37	39
25	53	41	40	44	11

TABLE 13

Cumulative weight gain and % weight gain of rats fed various diets at the end of adjustment and collection periods

Rat no.	Adjustment period		Period 1		Period 2		Period 3		Period 4		Period 5	
	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%
Diet I. Low nitrogen												
3	-1	2.38	2	4.76								
7	1	2.08	3	6.25								
24	1	2.17	3	6.52								
27	4	8.16	7	14.29								
28	3	6.38	7	14.89								
Diet II. Wheat												
1	2	3.85	6	11.54	15	28.85	19	36.54	24	46.15	29	55.77
4	2	4.17	3	6.25	9	18.75	16	33.33	22	45.83	26	54.17
11	2	4.44	8	17.78	14	31.11	19	42.22	24	53.33	29	64.44
21	1	2.17	6	13.04	14	30.44	18	39.13	23	50.00	26	56.52
23	1	2.56	4	10.26	10	25.65	13	33.33	19	48.72	26	66.66
Diet III. Wheat protein concentrate												
5	6	14.63	11	26.84	22	53.66	33	80.49	41	100.00	55	134.15
9	6	12.77	16	34.04	32	68.09	47	100.00	58	123.40	69	146.81
13	7	14.00	12	24.00	25	50.00	36	72.00	48	96.00	62	124.00
15	6	12.50	14	29.17	30	62.00	42	87.50	56	116.67	74	154.17
26	5	11.11	18	40.00	27	60.00	32	71.11	42	93.33	55	122.22
Diet IV. Sorghum												
2	-1	-2.17	-1	-2.17	3	6.52	5	10.87	9	19.57	14	30.44
16	2	4.44	3	6.67	7	15.56	10	22.22	12	26.67	16	35.56
17	0	0.00	2	4.08	7	14.29	13	26.53	16	32.65	19	38.78
20	-1	-1.92	2	3.85	6	11.54	7	13.46	12	23.08	17	32.69
22	3	7.32	4	9.76	5	12.20	8	19.51	10	24.39	14	32.15
Diet V. Sorghum and wheat protein concentrate												
8	0	0.00	5	10.20	14	28.57	19	38.78	27	55.10	32	65.31
14	2	4.55	6	13.64	14	31.82	19	43.18	23	52.27	31	70.46
19	0	0.00	3	6.52	9	19.57	14	30.44	20	43.48	27	58.70
29	4	8.33	9	18.75	13	27.08	18	37.50	23	47.92	28	58.33
30	1	2.13	6	12.77	13	27.66	19	40.43	24	51.06	29	61.70
Diet VI. Sorghum and lysine												
6	1	2.63	7	18.42	6	15.79	7	18.42	10	26.32	14	36.84
10	-1	-2.08	-1	-2.08	1	2.08	6	12.50	6	12.50	10	20.83
12	0	0.00	1	2.17	4	8.70	5	10.87	8	17.39	9	19.57
18	-2	-3.85	-1	-1.92	2	3.85	5	9.62	8	15.38	13	25.00
25	2	4.35	3	6.52	8	17.39	13	28.26	19	41.30	25	54.35

TABLE 14

Protein efficiency ratios of various test diets fed to rats during each collection period

Rat no.	Period 1			Period 2			Period 3			Period 4			Period 5		
	Gain in body weight (g)	Protein intake (g)	PER	Gain in body weight (g)	Protein intake (g)	PER	Gain in body weight (g)	Protein intake (g)	PER	Gain in body weight (g)	Protein intake (g)	PER	Gain in body weight (g)	Protein intake (g)	PER
Diet I.															
3	4	1.44	2.78												
7	5	1.23													
24	-3	1.14													
27	3	1.80													
28	4	1.59													
Diet II.															
1	4	3.42	1.17	9	3.60	2.50	4	4.59	0.87	5	4.05	1.23	5	3.87	1.29
4	1	2.32	0.04	6	2.97	2.02	7	3.42	2.05	6	3.96	1.52	4	4.23	0.95
11	6	3.15	1.90	6	3.15	1.90	5	3.87	1.29	6	4.23	1.42	5	3.87	1.29
21	5	2.97	1.68	8	3.33	2.40	4	3.96	1.01	1	3.33	0.30	8	4.32	1.85
23	3	2.52	1.19	6	2.79	2.15	3	3.87	7.75	6	3.69	1.63	7	4.05	1.73
Diet III.															
5	5	3.51	1.42	11	4.50	2.44	11	5.04	2.18	8	5.85	1.37	14	6.03	2.32
9	10	4.23	2.36	16	5.31	3.01	15	6.21	2.42	11	6.57	1.67	11	6.39	1.72
13	5	3.51	1.42	13	4.50	2.89	11	4.95	2.22	12	5.58	2.15	14	5.85	2.39
15	8	3.96	2.02	16	5.40	2.96	12	5.94	2.02	14	6.21	2.25	18	6.84	2.63
26	13	5.13	2.53	9	3.78	2.38	5	4.77	1.05	10	5.13	1.95	13	5.58	2.33

TABLE 14 (Concluded)

Rat no.	Period 1			Period 2			Period 3			Period 4			Period 5		
	Gain in body weight (g)	Protein intake (g)	PER	Gain in body weight (g)	Protein intake (g)	PER	Gain in body weight (g)	Protein intake (g)	PER	Gain in body weight (g)	Protein intake (g)	PER	Gain in body weight (g)	Protein intake (g)	PER
Diet IV.															
2	0	2.61	-2.61	4	2.32	1.72	2	3.33	0.60	4	3.06	1.31	3	3.24	0.93
16	1	2.88	0.34	4	2.61	1.53	3	3.24	0.93	2	2.88	0.69	4	2.88	1.39
17	2	2.70	0.74	5	3.06	1.63	6	3.69	1.63	3	3.51	0.85	3	3.51	0.85
20	3	2.88	1.04	4	3.15	1.27	1	3.24	0.31	5	3.78	1.32	5	3.60	1.39
22	1	2.61	0.38	4	3.06	1.31	3	2.61	1.15	2	2.79	0.71	4	3.24	1.23
Diet V.															
8	5	3.60	1.39	9	4.14	2.17	5	4.14	1.21	8	4.14	1.93	5	3.96	1.26
14	4	3.78	1.06	8	3.87	2.07	5	3.69	1.36	4	3.51	1.14	8	3.87	2.07
19	3	2.88	1.04	6	3.33	1.80	5	4.05	1.23	6	4.23	1.42	7	4.32	1.62
29	5	2.16	2.31	4	3.42	1.17	5	4.05	1.23	5	4.05	1.23	5	3.96	1.26
30	5	3.51	1.42	7	3.87	1.81	6	3.78	1.59	5	4.05	1.23	5	3.87	1.29
Diet VI.															
6	6	2.16	2.78	-1	2.52	-0.40	1	2.61	0.38	3	2.70	1.11	4	1.80	2.22
10	0	2.16	-2.16	2	2.16	0.93	5	2.97	1.68	0	2.79	-2.79	5	1.53	3.26
12	1	2.32	0.43	3	2.61	1.15	1	2.52	0.40	3	2.52	1.19	1	2.43	0.41
18	1	2.43	0.41	3	3.15	0.95	3	3.15	0.95	3	3.33	0.90	5	3.33	1.50
25	1	4.77	0.21	5	3.69	1.36	5	3.60	1.39	6	3.96	1.52	6	0.99	6.06

TABLE 15

Liver weight, liver weight/body weight and % fat protein and moisture in livers of rats fed various diets

Rat no.	Liver weight (g)	Liver weight/ body weight (g)	Fat %	Protein %	Moisture %
Diet I. Low nitrogen					
3	1.55	0.04	2.24	24.50	75.66
7	1.81	0.04	2.30	22.10	77.09
24	1.23	0.03	2.20	35.00	72.61
27	1.70	0.03	3.01	23.32	73.88
28	2.01	0.04	2.08	22.42	75.30
Diet II. Wheat					
1	2.83	0.04	0.74	21.26	78.34
4	2.29	0.03	0.94	23.42	74.68
11	2.39	0.03	0.76	24.47	74.58
21	2.35	0.03	0.99	23.48	75.64
23	2.20	0.03	1.34	22.04	77.04
Diet III. Wheat protein concentrate					
5	2.77	0.03	0.74	24.42	74.13
9	3.48	0.03	1.20	23.28	73.84
13	3.66	0.03	0.89	24.78	73.09
15	3.59	0.03	0.90	23.80	73.24
26	3.11	0.03	0.68	24.90	73.74
Diet IV. Sorghum					
2	2.12	0.04	1.14	21.18	77.16
16	2.49	0.04	1.54	21.04	74.65
17	2.27	0.03	3.65	20.74	77.84
20	2.16	0.03	2.68	21.62	75.62
22	2.19	0.04	4.10	17.94	76.36
Diet V. Sorghum and wheat protein concentrate					
8	2.52	0.03	0.96	23.32	75.26
14	2.40	0.03	1.39	22.87	74.63
19	2.25	0.03	1.24	23.58	74.92
29	2.29	0.03	1.09	17.98	75.75
30	2.21	0.03	1.37	22.40	74.74
Diet VI. Sorghum and lysine					
6	2.01	0.04	1.38	21.06	74.82
10	2.14	0.04	1.34	21.02	75.78
12	1.97	0.04	0.92	26.03	75.17
18	2.42	0.04	1.51	18.86	76.18
25	2.50	0.04	1.80	21.24	76.34

TABLE 16

Nitrogen intake, excretion and retention of rats during experimental periods fed various test diets

Rat no.	Experimental periods	Nitrogen intake (g)	Nitrogen excretion		Nitrogen retention
			Fecal (g)	Urinary (g)	
Diet II. Wheat					
1	1	0.5472	0.0951	0.2686	0.2818
	2	0.5760	0.1975	0.2903	0.1864
	3	0.7344	0.1422	0.3131	0.3774
	4	0.6480	0.2814	0.3116	0.1533
4	1	0.3712	0.0707	0.2370	0.1618
	2	0.4752	0.1200	0.2275	0.2260
	3	0.5472	0.2969	0.1584	0.1902
	4	0.6336	0.3564	0.1613	0.2142
11	1	0.5040	0.0619	0.1953	0.3451
	2	0.5040	0.1514	0.1878	0.2631
	3	0.6192	0.1715	0.2348	0.3112
	4	0.6768	0.2892	0.2815	0.2044
21	1	0.4752	0.1076	0.1829	0.2930
	2	0.5328	0.1153	0.1959	0.3199
	3	0.6336	0.1220	0.2003	-0.0754
	4	0.5328	0.3522	0.1416	0.2643
23	1	0.4032	0.0908	0.1650	0.2457
	2	0.4464	0.0955	0.2151	0.2341
	3	0.6192	0.1078	0.2341	0.3758
	4	0.5904	0.3382	0.2609	0.0896

TABLE 16 (Continued)

Rat no.	Experimental periods	Nitrogen intake (g)	Nitrogen excretion		Nitrogen retention
			Fecal (g)	Urinary (g)	
Diet III.	Wheat protein concentrate				
5	1	0.5616	0.1500	0.1051	0.4138
	2	0.7200	0.2298	0.2253	0.3720
	3	0.8064	0.1448	0.2595	0.5004
	4	0.9360	0.3596	0.2584	0.4163
9	1	0.6768	0.1025	0.2080	0.4646
	2	0.8496	0.1582	0.3050	0.4847
	3	0.9936	0.3465	0.3554	0.3900
	4	1.0512	0.4424	0.3565	0.3506
13	1	0.5616	0.0955	0.1845	0.3799
	2	0.7200	0.1565	0.2110	-0.0002
	3	0.7920	0.1422	0.2205	0.5276
	4	0.8928	0.2555	0.2786	0.4570
15	1	0.6336	0.1303	0.2190	0.3826
	2	0.8640	0.1893	0.2539	0.5191
	3	0.9504	0.2180	0.2778	0.5529
	4	0.9936	0.4120	0.2811	0.3988
26	1	0.8208	0.1327	0.1536	0.6328
	2	0.6048	0.2476	0.1746	0.2809
	3	0.7632	0.2009	0.2282	0.4324
	4	0.8208	0.3061	0.2668	0.3462

TABLE 16 (Continued)

Rat no.	Experimental periods	Nitrogen intake (g)	Nitrogen excretion		Nitrogen retention
			Fecal (g)	Urinary (g)	
Diet IV.	Sorghum				
2	1	0.4176	0.0737	0.2459	0.1963
	2	0.3712	0.1212	0.2161	0.1322
	3	0.5328	0.0871	0.2168	0.3272
	4	0.4896	0.1870	0.2745	0.1255
16	1	0.4608	0.0881	0.2705	0.2005
	2	0.4176	0.0828	0.3050	0.1282
	3	0.5184	0.0867	0.2852	0.2448
	4	0.4608	0.2770	0.2547	0.0274
17	1	0.4320	0.0836	0.1933	0.2534
	2	0.4896	0.0839	0.2150	0.2890
	3	0.5904	0.1407	0.2775	0.2705
	4	0.5616	0.3122	0.2348	0.1129
20	1	0.4608	0.0936	0.2367	0.2288
	2	0.5040	0.0892	0.2176	0.2955
	3	0.5184	0.0833	0.1622	0.3712
	4	0.6048	0.2665	0.2128	0.2238
22	1	0.4176	0.0827	0.3146	0.1186
	2	0.4896	0.0880	0.2609	0.2390
	3	0.4176	0.0754	0.2584	0.1822
	4	0.4464	0.2289	0.2506	0.0652

TABLE 16 (Continued)

Rat no.	Experimental periods	Nitrogen intake (g)	Nitrogen excretion		Nitrogen retention
			Fecal (g)	Urinary (g)	
Diet V.	Sorghum and wheat protein concentrate				
8	1	0.5760	0.0983	0.2411	0.3349
	2	0.6624	0.1072	0.3568	0.2967
	3	0.6624	0.1100	0.3109	0.3397
	4	0.6624	0.2173	0.3091	0.2344
14	1	0.6048	0.1041	0.2396	0.3680
	2	0.6192	0.1404	0.3454	0.2316
	3	0.5904	0.1130	0.3046	0.2711
	4	0.5616	0.2072	0.2892	0.1635
19	1	0.4608	0.1017	0.2330	0.2244
	2	0.5328	0.1118	0.2723	0.2470
	3	0.6480	0.1242	0.3165	0.3056
	4	0.6768	0.3649	0.3076	0.1026
29	1	0.3456	0.1038	0.2870	0.0531
	2	0.5472	0.0988	0.3530	0.1917
	3	0.6480	0.0936	0.3215	0.3312
	4	0.6480	0.2150	0.3010	0.2303
30	1	0.5616	0.0968	0.2055	0.3576
	2	0.6192	0.1166	0.2889	0.3120
	3	0.6048	0.1066	0.3330	0.2635
	4	0.6480	0.3125	0.3216	0.1122

TABLE 16 (Concluded)

Rat no.	Experimental periods	Nitrogen intake (g)	Nitrogen excretion		Nitrogen retention
			Fecal (g)	Urinary (g)	
Diet VI.	Sorghum and lysine				
6	1	0.3456	0.0456	0.2003	0.1980
	2	0.4032	0.0698	0.1698	0.2619
	3	0.4176	0.0708	0.2617	0.1834
	4	0.4320	0.1723	0.1628	0.1954
10	1	0.3456	0.1012	0.1735	0.1692
	2	0.3456	0.0750	0.2117	0.1572
	3	0.4752	0.0789	0.1926	0.3020
	4	0.4464	0.1753	0.2062	0.1632
12	1	0.3712	0.0810	0.1632	0.2253
	2	0.4176	0.0635	0.2367	0.2157
	3	0.4032	0.0683	0.2602	0.1730
	4	0.4032	0.2740	0.2150	0.0125
18	1	0.3888	0.0905	0.1455	0.2511
	2	0.5040	0.1024	0.2462	0.2537
	3	0.5040	0.0978	0.2687	0.2358
	4	0.5328	0.3198	0.2561	0.2552
25	1	0.7632	0.1015	0.0283	0.2474
	2	0.5904	0.0940	0.0257	0.6130
	3	0.5760	0.1590	0.0786	0.4367
	4	0.6336	0.2776	0.1360	0.3183

THE EFFECT OF WHEAT PROTEIN CONCENTRATE SUPPLEMENTATION UPON SORGHUM DIETS AS MEASURED BY RAT PERFORMANCE

by

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The effect of supplementing a sorghum diet with wheat protein concentrate (obtained from millfeed from wheat shorts) and with lysine was investigated, using a wheat diet as a standard. Thirty weanling rats of the Sprague-Dawley strain were randomly divided into six groups and assigned to one of six diets, namely a low nitrogen diet based on 3% protein from whole egg, and 9% protein diets based on wheat, wheat protein concentrate, sorghum, sorghum plus wheat protein concentrate, and sorghum plus lysine. The first five days of the thirty-day experimental period comprised the adjustment period, followed by five collection periods of five days each. Growth and protein efficiency ratios, nitrogen balance, biological value, net protein utilization, and estimation of liver and blood proteins were the parameters used to evaluate protein quality.

Rats on the wheat protein concentrate diet had markedly significantly ($P < 0.05$) higher weight gains over those on the other test diets, namely, wheat, sorghum, sorghum plus wheat protein concentrate, and sorghum plus lysine. Growth of rats on wheat and sorghum plus wheat protein concentrate diets did not differ significantly; however, the wheat diet was significantly different ($P < 0.05$) from the sorghum and sorghum plus lysine diets. The protein efficiency ratio, biological value, and net protein utilization were highest for the wheat protein concentrate diet, followed by the sorghum plus wheat protein concentrate diet, wheat diet, sorghum diet, and sorghum plus lysine diet. Liver protein content of rats on diets ranged, highest to lowest, in the following order: wheat protein concentrate, wheat, sorghum, sorghum plus wheat protein concentrate, and sorghum plus lysine. There was an inverse relationship between protein and fat in rat liver and the protein quality of the diet. The higher the

protein quality in the diet, the less liver fat; the lower the protein quality, the higher the liver fat value. Hemoglobin and hematocrit values were highest for sorghum and wheat protein concentrate diet followed by wheat protein concentrate, wheat sorghum, and sorghum plus lysine diets.

The addition of lysine to sorghum diet resulted in decreased growth rate and raises a question concerning lysine supplementation of this grain. The addition of wheat protein concentrate to sorghum markedly improved the response of test animals for all variables over those for sorghum alone.

On the basis of this study the supplementation of sorghum with wheat protein concentrate to improve protein quality is promising. The potential use of wheat protein concentrate in human diets merits further investigation.