PROTEIN VALUE OF POTATO AND NAVY BEAN POWDERS: NUTRITIONAL EVALUATION USING THE MEADOW VOLE (MICROTUS PENNSYLVANICUS)

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ABSTRACT

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The protein content of navy bean powders produced from beans cooked in atmosphere and in a retort was found to average 23.4 g/100 g. The protein contents analysed by the Kjeldahl and Biuret methods were similar, indicating that the Biuret method could be used to measure the protein of potatoes.

Methionine was the low level amino acid. Glutamic and aspartic acids were in higher amounts than their essential amino acids. The use of Chemical Score was successful when applied to nutritional studies with meadow voles. Potatoes at 5.28% protein permitted better growth than beans, indicating that the former had more balanced proteins. Inclusion of methionine improved the nutritional value, demonstrating that methionine was the first limiting amino acid and isoleucine the second limiting one. The absorption of nitrogen was also

increased when voles received the supplemented proteins.

The nutrive value of navy beans cooked in a retort was less than that of beans cooked in the atmosphere and may be attributed to intensity of heat applied. The use of meadow voles (Microtus pennsylvanicus) in nutritional studies deserves attention because of their sensitivity in responding to low level protein diets. Their low cost, availability and rapid growth could be important factors in promoting their use in developing countries.

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INTRODUCTION

One of the most serious problems found today in developing countries is the shortage of high quality protein foods. Protein malnutrition, especially among young children, has become a major concern (Senecal, 1958). This problem is particularly complex because the various regions of the world differ not only in topographic and climatic conditions but in social patterns and economic resources.

In some areas of developing countries the expense of protein of animal origin limits its consumption to a minority of people while the great majority get their protein food from plant origin.

When the body receives a deficient protein, the health is injured and particularly in growing children this insufficiency has deleterious effects (Allison et. al., 1960). Mortality rates rise considerably when resistance to infections is decreased (Scrimshaw, 1966). For those who survive, physical growth is impaired (Jackson, 1966) and mental ability lessened (Cravioto et al., 1967). The mind may be irreversibly damaged depending on the duration of the deficient food intake (Coursin, 1965).

Tizard (1968) emphasized that prolonged and severe malnutrition in infancy may cause changes in the chemical composition of the brain, as well as decreased stature and lack of stamina.

In young experimental animals, learning, memory and behavior are affected when malnutrition is severe (Nutrition Foundation, 1967-68). These authors reported that the brain acquires about 80 per cent of its adult weight by the age of three years, when the body has reached 20 per cent of its adult weight. Malnutrition in children was shown by psychological and behavioral changes, characterized by apathy (Pearson, 1967).

In adults the inadequacy of food intake creates an incapacity to work efficiently, which also can be aggravated by psychological changes rendering the mind apathetic to work. These affections can vary in intensity, depending upon the physiological state of the organism, which, in turn, is related to the amount of time of the individual's undernutrition or malnutrition. Keys et al. (1950), reported that prolonged undernutrition deteriorates not only general behavior but intelligence and personality. Brozek (1955) pointed out that food intake and behavior are complex and interdependent systems.

A world wide survey has demonstrated the magnitude of the problem of hunger and malnutrition. Half of the

world's population is suffering from undernutrition or malnutrition and probably both (FAO, 1963). Undernutrition refers to an inadequate intake of food and calories, whereas malnutrition refers to an inadequacy in the quality of food intake, notably in protein, vitamins and minerals.

If one considers the scarcity of good protein sources in some economically depressed areas of the world and the larger number of preschool children receiving subnormal levels of nutrients (70% according to Gyorgy, 1966), the campaign for averting the deleterious effect of protein deficiency is welcome (United Nations, 1968). Numerous investigators are dedicated to this important task of preventing and controlling malnutrition through the beneficial system of food supplementation. This supplementation increases the quantity and quality of available protein for metabolic purposes, covering in this way the shortage of animal protein.

Today there are several ways of enhancing the protein value of a food. A practical method is to mix two or three different vegetable foods in such a way that they will produce a higher quality protein supplying the required dietary protein (Bressani et al., 1961).

Protein concentrates are also excellent sources of protein. According to their utilization they may be arranged as follows: fish flour, soy products, peanut

flour, sesame flour, cotton seed flour, and coconut protein (Hundley, 1968).

The quality of the protein can be improved by the addition of the deficient amino acids. This method is being used today with success not only in animal feeding but also as a means of combating the protein malnutrition problem for human beings (Gomez et al., 1958 and Hansen et al., 1956).

According to Flodin (1953) proper supplementation of staple foods with amino acids doubles the value of the dietary protein.

The purpose of the present work is to become acquainted with principles involved in improving the quality of the protein in order to apply these principles to agricultural products native to Bolivia, with the objective of combating the protein malnutrition problems, especially in children at preschool and school age.

In this study the nutritive quality of drum dried potato flakes and navy bean powders was evaluated by determining protein content and amino acid composition and in feeding trials using nonsupplemented and supplemented proteins. Both chemical and bioassay procedures were used.

LITERATURE REVIEW

1. FACTORS DETERMINING PROTEIN MALNUTRITION IN PRESCHOOL CHILDREN.

A. PROTEIN DEFICIENCY.

Several scientists from different parts of the world working on the control of children's health agreed that protein malnutrition is mostly manifested under the age of five years in underdeveloped countries.

The high rates of morbidity and mortality in infants in the immediate postweaning period are principally due to the inadequate intake of protein. Breast feeding is dominant and the infants do not receive supplementation during lactation, which may be extended to twelve months (Scrimshaw et al., 1961). A marasmic condition could develop when the infant is nourished from a deficient mother, although a combination of parasitic and nutritional deficiency diseases may obscure the diagnosis (Behar et al., 1958). The infant's impaired clinical picture is mostly due to the shortage of dietary protein because family's food selection for post weaning is basically of farinaceous origin, thus eliminating the required protein so essential for growth and maintenance (Hansen and Howe, 1964). Bengoa et al. (1959) reported that malnutrition is more dominant in the postweaning period and the high mortality rate of children between the age of one and four years is indicative of the true dimension

of the problem of malnutrition. Over half of the population in developing countries dies before the age of fifteen and in many areas 50% or more of the infants die before reaching the age of five (Schaefer, 1963). The infant mortality rate in developing countries could reach ten to fifteen times more than in developed ones and the infant mortality rate between one and four years may be as much as fifty to sixty times higher (Williams, 1966).

B. PROTEIN CALORIE MALNUTRITION.

Today it is well known that protein calorie malnutrition is manifested in two ways: 1) Kwashiorkor resulting from the ingestion of low levels of protein with considerable amount of calorie intake and 2) Marasmus representing complete malnutrition having both protein and calorie deficiency (Altschul, 1965). Kwashiorkor was described by Scrimshaw et al. (1961), as a disease characterized by edema, dermatitis of the hyperkeratosis type accompanied by hyperpigmentation and desquamation of the skin. The child is psychologically affected and anorexia and anemia are symptoms of this disease. The fact than the hair falls or drops out spontaneously, plus the absence of uniform hair color was found to be a specific symptom of kwashiorkor. The diets of children developing kwashiorkor are deficient not only in protein, but also in other nutrients, particularly vitamins and

minerals (Behar et al., 1958). Marasmus is characterized by marked growth retardation, loss of weight, extreme muscular wasting and absence of subcutaneous fat.

C. SOCIO ECONOMIC FACTORS.

According to Scrimshaw (1961), Behar (1958), Rao et al. (1959), and Autret (1961), the families of children who manifested kwashiorkor or marasmus frequently come from less favoured social groups, where ignorance, food habits, attachment to the past, unsanitary conditions and apathetic attitudes toward child care are factors which precipitate the development of these diseases. On the other hand, the limited availability of protein can be due to the low fertility of soil, production of agricultural products that are not good sources of protein and lack of knowledge about correct selection of food for fulfilling the children's requirements (Burgess and Dean, 1962).

The availability of native foods creates food habits which are related to cultural patterns, and these in turn are influenced by technological, economic and social factors.

Soil and climatic conditions are factors leading to undernutrition or malnutrition. In developing countries the lack of modern technology limits food production. Lack of proper conditions of storage promotes food spoilage due

to contamination with rodents and insects. Food of high nutritive value like eggs, meat, fish, milk, fruits and vegetables need appropriate handling, transport and storage for preservation (Burgess and Dean, 1962). The stock of foods in the market also depends on the consumer's demand, influenced by socio-economic factors.

D. INTERRELATION BETWEEN ENERGY INTAKE AND NITROGEN RETENTION

Several investigators have shown that carbohydrate and fat have a protein sparing effect, with carbohydrate being the more efficient. Swanson (1959) and Howes and Spector (1954) pointed out that nitrogen utilization is influenced by both caloric and protein intake. These authors have observed that the negative nitrogen balance of active young men receiving no protein food decreased when about 700 calories were supplied, and the level of one thousand calories plus 3 grams of nitrogen increased the sparing effect similar to the ingestion of high quantities of nitrogen.

When a constant nitrogen intake of 3.82 grams per day and slightly decreasing carbohydrate diets were given to dogs, an increase of urinary nitrogen excretion and a constant balance index were observed. When the carbohydrate was severely restricted in the diet, nitrogen excretion was increased and the nitrogen balance index was

decreased (Rosenthal, 1951). This investigator has observed that responses varied with the physiological state of the animal.

Albino rats fed 4, 6 and 8 grams daily of a complete diet containing either 10% soybean oil meal or methionine supplemented fibrin had the lowest biological value at 4 grams. Forbes and Yohe (1955) attributed this to the use of protein for caloric needs.

The importance of consuming a high quality protein when levels of protein and calories are marginal was studied by Leverton et al. (1951) who used two groups of young women receiving 43 and 63 grams of protein at isocaloric intakes of 1800 calories. Those receiving lower levels of protein had a higher nitrogen excretion when 240 grams of milk were included at the noon meal; no difference in nitrogen excretion occurred with the second group. When calories were increased to 2400 with no supplement of milk protein, the nitrogen excretion of both groups decreased with the nitrogen sparing effect being higher for those with lower protein intake.

Differences in nitrogen retention and in the behavior of animals receiving plant and animal proteins have been observed. Puppies fed wheat gluten retained 0.18 g of nitrogen per gram of nitrogen consumed, whereas, with egg protein they showed a retention of 0.5 g per gram nitrogen intake. Animals receiving gluten were obese

and inactive and the others lean and active. However, the increase of body weight per g of nitrogen intake was approximately the same for both groups (Allison and Wannemacher, 1957). This study showed the importance of the quality of the protein fulfilling their nitrogen needs.

Gluten protein, being deficient, was not totally used and was converted to a caloric source, manifested in the obesity of the animals.

2. MEANS OF PROVIDING MORE AVAILABLE PROTEIN.

A. VEGETABLE MIXTURES.

A great part of the world's population subsists on vegetable crops, usually of low protein content. The majority of vegetable proteins are deficient or low in at least one amino acid. A mixture of foods having different amino acid deficiencies will produce a protein of better nutritional value than their components, due to the effect of supplementation.

In Central America, Incaparina, the low cost mixtures containing over 25% protein, were developed for preschool children at the Institute of Nutrition of Central America and Panama (INCAP). Mixture 8 contained 50% lime treated corn, 35% sesame flour, 3% torula yeast, 1% calcium carbonate and 4500 I.U. of vitamin A acetate. This formula was found to be equivalent to milk in treating kwashiorkor (Scrimshaw et al., 1961). Other formulas

gave similar results. Number 9 is composed of corn flour 29%, sorghum flour 29%, cotton seed flour 38%, torula yeast 3%, Ca CO_3 1% and vitamin A 4500 I.U.

Formula No. 15 is in commercial production; it contains corn flour 58%, cotton seed flour 19%, soya flour 19%, torula yeast 3%, Ca CO_3 1%, Vitamin A acetate 4500 I.U. (Behar et al., 1966). In Guatemala, Mexico and Colombia Incaparina formula 9b is in commercial production. This formula has 29% ground whole maize, 29% ground whole sorghum, 38% cotton seed flour, 3% torula yeast, 1% Ca CO_3, 4500 I.U. Vitamin A and its protein content is 27.5%. Incaparina can be drunk as "atole"; it is made by cooking 25 g of Incaparina in one glass of water for 25 minutes. Sugar is added to improve taste. It can also be incorporated into soups, breads and puddings. Incaparina is found commercially in polyethylene bags at a price of three U.S. cents per bag of 75 grams (Shaw, 1964).

B. MIXTURE OF FOODS AND PROTEIN CONCENTRATES

In South Africa Pronutro was developed as a food supplement of good quality protein balanced with vitamins and minerals for preventing kwashiorkor and other forms of malnutrition and undernutrition. The chemical composition at present is as follows: protein 22 g; fat, approximately 11.5 g; calories, 413; calcium, 460 mg; phosphorous, 580 mg; iron, 5.5 mg; Vitamin A, 3500 I.U.;

thiamine, 1.06 mg; riboflavin 1.5 mg; niacin, 14 mg and vitamin C 53 mg. The protein source is a mixture of soya beans, peanuts, yeast, wheat germ, and milk (Odendaal, 1966). Pronutro is found in the market in packages of one pound being sold at a price of 15 U.S. cents.

Cooked full fat soy products obtained by the extrusion cooking process give a high nutritive value, good oxidative stability, and mild flavor. Vitamins and the heat labile lysine are conserved, and the growth inhibiting factors are destroyed. With the cooperation of UNICEF this product was chemically tested in Taiwan where full fat soya flour was formulated with sugar and supplementary vitamins and fed to children from 4 to 12 months of age. Reports from these studies have indicated that this product supports excellent growth, the gains being comparable to those obtained in babies fed cow's milk (Mustakas et al., 1966).

Fish protein concentrates are primarily used in Peru and Chile. In Peru a group of children ranging in age from 6 to 41.5 months receiving wheat noodles enriched with 10% fish protein concentrate showed better growth and weight response than those on a diet of wheat noodles without supplementation (Graham et al., 1966).

The University of Chile is also engaged in studying the use of fish protein concentrate for human consumption and particularly for children. In 1964 production of

fish flour in Chile reached 400,000 tons, and it was expected that one million tons would be produced in 1965. With the cooperation of the UNICEF, a plant is working with the aim of obtaining odorless fish flour. Bread, spaghetti, and soup are being enriched with fish protein concentrate, and good results have been obtained in test groups (Moncksberg, 1966).

New protein foods from inexpensive products like the oil seeds can be used to repair the dietary deficiencies observed in malnourished people. These products are being produced as protein beverages and textured products (Altschul, 1967).

C. AMINO ACID SUPPLEMENTATION

Protein problems can be present in two ways; deficiency of total protein when related to consumption and deficiency of the protein itself, when referred to its amino acid composition (Jansen et al., 1964).

The value of the protein in the food is mainly measured by its amino acid content. The deficiency of the protein can be overcome by addition of small amounts of the needed amino acid. According to Jansen (1964), in a corn eating country, tryptophan and lysine will be missing in the diet. With diets consisting of casava and potato, the total protein is low, and methionine will be in

shortest supply. In rice eating countries there is a deficiency in lysine and threonine.

Teff (Eragrostis abysinica) is consumed in Ethiopia. A typical Ethiopian diet was calculated to furnish 65 grams of protein, of which 45 grams came from teff. This food is a good source of vitamins and minerals, including iron, but is deficient in lysine. The protein quality could be improved by supplementing with the deficient amino acid (Jansen, 1962).

Children recovering from protein malnutrition increased their nitrogen retention considerably when they were given an adequately adjusted diet of wheat protein supplemented with lysine. Bressani et al. (1960), and Barnes et al. (1961), have observed that nitrogen retention was improved when lysine and potassium were added to the diet. Better growth response was observed at protein levels of 1.75 to 3 grams per kilogram of body weight with 75 to 100 calories and more than 2 milliequivalents of potassium.

Bressani et al. (1958) have observed the importance of the physiological state of children. Children show varying responses to a given level of protein. For this study a simplified basal diet supplying two grams of protein per Kg of body weight was given to infants. The diet was supplemented by adding the level of a given amino acid listed in the FAO "reference protein." The

addition of either tryptophan or lysine did not give as good a response as when these two amino acids were added together. Nitrogen retention was considerably increased when isoleucine was included.

The nutritive value of polished rice protein was improved by the addition of two essential amino acids, lysine and threonine. When these amino acids were used separately, no change in the nutritive value of the protein was noticed. Albino rats were used in this experiment (Pecora, 1951).

The addition of 0.5% dl lysine hydrochloride, which corresponds to 0.2% l lysine, to a bread diet increased the average weight of weanling rats from 32% to 75%. The beneficial effect was similar when lysine was added to the bread before baking or when it was incorporated into the bread diet. Higher proportions of lysine e.g. 0.8% or more, gave a growth response similar to the stock diet (Rosenberg, 1952).

Corn varieties containing low and high levels of protein showed a difference in protein quality (Sauberlich et al., 1953). The low protein variety was deficient in lysine, tryptophan, isoleucine, threonine and valine, whereas the high protein variety was deficient only in lysine and tryptophan.

In diets where cereal grains are the chief staple food the deficiency in protein is one of quality rather

than quantity. Supplementation with lysine, tryptophan and threonine will improve the quality of the protein to such a degree that it can be compared with a milk protein (Howe et al., 1965). Casava, yams and bananas are dietary components of a large number of populations. Because of their low level of protein, the incorporation of fish protein concentrates and properly processed oil seed protein in the diet would be beneficial, and supplementation with amino acids will bring the protein level of the food to a satisfactory level (Howe et al., 1965).

D. MICROORGANISMS IN FOOD PRODUCTION

Yeast is a promising food for solving the problem of malnutrition. The potential for this food is mainly because it is rich in protein and vitamins. It can also be produced from inexpensive waste materials such as molasses, sulphite waste, corn steep liquor and wood residues.

Yeast is currently in greater use as a food source for humans and animals than are algae and bacteria which have also been considered as potential food sources of protein.

Yeast is added to Incaparina, the vegetable protein mixture manufactured in Central America for the treatment of children's protein malnutrition, to provide the B complex vitamins (Scrimshaw et al., 1961).

According to Peppler (1967), yeast production for 1964 was as follows: USA, 28,900 tons of dried yeast per year; Europe, 52,400 tons; USSR, 52,000 tons; Formosa about 13,000 tons per year.

Candida utilis contains more than 50% of protein on a dry weight basis and all the vitamins of the B group. However, its protein is low in methionine and cystine and requires supplementation. It could be an important protein and vitamin source for the depressed areas of the world (Prescott and Dunn, 1959). Frazier (1967) reported that food yeast may furnish, in varying amounts, thiamine, riboflavine, biotin, niacin, pantothenic acid, pyridoxine, choline, streptogenin, glutathione and probably folic and p-amino benzoic acids. From the literature it appears that considerable difficulties arise in promoting the consumption of food yeast on a large scale basis. Its incorporation in fairly low concentration in certain prepared foods such as biscuits, seems to be a good method. The important task would be to find strains which are not disagreeable to consumers.

China, Japan, and South East Asia produce and consume large amounts of fermented products. Molds are used for the production of exotic and flavorful foods.

Shoyu production is industrially significant in Japan. Aspergillus oryzae is the microorganism used in the preparation of shoyu, a salty, dark brown liquid with

a characteristic flavor. It is used as a seasoning agent for poultry, meat, fish and cooked vegetables. Miso also constitutes a big Japanese industry. It is produced by mold and yeast fermentation and is rich in glutamic acid. The fermentative organisms are Aspergillus oryzae and the yeast Saccharomyces rouxii. Miso is also used as a seasoning agent for meats and vegetables.

Sufu or Chinese cheese is a common food in China and Formosa. Its texture resembles that of cream cheese and its flavor is suggestive of anchovies. Soybeans are the substrate and the enzymes are produced by the mold Actinomucor elegans which is inoculated on the surface of expressed soybean curds called tofu (which are previously subjected to heat treatment). Once ripened, sufu cubes are salted in 5 to 10% brine.

Tempeh, another soybean food, is widely consumed in Indonesia and is produced by inoculating the beans with Rhizopus oligospurus. Ontjom is the name given to a fermented product made by inoculating peanut presscake with Neurospora sitophila.

Ang-Kak or red rice is an exotic colored food produced commercially in the Phillippines, Indonesia and China. The fermenting organisms are spores of Monascus purpureus.

The fermentation of these soybean products not only imparts variety to the diets, but it also improves the

digestibility of soybeans, an excellent source of protein (Nelson and Richardson, 1967).

A 1:1 mixture of wheat and soybeans supported growth comparable to that obtained when casein was fed. Rhizopus oligospurus was used as a fermenting agent. The P.E.R. of the rats fed fermented wheat was higher than that of those fed the unfermented wheat. The authors attribute the improvement in the protein to the mold's proteolytic enzyme action, increasing the availability of lysine (Wang et al., 1968).

3. AMINO ACID SUPPLEMENTATION

A. CLASSIFICATION OF AMINO ACIDS AND REQUIREMENTS

Rose (1937) showed that excellent growth was obtained in rats when non-essential amino acids were excluded from the diet. The essential amino acids were found to be tryptophan, lysine, histidine, phenylalanine, leucine, isoluecine, threonine, methionine, valine, and arginine. Rose and Rice (1939) found that the removal of any one of these amino acids except arginine from the diet, produced a nutritive failure in growing rats. They also stated that amino acids, not essential for the growing rat, were also dispensable for an adult dog. Klose et al. (1938) have shown that arginine, histidine and tryptophan were necessary for the chick.

Final classification of required amino acids for rats was presented by Rose and Womack (1948). Rose et al. (1957), working on the amino acids requirements of man, demonstrated that 8 were essential. Histidine was not considered critical for maintenance and growth of an adult male, but it was required by children. Holt and Snyderman (1967) and Swendseid and Dunn (1958) concluded that the amino acids required by men were also required by women.

Rose and Wixon (1955) have shown that 1 cystine is capable of replacing 80 to 89 per cent of the methionine needs of an adult man. These authors pointed out the importance of this finding for the areas of the world where several foods have methionine as the limiting amino acid.

B. CONCEPT OF PROTEIN'S FIRST LIMITING AMINO ACID

The concept of the first limiting amino acid was due to the work of Mitchell and Block (1946). According to these authors, "The amino acid limiting the nutritional value for maintenance and growth of the laboratory rat, for any particular food protein, would be that amino acid present in the least amount with reference to whole egg proteins i.e., that amino acid with the greatest percentage deficit."

Mitchell and Block (1946) have expressed the nutritive value of a protein in terms of a Chemical Score; subtracting from 100 the percentage deficit in the limiting essential amino acid. The greater this percentage deficit, the lower the nutritive value of the protein. Thus, the larger the Chemical Score, the better the protein quality. These data, in a way, predict the value of the protein.

For supplementary effects, the first limiting amino acid must be added in such amount to balance with the second limiting amino acid, and these two amino acids will be in balance when they cover the requirements of the organism for protein synthesis (Rosenberg, 1959).

C. AMINO ACID BALANCE AND IMBALANCE

To be well utilized for maintenance and growth of laboratory animals, all amino acids (essential and non-essential) must be present in the correct amount and at the proper time (Geiger, 1950). A balanced protein supplies the required amino acids and has a high biological value (Harper, 1959). Imbalance of amino acids in a protein is reflected in reduced animal growth. This effect is reversed by the addition of the most limiting amino acid (Flodin, 1953). Pellagra, for example, found where corn is the predominant food, is due to tryptophan and lysine deficiencies. It can be corrected by supplementing the diet with these amino acids (Jansen, 1964)

or the vitamin, niacin (Laguna and Carpenter, 1951). Sauberlich (1959) reported that the growth of weanling rats was depressed more than 50% when oxidized casein (methionine and tryptophan destroyed by H_2O_2) was used. Normal growth was restored by the addition of methionine.

When large amounts of gelatin were added to a low protein diet, the growth rate declined. The depressed growth was reversed by the addition of tryptophan (Salmon, 1964).

If amino acids are not supplied in the proper proportions, they can not be used for protein synthesis. Addition of excessive amounts of amino acids does not improve the efficiency of use of the dietary protein. If the imbalance is large, greater amounts of the most deficient amino acid will be required to restore normal growth (Natl. Acad. Sci., 1963).

In protein metabolism a relationship between the amino acids has been observed. Mitchell and Block (1946) reported that methionine can be converted to cystine, but the reverse does not occur. Graw and Almquist (1943) showed the sparing action of dietary cystine on methionine. The requirement for phenylalanine is decreased by the presence of tyrosine (Block and Bolling, 1944b).

To evaluate the nutritive value of a protein, balanced patterns of amino acids, either the whole egg

protein or the FAO Provisional Pattern, were used as references (FAO, 1957).

4. PROTEIN EVALUATION

A. BIOLOGICAL METHODS

The quality of a dietary protein is based on the amount and kind of its amino acids (Allison, 1959). Protein requirements are in relation to the needs of the organism for growth, tissue repair, maintenance, and enzyme replacement. A diet adequate for maintenance and repair may be inadequate for growth (Mitchell, 1947).

Many biological methods have been proposed for evaluating the nutritive value of a protein. Studies have been conducted in an attempt to set up a method that could measure growth and maintenance.

Animal assays have shown that there are two ways to measure the nutritive value of the protein:

1) Methods measuring the growth of the animal related to gain or loss in body weight.
2) Methods measuring the utilization of the nitrogen in terms of absorption and retention.

B. PROTEIN EFFICIENCY RATIO (P.E.R.)

One of the most common methods used for evaluating the protein quality is the P.E.R., defined as grams of weight gain per gram of protein consumed. Growing rats

are commonly used as the test animals. P.E.R. is a simple method, its efficiency depends on age, the sex of the rats, time of experimentation, and protein concentration.

Chapman et al. (1959) have observed that the younger the rat, the higher were the P.E.R. values. Female rats gave higher P.E.R. values at lower concentrations of protein than male rats (Morrison and Campbell, 1960). As the time of the assay was extended, P.E.R. values decreased in both sexes. Barnes et al. (1946) showed that a 10% protein level gave a maximum ratio. Morrison and Campbell (1960) reported that casein was more efficient at 7% than at 10% or 15%. These authors also proposed the incorporation of a control group to eliminate differences in P.E.R. values due to the use of different strains.

The addition of 20% water to a purified diet containing 9% protein significantly increased the P.E.R. values. The same effects were observed at levels of 6% and 12% (Keane, 1962).

In view of the usefulness of this technique, Derse (1960) proposed a standardization of the procedures used in determining the P.E.R., to allow comparison of results obtained.

From the literature one can see that P.E.R. values have been shown to correlate with the quality of the

protein. The more balanced the protein the better the growth response of the animal and vice versa.

C. DIETARY NITROGEN UTILIZATION

In order to obtain a satisfactory and simplified method for evaluating dietary protein quality, nitrogen balance methods were subjected to a series of modifications. Mitchell (1923-4) estimated the biological value of the proteins by the formula $B.V. = \frac{\text{Retained nitrogen}}{\text{Absorbed nitrogen}} \times 100$. Retained nitrogen is equal to absorbed nitrogen minus urinary nitrogen and absorbed nitrogen is equal to food nitrogen intake minus fecal nitrogen. Bender and Miller (1953a) determined the protein value by comparing the gain in body nitrogen of rats after 10 days on a protein diet with those on a non-protein diet.

Later (1953b) they measured the water content of the body instead of determining the nitrogen content. In 1955 they established the Net Protein Utilization (N.P.U.) as a ratio of retained nitrogen/food nitrogen X 100. Retained nitrogen equals the body nitrogen of the test group less body nitrogen plus nitrogen consumed by the non-protein group. Food nitrogen is equal to the nitrogen consumed by test group. Bender (1956) obtained a close correlation between the Net Protein Utilization and P.E.R. values.

Bender and Doell (1957) proposed the Net Protein Ratio, which is defined as the sum of the weight loss of the non-protein group and weight gain of the test group divided by the weight of protein consumed by the test group. Morrison et al. (1963) showed good correlation between Net Protein Ratio and P.E.R.

True digestibility is defined as the fraction of dietary nitrogen absorbed into the blood stream (Allison, 1955). Absorbed nitrogen is calculated by the following equation: A = I - (F-Fm) I = nitrogen intake, F = fecal nitrogen and Fm = endogenous nitrogen. True digestibility = A/I. If correction for fecal losses is not made the value is designated as Apparent Digestibility = I - F/I. Waterlow and Verity (1960) gave the following equation: Apparent nitrogen absorption = N intake - fecal N/N intake X 100.

D. THE MEADOW VOLE (MICROTUS PENNSYLVANICUS) IN BIOASSAY TECHNIQUE

Mice of the genus Microtus are the most abundant mammals around the world. They live in temperate and boreal zones, usually in farming fields. The species M.pennsylvanicus is one of the most wide spread among the several species. In the United States they can be found in the Northeastern parts of the country. These animals are easily captured by means of mouse traps. When housed in metal containers and fed foods with high

water content, they breed and exhibit normal behavior. Males of M. pennsylvanicus are sexually mature when they are five weeks old. The females have shown to be most prolific among mammals with a gestation period of three weeks.

The voles at birth are pink, hairless, and weigh 1.6 to 3 grams. Litters are composed of five to six animals. Growth is rapid from one to two days after birth. While the young mice are still nursing they supplement their milk food with vegetables, living on this diet until they are weaned. They are weaned before they reach the age of twelve days when they weigh approximately twelve grams. Once weaned, they grow rapidly, gaining about one gram per day (Hamilton, 1941).

Whitmoyer (1956) has observed certain characteristics of the growth rates of M. pennsylvanicus:

a) No statistical differences were observed in the instantaneous growth rates of males and females in a one month period.

b) During the first three weeks no distinct growth periods were observed.

c) While animals within litters were consistent in their rate of growth, the average rates for the litters have shown fluctuations during the first three weeks. Also, significant differences were observed between litters for short periods.

d) Heredity, parental history, size of litter, degree of disturbance, and month of birth are factors affecting growth rates.

Meadow voles are used as experimental animals mainly in studies with forage plants, which are characterized by their low protein and high content of fiber.

Studies done by Elliott (1963) have demonstrated that the meadow voles (M. pennsylvanicus) can be used as a bioassay organism. Specific growth responses were obtained when the meadow voles were placed on experimental diets for six days (Elliott, 1963). Schillinger and Elliott (1966) calculated the specific growth (Gsp) from the equation:

$$Gsp = \frac{Ge - Gc}{Gc}$$

Ge equals average percent weight gain on the experimental diet. Gc equals average percent weight gain on control diet.

5. POTATOES AND BEANS IN NUTRITION

A. POTATOES AS FOOD

Potatoes belong to the family of Solanaceae. They were first cultivated near lake Titicaca on the Peru-Bolivian border. This zone of the Andes is 9000 to 13500 feet above sea level (Cox, 1967). Interesting varieties that are difficult to recognize as potatoes exist in these

regions. Some are golden yellow, others are purple, blue, spotted or striped. The shape varies from round or oblong to cylindrical and the skin may be smooth (Talburt, 1967).

Potato production is spread over the world. Potatoes constitute one of the largest crops and are a valuable protein source for the human diet. An advantage of this food is its long storage time.

The potato was dried at least 200 years ago. The natives of the mountainous Andes allowed them to freeze at night, squeezed the free liquid from the thawed potato with their bare feet and then sun dried the potatoes. This operation was repeated until the potatoes were sufficiently dry to keep (Talburt, 1967). This dried product is still sold in the markets under the name of "chuño negro." Another type of dried potato, "chuño blanco," is also produced. It is dried under straw to prevent blackening.

At the present, the potato processing industry is expanding in the U.S.A. In 1940 less than 2% of the total potato crop was processed, in 1955, slightly over 15%, in 1962, 25% (Feustel et al., 1964). In 1966 the figure reached over 41% (Smith and Davis, 1968). Although the amount of potato chips produced has increased steadily per year, frozen products are increasing most rapidly. Frozen products represented over 41% of the processed volume, dehydrated potatoes (potato granules, shreds and

flakes) over 20% and the rest was processed as canned products, such as soups, stews, hash, etc. (Smith and Davis, 1968).

Although there are more than 350 varieties of potatoes, at present only a few are being commercially grown. About 50 varieties are being cultivated in the USA, and each variety has its own characteristic yield, shape, size, resistance to disease and degree of adaptation to climatic conditions. The United States Department of Agriculture, as well as various State Experimental Stations, are conducting breeding programs which have produced new varieties with greater resistance to disease and proper conditions for processing (Feustel et al., 1964).

The consistency of the finished product is an important factor in determining the quality of processed potato products. A potato variety with mealy texture also possesses a high solids content. Usually the higher the specific gravity or dry matter content, the more suitable the potato is for processing (Smith, 1968). Potato varieties such as Kennebeck, Russet Burbank, Cherokee, Haigh, Irish Cobbler, Katahdin, Norgold Russet and Superior are considered the best ones for processing (Thompson, 1967).

The white potato makes important contributions to human nutrition. As an energy source it ranks second

only to cereals among the products of the vegetable kingdom used for human food. The literature on the chemistry of the potato is extensive and from the data it is difficult to obtain a clear picture of the raw potato composition. The proximate composition of potato flakes per 100 grams of edible portion is: water, 5.2 g; protein, 7.2 g; fat, 0.6 g; carbohydrate, 84 g; fiber, 1.6 g; ash, 3 g; niacin, 5.4 mg; thiamine, 0.23 mg; and riboflavin 0.06 mg (USDA, 1963). Other vitamins present in potatoes are folic acid, panthotenic acid, and pyridoxine; which are present in relatively higher amounts than in other vegetables (USDA, 1957). Mineral content is: calcium, 35 mg; phosphorus, 173 mg; iron, 1.7 mg (USDA, 1963). Potatoes are one of the richest sources of potassium. The fact that potatoes are a good source of potassium may result in the efficient metabolism of protein. Barnes et al. (1961) have shown that adequate amounts of potassium were required for the good utilization of protein.

It is well known that the cooking method influences the vitamin content of potatoes. After processing by different household methods (baking, cooking in a saucepan and frying) the content of ascorbic acid, dehydro ascorbic acid, niacin and thiamine was measured. Thiamine was 85% retained when potatoes were boiled unpared, with no reduction of the other vitamins. Seventy to 80% retention of ascorbic acid and somewhat higher retention for

the other vitamins was observed when potatoes were pared. Pared, quartered potatoes cooked in a saucepan retained about 80% ascorbic acid with no destruction of the other nutrients. Potatoes baked in their skins kept 70% of the thiamine and the other vitamines were not affected. Frying of the raw product gave a retention of 60% ascorbic acid, thiamine being almost completely destroyed (Hewston et al., 1948).

The length of storage was shown to have an effect on the ascorbic acid content. In a period of seven months, the ascorbic acid content of raw, fresh mashed and reconstituted dehydrated potatoes decreased from 29.3; 18.8; and 8 mg/100 grams respectively to 10.6; 6.8; and 2.8 mg/100 grams (Bring et al., 1963). During the process of potato flaking, 71 to 73% of the natural and added ascorbic acid was retained. Flakes containing 5% moisture and antioxidant retained 70 to 76% of the natural and added vitamin C. When stored under nitrogen at 75° F for 28 weeks, no vitamin destruction was observed (Cording et al., 1961). The destruction that occurred during cooking was greater for pyridoxine than for niacin; 4.8 and 8.8% losses of pyridoxine for boiled and baked potatoes in relation to 1.5 and 4.2% for niacin (Page and Hunning, 1963).

Potatoes have substantially more of all essential amino acids except histidine than whole wheat. The

non-protein nitrogen of boiled potatoes showed appreciable amounts of lysine and arginine and poor levels of the remainder of the essential amino acids (Hughes, 1958).

In the table of amino acids published by Hopper (1958) white potato contains more tryptophan, threonine and methionine than the other vegetable proteins.

From studies done with rats, Mitchell (1924b) reported that at a concentration of 5% protein, the biological value of potato was lower than that of casein, corn, oat, rice or yeast. However, when the level was raised to 10% protein, the biological value of potato protein was the highest. Mitchell pointed out that the nitrogen balance method could have failed to measure adequately the yield of the potato protein, since it contained significant amount of non-protein nitrogen.

The biological value of potato protein (50% boiled potato, 50% commercial flakes) was compared with the biological value of protein of ground whole barley. Two levels of nitrogen were used: 1.5% with no casein and 2.4% with 6% casein. The results showed lower values for potatoes at both protein levels. The authors stated that the heat from processing could have damaged the protein (Hutchinson et al., 1943). Rats fed potato protein gave higher results than those fed wheat, but its apparent digestibility was lower (Chick and Cutting, 1943).

In a theoretical comparative study, Hegsted (1957) demonstrated that the protein of potato can easily furnish the required protein for a child with an 800 calorie intake, as related to corn grits and white bread which cannot be used as the sole protein source.

Flodin (1953) stated that a combination of potatoes and cereals, especially corn, makes desirable food mixtures because of the level of lysine and tryptophan in potatoes.

Non-protein nitrogen in potatoes increases more than protein nitrogen when nitrogen fertilization is increased. However, the nutritive value of protein is not impaired due to the greater gain in protein nitrogen.

During storage the nutritive value of the tubers is enhanced due to a decrease in both total solids and non-protein nitrogen. Protein nitrogen decreases in sprouting tubers (Pol and Labib, 1963).

B. BEANS AS FOOD

Legumes are generally of high protein content but their nutritional value has to be improved by adequate heat treatments and by the addition of one or two amino acids. Legumes also contain high amounts of carbohydrate material and low amounts of fat except for soybeans and peanuts.

Beans are considered among those foods which can help to solve the big gap of protein deficiency by increasing the protein supply in those parts of the world where animal protein is scarce or expensive.

In some places beans are a staple food. Individuals in certain communities in Rwanda and Uganda consume as much as 400 to 500 grams of beans per day. Beans are said to be the "poor's meat" (Aykroyd and Doughty, 1964).

Nutritional surveys in the developing countries have shown that consumption of beans ranges from 10 to 50 grams daily. This food fits well in the budget of the economically unfavoured people. Beans also have the great advantage of a long storage period, without the necessity of refrigeration.

Most of the studies have been made on the bean varieties of the species Phaseolus vulgaris, since they are most commonly consumed. Kakade and Evans (1964) reported the chemical composition of navy beans (Sanilac) expressed in grams per 100 g of beans to be: Moisture, 8.96; ash, 3.71; crude fiber 4.18; carbohydrate, 58.07; protein 24.0; fat, 1.02; calcium, 0.15; phosphorus, 0.46 and iron 0.007. The same authors in 1965 reported the amino acid composition of five varieties of navy beans. Seaway contained more of the essential amino acids and more glutamic and aspartic acids than the other varieties, but methionine was lower in Seaway.

Variety and location are factors that influence the nutritive value of a food. Tandon et al. (1957) analyzed 25 varieties of kidney beans, produced in two different areas in Guatemala, for contents and levels of nitrogen, methionine, lysine, tryptophan, niacin, thiamine and riboflavin. The protein content ranged from 20.1 to 27.9%, with an average of 24.1%. The remaining values are listed below:

Methionine	from 0.17 to 0.33%	average 0.25%
Lysine	from 1.69 to 2.41%	average 1.98%
Tryptophan	from 0.14 to 0.22%	average 0.17%
Niacin	from 1.68 to 2.95%	average 2.22%
Riboflavin	from 0.16 to 0.23%	average 0.18%

Osborne and Clapp (1907) reported that phaseolin, the principal protein of navy beans, contained all the essential amino acids. A starch free product made from white beans did not support the growth of rats. Lafayette et al. (1912) attributed this to the presence of hemicellulose and cellulose in the product. McCollum and Simonds (1917) found that the protein of navy beans did not promote rat growth, despite the fact that the vitamin and mineral content of the diet was adequate. The mortality rate of the rats was also high. A diet of 70% or 45% bean meal, supplemented with 9% casein, supported growth. The 45% level of bean meal was shown to promote greater growth than the 70% level. McCollum and Simmonds

theorized that the gas liberated produced distention of the digestive tract or that some unknown chemical complex which is harmful to the young rat is present in the bean meal. The results obtained by Lafayette et al. and McCollum and Simmonds were confirmed by Johns and Fink (1920), who thought that the failure of the raw protein of navy bean to support growth was due to the low cystine in the diet. Addition of this amino acid to the diet resulted in maintenance of weight, but growth responses were not satisfactory. Johns and Fink (1920) also observed that heating the navy beans increased the efficiency of protein utilization and the protein quality was further enhanced when supplemented with cystine.

Everson and Meckert (1944) compared the nutritive value of raw and heated sources of protein, including navy, kidney, and pinto beans. They reported that the proteins from P. vulgaris were improved the most by the cooking.

Feeding raw beans to rats at a concentration of 15% protein, caused death sooner than feeding a diet of raw beans at a 10% protein level.

It has long been known that legumes contain substances toxic to the organism that can be destroyed by heat. These were found to be trypsin inhibitors and hemmagglutinins. The trypsin inhibitor blocks the

enzyme trypsin present in the digestive tract, and hemmagglutinins agglutinate the red blood cells (Liener, 1962).

Bowman (1944) noticed that one of the fractions of navy beans containing trypsin inhibitor retarded the in vitro digestion of milk casein. Jaffe (1949) reported that soaking beans prior to autoclaving improved their nutritive quality. The addition of methionine markedly improved the biological value of kidney bean protein and made it comparable to that of casein. In 1950 Jaffe demonstrated that heated beans had greater nutritive value and these observations were confirmed by Honavar et al. (1962). He also reported that fractions containing hemmagglutinin factors, free from trypsin inhibitors, were isolated from black beans and fed to rats in diets containing 10% casein. The growth was strongly affected and 0.5% of hemmagglutinin was reported to be a lethal dose.

Kakade and Evans (1963) found that properly heated navy bean protein was almost completely digested by trypsin; however, beans autoclaved for prolonged periods were poorly digested by trypsin. In 1964, they reported that a diet of raw navy beans supplemented with methionine, vitamin B12 and antibiotics barely promoted rat growth. Autoclaved navy beans supplemented with methionine supported growth to the same extent as a casein diet.

The fact that autoclaving destroys the trypsin inhibitor and hemmagglutinin in navy beans was confirmed by Kakade and Evans (1965). One hundred percent mortality occurred when rats were fed navy beans and a very high mortality was observed when autoclaved beans with 2% and 3% trypsin were fed. The same investigators (1966) found that soaking and germinating the beans did not eliminate the toxic factors. They believe that soaking prior to autoclaving was not necessary since heat alone destroyed the toxic factors.

Bressani and Valiente (1962) reported that maximum growth in rats was obtained by feeding a combination of rice and black beans, with rice supplying 60% of the protein. Evans and Bandemer (1967) found that a mixture of navy beans and Gary oats, each supplying 5% protein, was superior to either source alone, at a level of 10% protein. They also reported that methionine supplementation of legume diets improved growth rate.

Powrie and Lamberts (1964) reported that the apparent digestibility of navy beans canned in water was decreased from 69 to 63% when processing times at 121°C were increased from 20 to 70 minutes. Inclusion of glucose decreased the digestibility to 47%. Weight gains, P.E.R. and biological values were also reduced.

METHODS AND MATERIALS

Potato flakes and drum dried navy bean powders prepared in the Department of Food Science laboratory were used.

Sample designation. Potatoes: Numerically designated clones 709, 58, 1111-2, 711-3, 711-8, 706-32, 706-34, Russet Burbank and the Solanum tuberosum-S. stoloniferum species hybrids 321-65, 322-6 were used.

Potato flakes were processed as described by Pope (1969). The procedure is given in the appendix. After processing the potato flakes were powdered in a micromill (Chemical Rubber Co.).

Beans: Navy beans, Phaseolus vulgaris (Var. Sanilac), which had been atmosphere or retort cooked, then drum dried, were used. The powders were prepared as described by Counter (1969) and the procedure is given in the appendix.

Determination of proteins. The protein content of both the navy bean powders and potato flakes was determined by the Kjeldahl method as described by A.O.A.C. (1965). The protein content of the potatoes was also determined using the Biuret procedure (Layne, 1955). The potato protein extract was prepared as follows: 40 ml centrifuge tubes containing 20 ml of deionized water were placed in a 0° F freezer until the freezing point was almost reached. Two grams of powdered potato flakes

were very slowly added to the tube while it was being constantly agitated with a test tube mixer (Deluxe mixer-Scientific products) to avoid the formation of lumps. When the total sample was mixed, the walls of the tubes were washed down with five ml of cold water and shaking was continued for another three minutes. The tubes were again placed in the freezer, cooled almost to the freezing point and then centrifuged for 30 minutes at 2800 r.p.m. in an International model U centrifuge. The clear supernatant liquid was decanted into labeled test tubes.

Analysis of protein. One ml of the extract solution was transferred to small centrifuge tubes (1/2 x 4 inches). Four ml of Biuret reagent was added and the tubes were inverted five times to mix thoroughly. Tubes were allowed to stand 30 minutes at room temperature (70° F) for color development and then were centrifuged for 10 minutes at 2600 r.p.m. in a clinical centrifuge, model C.L. The clear supernatant liquid was transferred to a Bausch and Lomb Spectronic 20 Cuvette (1/2 x 4 inches) and absorbance determined at 545 mu. The instrument was set at 0 absorbance using 1 ml H_2O plus 4 ml Biuret reagent (E. and M. Chemicals, Brinckmann Instruments, Westbury, New York). The amount of protein present was determined from a standard curve or formula (Fig. 3). The curve was obtained as follows: representative aliquots (0.2, 0.4, 0.6, 0.8 and 1 ml) of a Standard E.M. protein

serum containing 6 g protein/100 ml were transferred to the cuvettes and made up to 1 ml with distilled water. Four ml of Biuret reagent were added and the color development and absorbance measurement were the same as described above.

A straight line relationship was obtained (Fig. 3, appendix), which gave a factor of 15.69 (concentration of protein mg per ml/absorbance). The protein concentration of the samples was determined by the formula:

$$\frac{\text{Sample absorbance X factor X } 100^{1}}{\text{weight of sample}} \text{ X } \frac{1^{2}}{1000} \text{ X } 100$$

1 -- sample diluted to 25 x 4

2 -- conversion of mg to grams.

Amino acid analysis. Amino acid determinations were made using a Beckmann amino acid analyzer, model 120 C.

The samples were prepared for amino acid determination as follows:

1. 10 mg bean powder or 30 mg potato powder was weighed into 10 ml ampoules, 6 ml 6 N HCl was carefully added and the contents thoroughly mixed.

2. The ampoules were placed in a dry ice ethanol bath and evacuated with a vacuum pump until they froze.

3. They were removed, allowed to slowly melt and examined for the presence of gas.

4. If free of gas the ampoules were refrozen in the dry ice-ethanol bath under vacuum and sealed with a gas flame.

5. The sealed ampoules were transferred to an oven at 110°C and held for 22 hours to hydrolyze the proteins.

6. The ampoules were then opened and 1 ml of norleucine solution containing 2.5 micromoles was added. The addition of a known amount of norleucine permits correction for losses in transfer.

7. The hydrolyzate was transferred to a 25 ml pear shaped flask and evaporated to dryness on a rotatory evaporator.

8. The residue was dissolved with 0.5 ml of deionized water and redried.

9. The residue was dissolved in dilutor buffer (pH 2.2) as described in the manual for the Beckmann amino acid analyzer, model 120 C, transferred to a 5 ml volumetric flask and made to volume with buffer.

10. From this sample an aliquot of 0.2 ml for basic amino acids and 0.3 ml for acidic and neutral amino acids was transferred to the amino acid analyzer for analysis.

Since the amino acid analysis has been made on acid hydrolyzates, tryptophan was not determined.

Protein evaluation by animal assays. Two bioassay techniques were used, namely growth methods, expressed as Protein Efficiency Ratio (P.E.R.) and gain in body weight. The absorption of nitrogen was measured by apparent absorbability, which does not consider endogenous nitrogen. Litters of weanling voles, Microtus pennsylvanicus, were used. These were obtained from the Department of Crop and Soil Sciences Colony at Michigan State University. The voles were weaned at two weeks and only those attaining a weight of 11 grams were used. The voles were assigned randomly to individually disposable plastic laboratory cages equipped with a vole feeder (Shenk and Elliott, 1969) as described in the appendix. The voles were fed semi-synthetic diets (starter) for two days before they were given the experimental diet. Five voles were used for each diet. The experimental diet was fed for six days. The voles were weighed every two days, four weighings in all. Both the water and food were supplied ad libitum. The food consumption values were determined by the loss in weight of the entire feeder. The feces were collected at the end of the experiment from the sheets of filter paper placed underneath the wire mesh, dried at 105° F for 22 to 24 hours, weighed and ground for total nitrogen determination using the microkjeldahl technique (A.O.A.C., 1965).

Composition of diets. The composition of the control, bean powder and potato powder diets is given in Table VII (appendix). "Vitamin free casein" was used as the protein source in the control diet. The diets were mixed with the amount of water necessary to obtain a dough like consistency, then molded into wafers of approximately 1/2 x 2 x 6 inches that would fit into the vole feeder (Shenk and Elliott, 1969). They were dried at 105° F for 48 hours, wrapped in aluminum foil and stored at 0° F until needed. They were thawed at room temperature for 16 to 18 hours before weighing and feeding. The moisture content of the wafered diets at the time of feeding was determined using the vacuum oven method (A.O.A.C., 1965) and values obtained are given in Table I.

In the first experiment, two processed samples of navy beans and six samples of potatoes were used. These included atmosphere cooked navy bean powder and powders prepared from Russet Burbank potato, potato 58, potato 321-65, potato 322-6, potato 711-3, and potato 709. These powders were incorporated into the diets so as to provide 5.28% protein.

In the second experiment, atmosphere cooked navy bean powder and potato powders 58, 321-65, 322-6, 711-3, were used. These diets also had 5.28% protein and were supplemented with methionine. Methionine was added to

Table 1.--Moisture content of samples.

	As processed	Experiment I	Experiment II
	g/100 g		
Control casein	6.00*	16.25	15.31
Atm. cooked bean	5.32	19.85	16.85
Retot cooked bean	6.88	18.45	-----
Potato R. Burbank	8.98	21.19	-----
Potato 58	6.52	12.51	32.42
Potato 321-65	6.10	20.45	28.98
Potato 322-6	6.42	21.78	35.92
Potato 711-3	4.82	17.48	27.30
Potato 709	6.38	23.56	-----

*Data obtained from Nutritional Biochemical Corporation.

each 100 grams of atmosphere cooked bean diet at a level of 0.11g and 0.066g of methionine was added to each 100 grams of potato powder diet. Sodium propionate at 0.3% level was added to these diets as a fungicide.

The Chemical Score (C. S.) method (Mitchell and Block (1946) was used to determine the amount of methionine required where:

1. The amino acid content of the sample was expressed as the per cent of the same amino acid in whole egg protein.

2. 100 - percent found = per cent deficit.

3. 100 - per cent deficit = Chemical Score.

For example, in atmosphere cooked navy bean powder, the methionine content of 0.57g/100 g protein was 14 per cent of that of whole egg protein. Therefore, the Chemical Score from the above formula is 14.

To determine the amount of methionine to add as a diet supplement, it is necessary to determine the Chemical Score of the second limiting amino acid; in both navy bean powder and potato flakes, this amino acid was isoleucine (Table II). The Chemical Score for isoleucine in bean powder was 66. The methionine required was calculated as follows:

$$= \frac{\text{C.S. isoleucine x g/100 g protein methionine}}{\text{C.S. methionine}}$$

$$= \frac{66 \times 0.57}{14} = 2.68 \text{ g/100 g protein}$$

The diet used contained 5.28 g protein/100 g. Since there was 0.57 g methionine present, 2.68 - 0.57 = 2.11 g methionine/100 g protein will be required and the amount of methionine to add to the diet will be:

2.11 x 5.28/100 g diet.

For the potato flake diets, the methionine required to supplement the diets was determined using the average Chemical Score of the six varieties. Values for methionine and isoleucine were 27 and 57.3, respectively.

Table II.--Chemical Score of navy beans and potatoes as related to the amino acids of whole egg

	Whole Egg % Protein	NAVY BEANS		POTATOES					
		Atmp. Cooked	Retort Cooked	Russet Burbank	58	321-65	322-6	711-3	709
Arginine	6.4	100	100	99	87	66	92	66	82
Histidine	2.1	100	100	100	98	96	100	100	98
Lysine	7.2	100	100	99	86	100	100	76	85
Tyrosine	4.5	82	80	96	88	98	100	96	82
Tryptophan	1.5	---	---	--	--	--	--	--	--
Phenylalanine	6.3	99	100	77	62	87	90	78	64
Methionine	4.1	14	14	28	30	19	24	31	30
Threonine	4.9	100	100	68	76	100	100	96	85
Leucine	9.2	96	98	70	64	94	90	77	68
Isoleucine	8.0	65	66	50	50	74	62	56	52
Valine	7.3	80	88	88	85	92	82	73	74

RESULTS AND DISCUSSION

PROTEIN CONTENT OF BEAN AND POTATO POWDERS

The total protein content of the bean and potato powders is given in Table III. The protein content of the atmosphere and retort cooked bean powders did not differ significantly and averaged 23.4 g per 100 g. The protein content of the potato powders determined by the Kjeldahl procedure ranged from 5.9 to 9.1 g per 100 g, and by the Biuret method from 5.5 to 9.3 g per 100 g. The differences in protein content obtained by the two methods were not significant and indicated that the Biuret method could be used as a more rapid procedure for determining the protein content. The ten values obtained for each sample of potato were tabulated and the degree of relationship among them was estimated by statistical parameters (standard deviation and standard error) and these values are reported in the appendix (Table VIII).

The extraction of the potato flake powder with ice cold water and centrifugation of the extract after reaction with the Biuret reagent eliminated the cloudiness caused by the presence of starch in the solution.

The amino acid composition of the two samples of navy beans and the six varieties of potatoes was calculated following instructions given in section 8 of the manual for the Beckman amino acid analyzer, model 120 C. The

Table III.--Protein content of bean powder and potato flakes.

SAMPLE	KJELDAHL METHOD[1] g/100g[3]	BIURET METHOD[2] g/100g[4]
NAVY BEANS		
Atmosphere cooked	23.6	--
Retort cooked	23.2	--
POTATOES		
321-65	5.9	5.5
Russet Burbank	6.9	6.7
322-6	6.7	6.8
58	7.7	7.9
709	7.6	8.0
711-3	8.7	8.2
711-8	7.9	8.3
706-34	7.9	8.4
706-32	8.3	8.7
1111-2	9.1	9.3

[1]Mean value of two determinations.

[2]Mean value of ten determinations.

[3]Protein (N x 6.25).

[4]Total protein.

calculated results are reported in g/16g nitrogen (appendix Table IX).

No meaningful differences were found in the amino acid content of the bean powders, indicating that the amino acids were not altered by the method of cooking.

The various strains of potatoes analysed vary considerably in amino acid content. The lysine, threonine, and tyrosine content of strains 321-65 and 322-6 was higher than that of whole egg. Histidine, except for strain 321-65, was at optimum levels and leucine, phenylalanine, arginine, isoleucine, and methionine levels were all lower than those of whole egg.

If the potato strains are arranged according to decreases in the number of essential amino acids (plus tyrosine, a non-essential) that were below the level found in whole egg, the following order is obtained: 58 > 709 > Russet Burbank > 711-3 > 321-65 > 322-6. None of the amino acids of strain 58 were as high as those in whole egg.

Comparison of the data obtained in this study with the amino acid composition pattern for potatoes published by Hopper (1958) shows several differences. Lysine, threonine, methionine, leucine and phenylalanine were lower in this study than reported by Hopper and isoleucine was present in higher proportion. Levels of the rest of the essential amino acids were similar. The amino acids

methionine, isoleucine, valine and tyrosine were present in lower amounts in bean powder than in whole egg. Amino acid values obtained, except for methionine, were higher than those previously reported by Kakade and Evans (1965) for the Sanilac variety.

Tandon et al. (1957) have shown that soil conditions influence the nutritive value of food. This could explain the differences between the results obtained in this study and those reported in the literature.

Aspartic and glutamic acids were present in considerably higher quantities than the essential amino acids in both navy beans and potatoes. They ranged from 12.2 to 26.4 and 13.4 to 23.0 g/100 g N in beans and potatoes respectively. These two dispensable amino acids were markedly greater in potato 58.

Chemical Score. The chemical scores of the different amino acids are given in Table III. In both bean and potato powders the lowest chemical score was found for methionine, being 14 for beans and 19 to 31 for potatoes. In the bean powder, in addition to methionine, isoleucine, valine, and tyrosine also had low scores. The chemical score of potatoes can be arranged in an ascending order as follows: 321-65 < 322-6 < R. Burbank < 58 < 709 < 711-3. This shows that potato 711-3 has the highest chemical score for the amino acids. If the Mitchell and Block (1946) chemical score is used as a

means of predicting the efficiency of the protein utilization for growth it may be said that potato powders could be better protein sources than bean powders.

Bio-assay using meadow voles. The food consumption of voles was reported on a dry matter basis. The results of both experiments are expressed as gain in body weight, and Protein Efficiency Ratio (P.E.R.). The Protein Efficiency Ratio is calculated as the ratio of weight gain/protein intake.

The digestive efficiency of the proteins was calculated as the ratio of nitrogen utilized to the nitrogen intake and calculated by the following formula:

$$\text{Apparent absorbability} = \frac{\text{N. Intake - Fecal N}}{\text{N. Intake}} \times 100$$

In the first experiment with the non-supplemented protein diet, the voles all gained weight. The weight gain ranged from 0.5g to 1.3g, with potato 58 giving the highest weight gain, and was greater than that of casein diet (4.77% protein). These results (Table IV, Fig. 1, appendix Table X) indicated that with a protein concentration of 5.28% the essential amino acid levels were high enough and sufficiently balanced to support vole growth. The presence of high levels of glutamic and aspartic acids may have contributed to the increased efficiency of protein utilization. However, the P.E.R. values

Table IV.--Vole growth with navy bean and potato powders as sole protein source (6 days).

Diet	Non-supplemented			Supplemented		
	Food consumed g	Wt. gain g	P.E.R.	Food consumed g	Wt. gain g	P.E.R.
Casein	12.90	1.10	1.51[a]	15.24	1.60	1.68[b]
Bean						
Atm. cooked	13.47	1.10	1.25	14.81	2.50	2.66
Retort cooked	15.49	0.70	0.70	---	--	--
Potato						
58	17.94	1.30	1.20	17.09	1.80	2.00
322-6	16.14	1.17	1.07	22.30	2.60	1.41
321-65	14.16	1.00	1.06	15.84	1.90	1.27
711-3	13.54	0.80	0.93	16.58	2.20	1.80
709	13.61	0.60	0.64	---	--	--
R. Burbank	10.09	0.50	0.76	---	--	--

a = 4.77% protein; b = 5.28% protein.

Table IV.--Continued.

Statistical Significance 5% non-supplemented diets.

58	322-6	Atmp. cooked bean	321-65	711-3	Retort cooked bean	709	Russet Burbank
1.30	1.17	1.10	1.00	0.80	0.70	0.60	0.50

(Table IV, Fig. 2) of the vegetable protein diets with 5.28% protein content were all lower than that of the casein diet (4.77% protein).

Food intakes were higher when potatoes 58 and 322-6 were fed, and this decreased their P.E.R. values.

Lowest weight gains and P.E.R. values were obtained with retort cooked bean powder, R. Burbank and potato 709.

Although the chemical scores of all essential amino acids of potato 58 were below 100, the higher weight gain indicated that they were in better balance than in the other samples. The lower weight gain with R. Burbank could be attributed to the low intake of food. The average food consumed was 10.09 g in 6 days which was one of the lowest among the nine groups (Table IV).

The lower weight gain with diet 709 may have been due in part to an imbalance of the essential amino acids. The lower weight gain obtained with retort cooked bean powder may have been due to the method of cooking.

In the second experiment, the vole weight gain on the supplemented diets with the exception of those on the potato 58 diet, were greater than those obtained on the non-supplemented diets. The greatest vole weight gains were obtained with diets using atmosphere cooked beans and potatoes 322-6 and 711-3. The range of the weight gain was from 1.5 to 2.6 g, as compared to 1.6 g for the casein

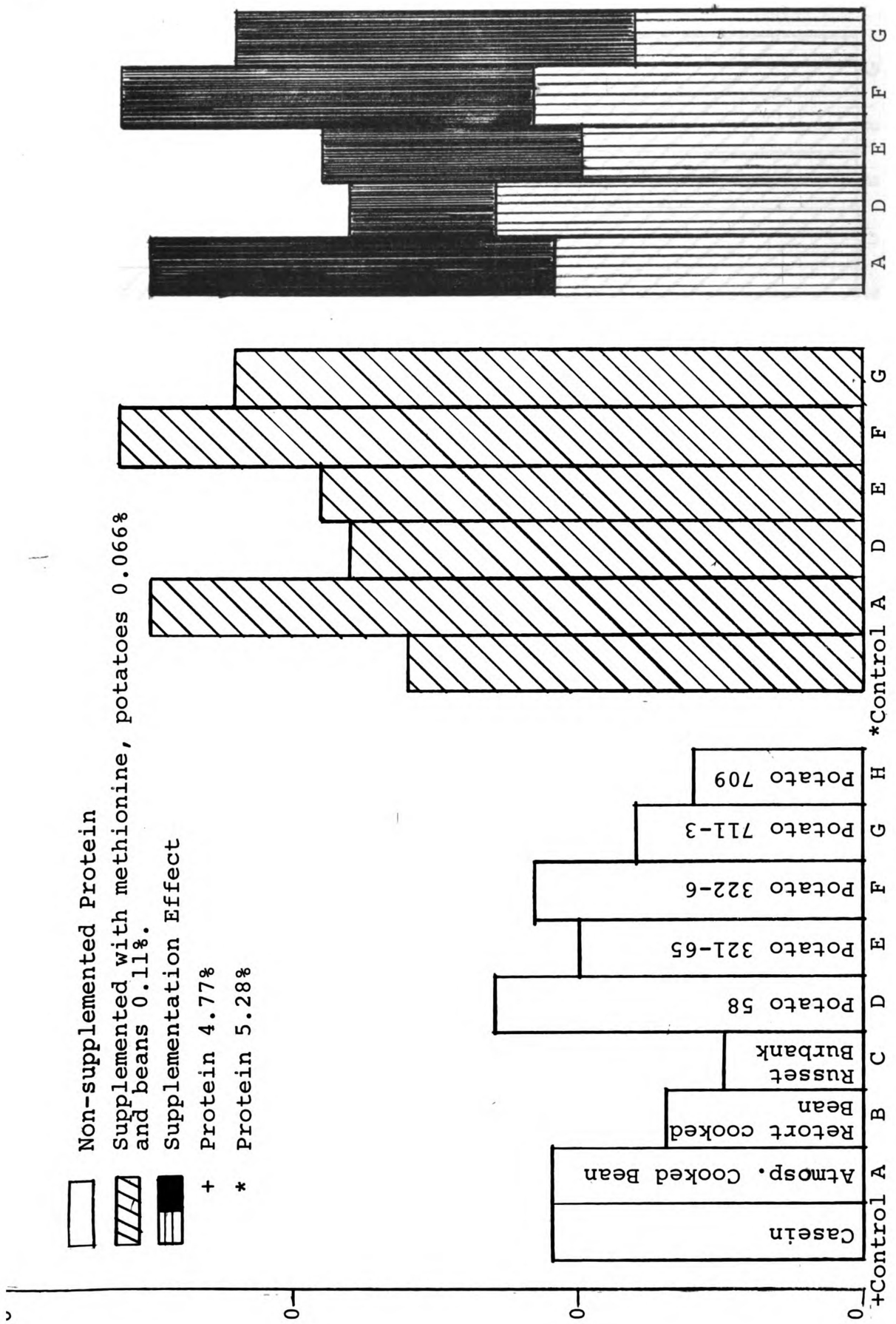


Figure 1.--Growth response in voles when supplied potato and bean proteins.

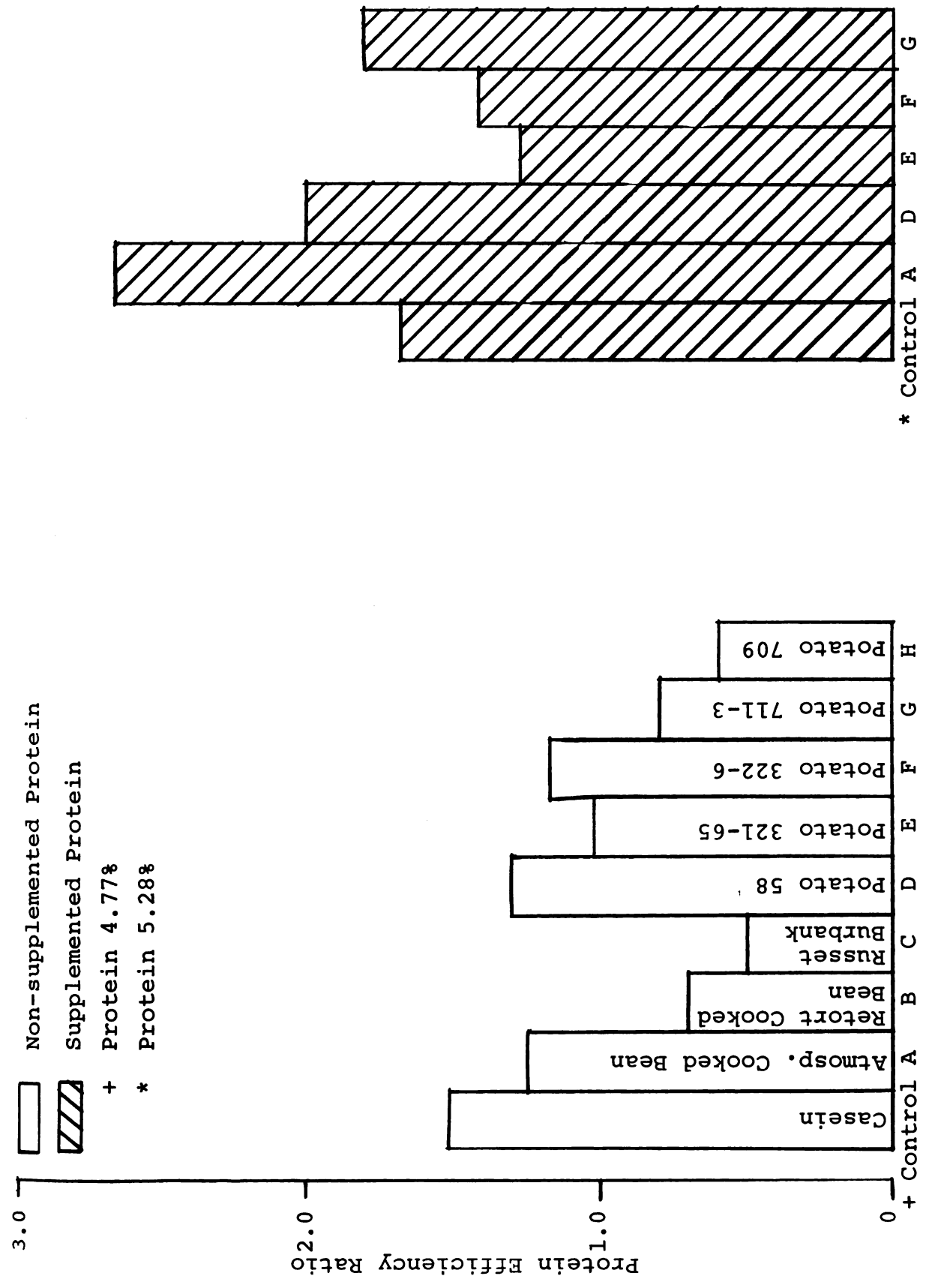


Figure 2.--Nutritional quality measured as protein efficiency ratio.

diet (Table IV, Fig. 1). The P.E.R. values were also greater with the supplemented diets; they ranged from 1.27 to 2.66 as compared to 1.68 for casein (Table IV, Fig. 2). Potato 322-6 and 58 diets were consumed by the voles in greater amounts than other potato or bean diets.

No significant differences were found in the weight gains on the different supplemented diets (Table XI, appendix). Since feeding trials demonstrated nutritional efficiency, the six comparable diets of the two experiments were statistically analysed using the orthogonal contrast method (Mendenhall, 1968) (Table XII and XIII, appendix). The results showed that the weight gains on diets of the supplemented, atmosphere cooked navy beans and potatoes 321-65, 322-6 and 711-3 were significantly greater than those of the non-supplemented diets.

Digestibility is considered to be a good index in evaluating the nutritive value of proteins. In the unsupplemented diets the apparent absorbability of the potato proteins ranged from 55.34 to 63.82% (711-3 < R. Burbank < 709 < 321-65 < 322-6 < 58) and that of casein was 64.52%. Atmosphere cooked navy bean powder had 62.50% absorbability compared to 57.28% for retort cooked bean powder (Table V). The greatest vole weight gains were observed when diets of potatoes 58 and 322-6,

atmosphere cooked navy beans and potato 321-65 were fed, which correlates with the values found for nitrogen absorbability.

In the methionine supplemented diets the apparent absorbability of the protein was increased and ranged from 69.5 to 88.6%. Apparent absorbability increased in potatoes as follows: 58 < 321-65 < 711-3 < 322-6. Only potato 58 (69.5%) and atmosphere cooked bean powder (66.3%) were lower than the control casein diet (77.5%). The greatest increase in absorbability occurred for the potato 711-3 and 322-6 diets (Table VI).

The results of these studies are in agreement with the statement of Flodin (1953) that the nutritive value of proteins can be doubled if proper amounts of the deficient amino acids are added to the protein. The addition of methionine to atmosphere cooked navy beans, potatoes 322-6 and 711-3 markedly enhanced their nutritive value.

Keane (1962) reported that the addition of 20% water to the diet improved the P.E.R. values at protein levels of 6% and 12%. Since sufficient water was added to both diets in this study, the improvement in the non-supplemented diet can not be attributed to added water.

Both navy bean powders were similar in their amino acid composition. However, the results from feeding experiments have shown that the protein of retort

Table V.--Absorbability of nitrogen (non-supplemented protein)

Protein Source	Food intake (g/6 days)	Nitrogen intake (g N/100g food)	Dry weight of feces (g/6 days)	Fecal Nitrogen (g N/100g food)	Apparent Absorb-ability (%)
Control casein	12.90	0.93	1.56	0.33	64.52
Atm. cooked bean	13.47	1.04	1.82	0.39	62.50
Retort cooked bean	15.49	1.03	1.99	0.44	57.28
Potato R. Burbank	10.09	1.09	1.38	0.43	60.55
Potato 58	17.94	0.94	2.12	0.34	63.82
Potato 321-65	14.16	1.06	2.05	0.40	62.26
Potato 322-6	16.14	1.05	2.44	0.38	63.81
Potato 711-3	13.54	1.03	1.94	0.46	55.34
Potato 709	13.61	1.10	1.80	0.42	61.82

Table VI.--Absorbability of nitrogen (supplemented protein).

Protein Source	Food intake (g/6 days)	Nitrogen intake (g N/100g food)	Dry weight of Feces (g/6 days)	Fecal Nitrogen (g N/100g food)	Apparent Absorb-ability (%)
Control casein	15.24	0.98	1.37	0.24	75.51
Atmosphere cook beans	14.81	1.01	1.80	0.34	66.33
Potato 58	17.09	0.82	1.66	0.25	69.51
Potato 321-65	15.84	1.14	1.80	0.27	76.32
Potato 322-6	22.30	1.40	1.47	0.16	88.57
Potato 711-3	16.58	1.14	1.52	0.24	78.94

cooked navy beans was impaired. The growth failure as compared to the value for atmosphere cooked beans could be attributed to the influence of heat while processing. Everson and Mackert (1944), Patton (1948), Jaffe (1950), Honavar (1962) and Kakade and Evans (1965) have shown that heat is beneficial in the destruction of anti-nutritional factors but over-heating of the food can be deleterious. Destruction of amino acids was attributed to non-enzymatic browning reactions, in which free amino acids interact with reducing sugars. Amino acids having a functional nitrogen which is not attached in a polypeptide linkage are especially prone to destruction by non-enzymatic browning (Patton, 1948).

Since beans and potatoes were subjected to heat treatment while processing (Counter, 1969 and Hope, 1969), the intensity of heat applied may have reduced the nutritive value of the proteins as, for example, in the case of retort cooked beans. Kakade and Evans (1963) observed that prolonged autoclaving of navy beans decreased the amount of lysine and methionine.

Rats fed autoclaved navy beans supplemented with methionine responded as well as those fed on 10% milk protein (Kakade and Evans, 1964). This study using voles (Microtus pennsylvanicus) as bioassay organisms has shown that supplementation of processed navy beans with methionine doubled the nutritive value, as compared to the

non-supplemented beans. This fact shows the sensitivity of the animal's response to the quality of the protein fed. Furthermore, it seems that meadow voles are sensitive to small differences in the concentration of the proteins in the diets, since different results were obtained with casein at 4.77 and 5.28%. Growth and nitrogen absorption were improved at 5.28%.

This study has shown that the protein quality of potato and navy bean powders was improved when methionine was added to the diets. Protein quality was assessed by growth methods (weight gain and P.E.R.) and by apparent nitrogen absorption.

SUMMARY AND CONCLUSIONS

The protein content of navy bean powders produced from beans cooked in the atmosphere and in a retort was found to average 23.4 g/100g. The protein content of powders prepared from a number of potato strains ranged from 5.5 to 9.3g/100g as determined by the Biuret method and from 5.9 to 9.1 by the Kjeldahl method. The protein contents determined by the Kjeldahl and Biuret procedures were not significantly different, indicating that the Biuret method could be used for the determination of potato protein.

The amino acid analyses showed considerable variation in the levels of amino acids in the various potato strains. However, in every case, methionine was the low level amino acid. This was also true for the bean powder. Glutamic and aspartic acids were present in greater amounts than the essential amino acids in both bean and potato powders. Potato 58 contained more of these two dispensable amino acids than the other strains.

Chemical scores were successfully used in the studies with Meadow voles, whose amino acid requirements are not known.

In the non-supplemented plant protein diet containing 5.28% protein, the amino acid content of potato was better than that of beans and supported growth more efficiently than beans. The weight gains on potato diets

ranged from 0.50 g (R. Burbank) to 1.30 g (potato 58). The vole weight gain on the casein (4.77%) and atmosphere cooked bean diets averaged 1.10 g and for retort cooked beans, 0.70 g. Milk protein showed a higher P.E.R. (1.51) than vegetable proteins, which ranged from 0.76 (R. Burbank) to 1.25 (atmosphere cooked beans).

Potatoes and beans at a 5.28% level of protein gave better weight gains when fortified with methionine, indicating that methionine was the first limiting amino acid in the atmosphere cooked navy beans and in potato clones 322-6, 711-3 and 321-65. With potato 58, methionine supplementation did not significantly improve the growth rate, indicating that methionine was near the optimum level and additional methionine may have disturbed its nutritional balance.

Chemical analyses of the atmosphere and retort cooked beans showed no meaningful differences in their proteins. However, feeding trials showed that the nutritive value of retort cooked beans was impaired, indicating the retort or high cooking temperatures decreased the nutritional value. The low nutritive value observed with the Russet Burbank potato could be attributed to the small amount of food consumed on this diet. With potato 709, an imbalanced protein could have caused the low growth response.

Digestibility studies have demonstrated that the addition of methionine increased the nitrogen absorption with all diets.

The use of Meadow voles in nutritional studies merits attention due to some advantages of voles over other experimental animals. Voles are: a) inexpensive, b) easily obtained, c) grow rapidly and d) show a sensitive response to different diets at low levels of protein. These could be factors of primary importance in biological research in developing countries.

LITERATURE CITED

LITERATURE CITED

Allison, J. B. 1955. Biological value of proteins. Physiol. Rev. 35:664.

Allison, J. B. and Wannemacher, R. W., Jr. 1957. Repletion of reserves in animals, pp. 1-13. In Amino Acid Malnutrition, ed. by William H. Cole. Rutgers Univ. Press, New Brunswick, New Jersey.

Allison, J. B. 1959. The efficiency of utilization of dietary proteins. In Protein and Amino Acid in Nutrition, ed. by A. A. Albanese, P. 97. Acad. Press, New York.

Allison, J. B. and Fitzpatrick, W. H. 1960. Protein malnutrition. In Dietary Proteins in Health and Disease, P. 13. Thomas; Springfield, Illinois.

Altschul, A. M. 1967. Food proteins. New Sources from Seeds. Science. 158:221-226.

Altschul, A. M. 1965. Kwashiorkor and Marasmus, pp. 184-188. In Proteins, their Chemistry and Politics. Basic Books, Inc. publishers, New York.

Association of Official Agricultural Chemists, 1965. Official Methods of Analysis, 10th ed. Washington, D.C.

Autret, M. 1961. Protein malnutrition and the FAO viewpoint, pp. 537-541. In N.A.S./N.R.C. Meeting Protein Needs of Infants and Children. Pub. 843.

Aykroyd, W. R. and Doughty, J. 1964. Legumes in human nutrition. FAO Nutritional Studies No. 19. Food and Agriculture Organization of the United Nations. Rome, Italy.

Barnes, R. H. and Borshardt, D. K. 1946. The evaluation of protein quality in the normal animal. Ann. N. Y. Acad. Sci. 47, 273.

Barnes, L. A., Kaye, R. and Valiasevi, A. 1961. Lysine and potassium supplementation of wheat protein. Am. J. Clin. Nutr. 9, 331.

Behar, M., Ascoli, W. and Scrimshaw, N. S. 1958. An investigation into the causes of death in children in rural communities in Guatemala, Central America. Bull. World Health Organization. 19, 1093.

Behar, M., Viteri, F., Bressani, R., Arroyave, G., Squib, R. L. and Scrimshaw, N. S. 1958. Principles of treatment and prevention of severe protein malnutrition in children. Ann. N. Y. Acad. Sci. 69, 954.

Behar, M. and Bressani, R. 1966. Experience in development of INCAPARINA for the preschool child, pp. 213-218. In Preschool Child Malnutrition. N.A.S./N.R.C. Pub. 1282.

Bender, A. E. and Miller, D. S. 1953a. A new brief method of estimating net protein value. Biochem. J. 53, VII.

Bender, A. E. and Miller, D. S. 1953b. Constancy of the N/H_2o ratio in the rat and its use in the determination of the net protein value. Biochem. J. 53, VII.

Bender, A. E. and Miller, D. S. 1955. The determination of the net value of protein by shortened method. Brit. J. Nutr. 9, 382.

Bender, A. E. 1956. Relation between protein efficiency and net protein utilization. Brit. J. Nutr. 10, 135.

Bender, A. E. and Doell, B. H. 1957. Biological evaluation of protein: A new aspect. Brit. J. Nutr. 11, 140.

Bengoa, J. M., Jellife, D. B. and Perez, C. 1959. Some indicators for a broad assessment of the magnitude of protein calorie malnutrition in young children in population groups. Am. J. Clin. Nutr. 7, 714.

Bowman, D. E. 1944. Fractions derived from soybeans and navy beans which retard the tryptic digestion of casein. Proc. Soc. Exptl. Biol. Med. 75, 219.

Bressani, R., Scrimshaw, N. S., Behar, M. and Viteri, F. 1958. Supplementation of cereal proteins with amino acids. Effect of amino acid supplementation of corn masa at intermediate levels of protein intake on the nitrogen retention of young children. J. Nutr. 66, 501.

Bressani, R. Wilson, D. L., Behar, M. and Scrimshaw, N. S. 1960. Supplementation of cereal proteins with amino acids. III. Effect of amino acid supplementation of wheat flour as measured by nitrogen retention of young children. J. Nutr. 70, 176.

Bressani, R. and Scrimshaw, N. S. 1961. The development of INCAP vegetable mixture. I. Basic animal studies, pp. 35-48. In Meeting Protein Needs of Infants and Preschool Children. N.A.S./N.R.C. Pub. 843.

Bressani, R. and Valiente, A. T. 1962. All vegetable mixtures for human feeding, VII. Protein supplementation between polished rice and cooked black beans. J. Food Sci. 27, 401.

Bring, S. V., Grass, L. C., Hofstrand, J. T. and Williard, M. J. 1963. Total ascorbic acid in potatoes. J. Am. Diet. Assoc. 42, 320.

Brozek, J. 1955. Nutrition and psyche with special reference to the experimental psychodietetics. Am. J. Clin. Nutr. 3, 101.

Burgess, A. and Dean, R.F.A. 1962. Malnutrition as public health problem, pp. 3-8. In Malnutrition and Food Habits, ed. by A. Burgess and R. F. A. Dean. The Macmillan Co., New York.

Burgess, A. E. and Dean, R. F. A. 1962. Food supply and consumption, pp. 9-18. In Malnutrition and Food Habits, ed. by A. Burgess and R. F. A. Dean. The Macmillan Co., New York.

Cording, J., Eskew, R. K., Salinard, G. J. and Sullivan, J. F. 1961. Vitamin stability in fortified potato flakes. Food Technol. 15, 279.

Coursin, D. B. 1968. Effects of undernutrition in central nervous system. Nutr. Rev. 23, 65.

Counter, B. T. 1969. Storage Stability of Drum Dried Navy Bean Powders. M.S. Thesis, Michigan State University, East Lansing.

Cox, A. E. 1967. The potato plant and its development, p. 11. In The Potato: A Practical and Scientific Guide, ed. by W. H. L. Collingridge LTD, London.

Cravioto, J., Roca de Licardie, E. and Vega, L. 1967. Amino acid protein malnutrition and mental development, pp. 449-457. In Amino Acid Metabolism and Genetic Variation, ed. by William L. Nyhan. New York, MacGraw Hill.

Chapman, D. G., Castillo, R. and Campbell, J. A. 1959. Evaluation of protein in foods. I. A method for determining protein efficiency ratio. Can. J. Biochem. Physiol. 37, 679.

Chick, H. and Cutting, M. E. 1943. Nutritive value of the nitrogenous substances in the potato. Lancet. 245, 667.

Derse, P. H. 1960. Evaluation of protein quality. J. Assoc. Offic. Agric. Chem. 43, 38.

Elliott, F. C. 1963. The Meadow Vole (Microtus pennsylvanicus) as bioassay test organism for individual forage plants. Mich. Agric. Exptl. Sta. Quart. Bull. 46, 58-72.

Evans, R. J. Bandemer, S. L. 1967. Nutritive value of legume seed proteins. J. Agric. Food Chem. 15, 439.

Everson, G. and Meckert, A. 1944. The biological value of some leguminous sources of proteins. J. Am. Diet. Assoc. 20, 81.

F. A. O. 1957. Protein requirements. In FAO nutritional studies. 16, 26. Report of the FAO Committee. Rome, Italy.

F. A. O. 1963a. The third world food survey. F. F. H. C. Basic study no. 11. Rome, Italy.

Feustel, I. C., Hendel, C. E. and Juilly, N. E. 1964. Potatoes, p. 303. In Food Dehydration, Vol. II, ed. by W. B. Van Arsdel and M. J. Copley. AVI Publishing Co., Inc. Westport, Conn.

Flodin, N. W. 1953. Amino acid and proteins, their place in human nutrition problems. J. Agric. Food Chem. 1, 222.

Forbes, R. M. 1955. Effect of energy intake and the biological value of protein fed to rats. J. Nutr. 55, 499.

Frazier, W. C. 1967. Foods and enzymes from microorganisms, p. 418. In Food Microbiology. McGraw Hill Second Ed.

Geiger, E. 1950. The role of the time factor in protein synthesis Science 111, 594.

Gomez, F., Ramos Galvan, R., Cravioto, J. and Silvestre, F. 1958. Prevention and treatment of cronic severe infantile malnutrition. Ann. N. Y. Aca. Sci. 69, 969.

Graham, G., Cordano, A., Baertl, J. and Morales, E. 1966. Programs for combating malnutrition in preschool child in Peru, pp. 163-167. In Preschool Children Malnutrition. N.A.S./N.R.C. Pub. 1282.

Graw, C. R. and Almquist, H. J. 1943. The utilization of the sulfur amino acids by the chick. J. Nutr. 26, 631.

Hamilton, W. J., Jr. 1941. Reproduction of the field mouse Microtus pennsylvanicus.(ord). Cornell Univ. Agric. Exptl. Sta. Memoir 237.

Hansen, J. D. L., Howe, E. E. and Brock, J. F. 1956. Amino acids and kwashiorkor. Lancet 2, 911.

Harper, A. E. 1959. Amino acid balance and imbalance. I. Dietary level of protein and amino acid imbalance. J. Nutr. 68, 405.

Hegsted, D. M. 1957. Theoretical estimates of the protein requirements of children. J. Am. Diet. Assoc. 33, 225.

Hewston, E. M., Dawson, E. H., Alexander, L. M. and Orent, K. E. 1948. Vitamin and mineral content of certain foods as affected by home preparation. U. S. Dept. Agric. Misc. Publication 682, 76.

Holt, E. R. Jr., and Snyderman, S. E. 1967. The amino acid requirements of children, pp. 381-390. In Amino Acid Metabolism and Genetic Variation, ed. by W. L. Nyhan. New York: McGraw Hill.

Honavar, P. M., Shin, C. V. and Liener, I. 1962. The inhibition of the growth of rats by purified hemmagglutinin fractions isolated from Phaseolus vulgaris. J. Nutr. 12, 188.

Hopper, T. H. 1958. Amino acid composition of foodstuffs, p. 890. In Processed Plant Protein Foodstuffs, ed. by A. M. Altschul. Academic Press, Inc., N. Y.

Howe, H. E., Jansen, G. R. and Gilfillan, E. W. 1965. Amino acid supplementation of cereal grains as related to the world food supply. Am. J. Clin. Nutr. 16, 315.

Howe, H. E., Gilfillan, E. W. and Milner, M. 1965. Amino acid supplementation of protein concentrates as related to the world protein supply. Am. J. Clin. Nutr. 16, 321.

Howes, C. D. and Spector, C. H. 1954. Nitrogen balance as related to caloric and protein intake in active young men. Am. J. Clin. Nutr. 2, 405.

Hughes, R. P. 1958. The amino acid composition of potato protein and of cooked potato. Brit. J. Nutr. 12, 188.

Hundley, J. M. 1958. Enrichment of foods with protein. Ann. N. Y. Acad. Sci. 69, 1042.

Hutchison, J. C., Bacon, J. S. D., Macrae, T. F. and Worden, A. N. 1943. The nutritive value of potato protein for the pig. J. Biochem. 37, 550.

Jackson, R. L. 1966. Effect of malnutrition on growth of the preschool child, pp. 9-21. In Preschool Children Malnutrition. N.A.S./N.R.C. Pub. 1282.

Jaffe, W. G. 1949. Limiting essential amino acids of some legume seeds. Proc. Soc. Exptl. Biol. Med. 71, 398.

Jaffe, W. G. 1950. Protein digestibility and trypsin inhibitor activity of legume seeds. Proc. Soc. Exptl. Biol. Med. 75, 219.

Jansen, G. R., Dimaio, L. R. and Hanse, L. N. 1962. Amino acid composition and lysine supplementation of Teff. J. Agric. Food Chem. 10, 62.

Jansen, G. R. and Howe, E. E. 1964. World problems in nutrition. Am. J. Clin. Nutr. 15, 262.

Johns, C. O. and Finks, A. J. 1920. Studies in nutrition. II. The role of cystine in nutrition, experiments with the proteins of navy bean (Phaseolus vulgaris) J. Biol. Chem. 41, 379.

Kakade, M. L. and Evans, R. J. 1963. Effect of heat on the in vitro digestion of navy beans (P. Vulgaris). Mich. Agric. Exptl. Sta. Michigan State University. 46 (1) 87.

Kakade, M. L. and Evans, R. J. 1964. Effect of methionine, vitamin B 12, and antibiotics supplementation on protein nutritive value of navy beans. Proc. Soc. Exptl. Biol. Med. 115, 890.

Kakade, M. L. and Evans, R. J. 1965. Nutritive value of navy beans (P. vulgaris). Brit. J. Nutr. 19, 269.

Kakade, M. L. and Evans, R. J. 1965. The nutritive value of different varieties of navy beans. Mich. Agric. Exptl. Sta. Michigan State University. 48 (1) 89.

Kakade, M. L. and Evans, R. J. 1966. Effect of soaking and germinating on the nutritive value of navy beans. J. Food Sci. 31 (5), 781.

Keane, K. W., Smutko, C. J., Kreiger, G. H. and Denton, A. E. 1962. The addition of water to purified diets and its effect upon growth and P.E.R. in the rat. J. Nutr. 77, 18.

Keys, A., Brozek, J., Henschel, A., Mickelsen, O. and Taylor, H. L. The Biology of Human Starvation. II. Univ. Minnesota Press, Minneapolis, Minnesota.

Klose, A. A., Stokstad, E. L. R. and Almquist, H. J. 1938. The essential nature of arginine in the diet of the chick. J. Biol. Chem. 123, 691.

Lafayette, B., Mendel, L. B. and Morris S. Fine. 1911-1912. Studies in nutrition. IV. The utilization of the proteins of legumes. J. Biol. Chem. 10, 433.

Laguna, J. and Carpenter, K. J. 1951. Raw versus processed corn in niacin deficient diets. J. Nutr. 45, 21.

Layne, E. 1955. Biuret method. In Spectrophotometric and Turbidimetric Methods for Measuring Proteins. Enzymology, III. ed. by Sidney P. Colowick and O. Kaplan. Academic Press, New York.

Le Clerg, E. L., Warren, L. H. and Andrew, C. G. 1962. Field plot technique. Second Ed. Burgess Publishing Co., Minneapolis, Minnesota.

Leverton, R. M., Gram, M. R. and Chaloupka, M. 1951. Effect of the time factor and calorie level on nitrogen utilization of young women. J. Nutr. 45, 21.

Liener, I. E. 1962. Toxic factors in edible legumes and their elimination. Am. J. Clin. Nutr. 11, 281.

McCollum, E. V., Simmonds, N. and Pitz, W. 1917. The dietary deficiency of the white bean (P. vulgaris). J. Biol. Chem. 29, 521.

Mendenhall, W. 1968. Orthogonal lineal contrasts for main effects in one way classification analysis, p. 237. In Introduction to Linear Models and the Design and Analysis of Experiments. Wadsworth Publishing Co., Inc., Belmont, California.

Mitchell, H. H. 1923-24. A method for determining the biological value of proteins. J. Biol. Chem. 58, 873.

Mitchell, H. H. 1924b. Biological value of proteins at different levels of intake. J. Biol. Chem. 58, 905.

Mitchell, H. H. and Block, R. J. 1946. Some relationship between the amino acid contents of proteins and their nutritive value for the rat. J. Biol. Chem. 163, 599.

Mitchell, H. H. 1947. Protein utilization by the adult rat. The lysine requirement. Arch. Biochem. 12, 293.

Monckeberg, F. 1966. Programs for combating malnutrition in the preschool child in Chile, pp. 168-177. In Preschool Children Malnutrition. N.A.S./N.R.C. Pub. 1282.

Morrison, A. B. and Campbell, J. A. 1960. Evaluation of protein in foods. V. Factor Influencing the P.E.R. of foods. J. Nutr. 70, 112.

Morrison, A. B. and Zabry, Z. I. 1963. Factors influencing the nutritive value of fish flour. II. Availability of lysine and sulfur amino acids. Can. J. Biochem. Physiol. 41, 469.

Mustakas, G. C., Griffin, E. L., Jr. and Sohns, V. E. 1966. Full fat soybean flours by continous extrusion cooking, pp. 101-108. In World Protein Resources, ed. by Robert F. Gould. Advances in Chemistry Series 57. American Chemical Society Publication.

National Academy of Sciences. National Research Council. 1963. Evaluation of protein quality. Pub. 1100. Washington, D. C.

Nelson, J. H. and Richardson, G. H. 1967. Molds in flavor production, pp. 94-105. In Microbial Technology, ed. by H. J. Peppler. Reinhold Publishing Corporation, New York.

Odendaal, W. A. 1966. Experiences in development of Pronutro in South Africa, pp. 224-230. In Preschool Child Malnutrition. N.A.S./N.R.C. Pub. 1282.

Osborne, T. B. and Clapp, S. H. 1907. The hydrolysis of phaseolin. Am. J. Physiol. 18, 295.

Page, E. and Hanning, F. M. 1963. Retention after storage and cooking vitamin B_6 and niacin in potatoes. J. Am. Diet. Assoc. 42, 45.

Patton, A. R., Hill, E. G. and Foreman, E. M. 1948b. The effect of browing on the essential amino acid content of soy globulin. Science, 108, 659.

Pearson, P. B. 1967. Scientific and technical aims, pp. 10-14. In Malnutrition Learning and Behavior. Massachussetts Institute of Technology. The M.I.T. Press.

Pecora, L. J. and Hundley, J. M. 1951. Nutritional improvement of white polished rice by the addition of lysine and threonine. J. Nutr. 44, 101.

Peppler, H. J. 1967. Yeast Technology, p. 149. In Microbial Technology, ed. by H. J. Peppler. Reinhold Publishing Corp., N. Y.

Pol, G., and Labib, A. I. 1963. Effects of fertilization and storage conditions on the nutritive value of potatoes of different varieties. In Potatoes: Production, Storing, Processing, p. 538. ed. by Smith, O. Westport, Conn., AVI Publishing Co., 1968.

Pope, L. R. 1969. Processing characteristics of selected potato clones. M. S. Thesis. Michigan State University, East Lansing.

Powrie, W. D. and Lamberts, E. 1964. Nutritive value of proteins in canned navy beans. Food Technol. 18, 111.

Prescott, S. C. and Dunn, C. G. 1959. Industrial Microbiology, New York, McGraw Hill.

Rao, K. S., Swaminathan, M. C., Swarup, S. and Patwardham, V. N. 1959. Protein malnutrition in South India. Bull. W.H.O. 20, 603.

Report of Nutrition Foundation Inc. 1967-68.

Rose, W. C. 1937. The nutritive significance of amino acids and certain related compounds. Science 86, 298.

Rose, W. C. and Rice, E. E. 1939. The significance of amino acids in canine nutrition. Science 90, 186.

Rose, W. C., Oesterling, L. M. and Womack, M. 1948. Comparative growth on diets containing ten and nineteen amino acids, with further observations upon the role of glutamic and aspartic acids. J. Biol. Chem. 176, 753.

Rose, W. C. and Wixon, R. L. 1955. The amino acid requirements of man. The sparing effect of cystine on the methionine requirement. J. Biol. Chem. 216, 763.

Rose, W. C. 1957. The amino acid requirement of adult man. Nutr. Abstr. and Rev. 27, 631.

Rosenberg, H. R. and Rodenburg, E. L. 1952. The fortification of bread with lysine. II. The nutritional value of fortified bread. Arch. Biochem. and Biophysics. 37, 461.

Rosenberg, H. R. 1959. Supplementation of foods with amino acids. J. Agric. and Food Chem. 7, 316.

Rosenthal, H. L. and Allison, J. B. 1951. Some effects of calorie intake on nitrogen balance in dogs. J. Nutr. 44, 423.

Salmon, W. D. 1954. The tryptophan requirement of the rat as affected by niacin and level of dietary nitrogen. Arch. Biochem. Biophys. 51, 30.

Sauberlich, H. E., Wan Yuim Chang and Salmon, W. D. 1953. The comparative nutritive value of corn of high and low protein content for growth in the rat and chick. J. Nutr. 51, 623.

Sauberlich, H. E. 1956. Amino acid imbalance as related to methionine, isoleucine, threonine and tryptophan requirement of the rat or mouse. J. Nutr. 59, 353.

Schaefer, A. E. 1963. Nutritional deficiences in developing countries. J. Am. Diet. Assoc. 42, 295.

Schillinger, J. A., Jr., and Elliott, F. C. 1966. Bioassays for nutritive value of individual alfalfa plants. Mich. Agric. Exptl. Sta. Quart. Bull. 48, 580. Michigan State University.

Senecal, J. 1958. The treatment and prevention of Kwashiorkor in French West Africa. Ann. N. Y. Acad. Sci. 69, 916.

Shaw, R. L. 1964. INCAPARINA. The third year, pp. 34-46. In W.H.O./F.A.O./U.N.I.C.E.F. PAG. News Bull. No. 4.

Shenk, S. J. and Elliott, F. C. 1969. Technical notes. A diet feeder for weanling meadow voles (Microtus pennsylvanicus) Am. Assoc. Lab. Animal Science. 19 (4), 522.

Smith, O. and Davis, C. 1968. Potato processing, p. 558. In Potatoes: Production, Storing, Processing, Westport, Conn., AVI Publishing Co.

Smith, O. 1968. Potatoes: Production, Storing, Processing. Westport, Conn., AVI Publishing Co.

Scrimshaw, N. S., Behar, M., Wilson, D., Viteri, F., Arroyave, G. and Bressani, R. 1961. All vegetable mixtures for human feeding. V. Clinical trials with INCAP mixtures 8 and 9 and with corn and beans. Am. J. Clin. Nutr. 9, 196.

Scrimshaw, N. S. and Behar, M. 1961. Protein malnutrition in young children. Science 133, 2039.

Scrimshaw, N. S. 1966. The effect of the interaction of nutrition and infection of the preschool child, pp. 63-71. In Preschool Child Malnutrition. N.A.S./N.R.C. Pub. 1282.

Swanson, P. 1959. Food energy and the metabolism of nitrogen, p. 195. In Protein and Amino Acid Nutrition, ed. by A. A. Albanese. Academic Press, New York.

Swendseid, M. E. and Dunn, M. S. 1958. Amino acid requirements of young women based on nitrogen balance data. J. Nutr. 58, 507.

Talburt, W. F. 1967. History of potato processing. In Potato Processing, 2nd ed. Ed. by W. F. Talburt and O. Smith. AVI Publishing Co., Inc., Westport, Conn.

Tandon, B., Bressani, R., Scrimshaw, N. S. and Le beau, F. 1957. Nutritive value of beans. Nutrients in Central American beans. J. Agric. Food Chem. 5, 137.

Thompson, N. R. 1967. Potato varieties. In Potato Processing. 2nd ed. Ed. by W. F. Talburt and O. Smith. AVI Publishing Co., Inc. Westport, Conn.

Tizard, J. 1968. A fresh look at retarded children. World Health, October-November, pp. 12-21.

United Nations, 1968. International action to avert the impending protein crisis.

United States Department of Agriculture. 1957. Potatoes: Facts for consumer education. Bulletin No. 178. Washington, D.C.

United States Department of Agriculture. 1963. Composition of foods; raw, processed, prepared. Handbook No. 8. Washington, D. C.

Whitmoyer, T. F. 1956. A laboratory study of growth rate in young Microtus pennsylvanicus. Thesis for degree of M.S., Michigan State University, East Lansing.

Williams, C. D. 1966. Malnutrition and mortality in the preschool child, pp. 3-8. In Preschool Children Malnutrition. N.A.S./N.R.C. Pub. 1282.

APPENDIX

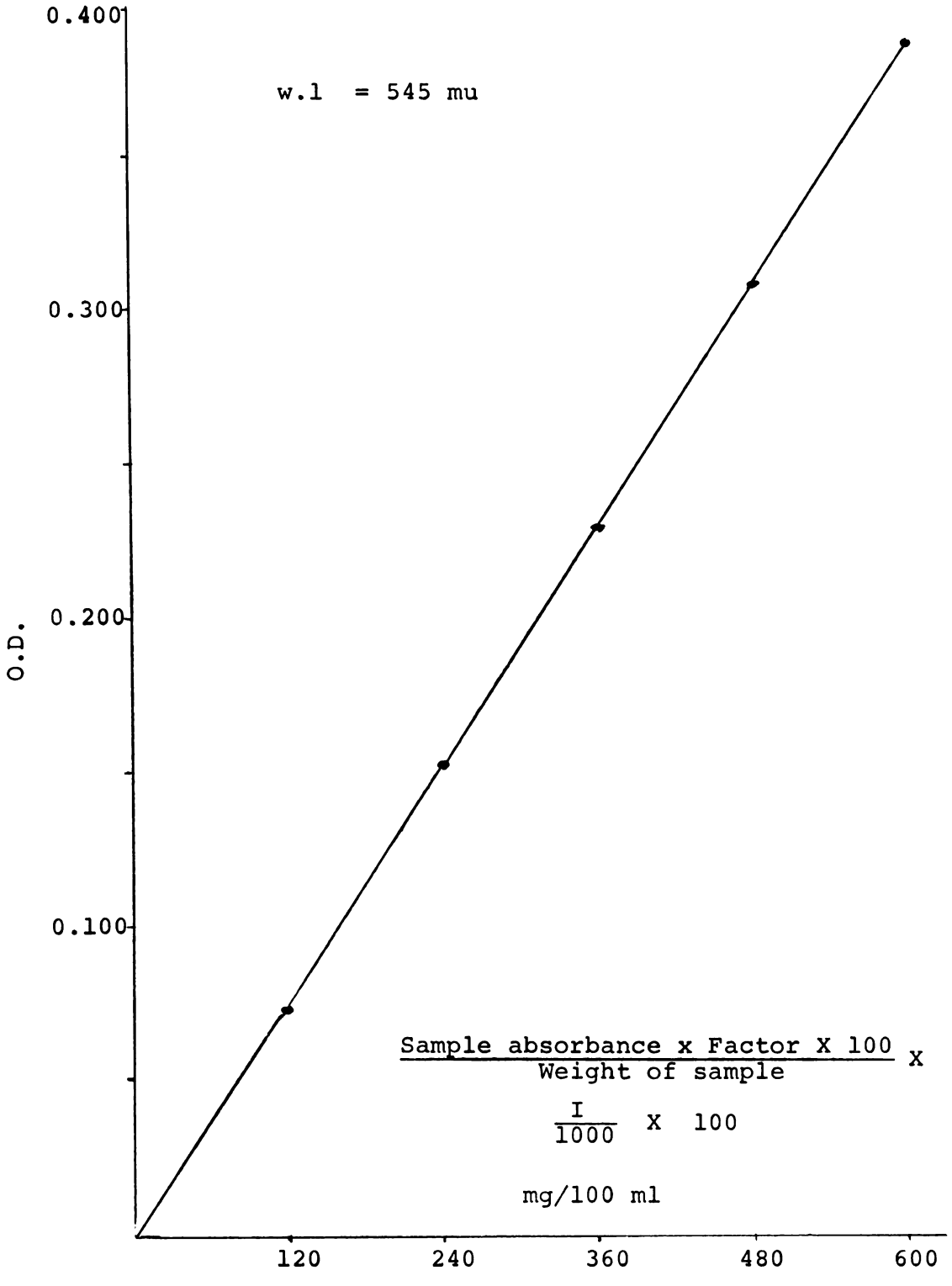


Fig. 3. Protein determination: Biuret Method.

Table VII.--Diet composition.

Protein source	5.28% protein g	Fiber[a] g	Corn oil[b] g	Minerals[c] g	Vitamins[d] g	CHO mixture[e] g
Control casein	5.30	5.00	1.00	3.00	2.00	83.70
Atm. cooked beans	22.39	5.00	1.00	3.00	2.00	66.61
Ret. cooked beans	22.78	5.00	1.00	3.00	2.00	66.22
R. Burbank	76.52	5.00	1.00	3.00	2.00	12.48
58	68.75	5.00	1.00	3.00	2.00	20.25
321-65	89.00	5.00	1.00	3.00	2.00	0.00
322-6	78.57	5.00	1.00	3.00	2.00	10.43
711-3	60.41	5.00	1.00	3.00	2.00	28.59
709	69.56	5.00	1.00	3.00	2.00	19.44

[a]Alphacel Non-Nutritive Bulk from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[b]Commercial oil "Mazola."

[c]Salt mixture W. detailed next page.

[d]Diet fortification mixture Nutritional Biochemicals Corporation, Cleveland, Ohio. Details next page.

[e]1 part sugar, 1 part dextrine, 2 parts corn starch.

VITAMIN DIET FORTIFICATION MIXTURE IN DEXTROSE

(Grams/100 lbs. diet)

	gm.
Vitamin A concentrate (200.000 units per gram)	4.5
Vitamin D concentrate (400.000 units per gram)	0.25
Alpha Tocopherol	5.0
Arcorbic Acid	45.0
Inositol	5.0
Choline chloride	75.0
Menadione (K)	2.25
P-aminobenzoic acid	5.0
Niacin	4.5
Riboflavin	1.0
Pyridoxine Hydrochloride	1.0
Thiamine Hydrochloride	1.0
Calcium pantothenate	3.0

	mgms/100 lbs. diet
Biotin	20
Folic acid	90
Vitamin B12	1.35

COMPOSITION OF SALT "W"

Calcium carbonate	21.000%
Copper sulfate (5H20)	0.039%
Ferric phosphate	1.470%
Manganous sulfate (anhyd)	0.020%
Magnesum sulfate (anhyd)	9.000%
Potassium aluminum sulfate	0.009%
Potassium chloride	12.000%
Potassium dihydrogen phosphate	31.000%
Potassium Iodide	0.005%
Sodium chloride	10.500%
Sodium Fluoride	0.057%
Tricalcium phosphate	14.900%

Table VIII.--Protein content of 10 strains of potatoes measured by the Biuret method.

Variety	protein g/100 g sample										Average	Standard Deviation	Standard error
Russet Burbank	6.56	6.40	6.76	6.76	6.96	6.76	6.76	6.76	6.96	6.96	6.74	0.16	0.05
709	8.44	8.90	8.04	8.20	7.60	7.60	8.04	7.80	8.70	8.70	8.08	0.44	0.16
706-34	8.44	8.44	8.44	8.70	8.04	8.44	8.44	8.20	8.70	8.44	8.42	0.18	0.06
1111-2	9.76	9.56	9.36	9.36	9.36	9.36	9.12	9.36	9.12	9.12	9.34	0.20	0.06
711-8	8.20	8.20	8.20	8.20	8.70	8.44	8.20	8.20	8.44	8.44	8.32	0.16	0.05
58	8.04	7.80	8.04	8.04	8.04	7.60	8.04	8.20	7.60	7.60	7.90	0.22	0.06
322-6	6.76	6.76	6.76	6.76	6.76	6.96	6.96	6.96	6.96	6.76	6.84	0.32	0.10
711-3	7.60	7.60	8.44	8.44	7.70	7.80	8.44	8.44	8.70	8.90	8.21	0.48	0.15
321-65	5.30	5.30	5.20	5.30	6.10	6.10	5.32	5.56	5.56	5.30	5.50	0.10	0.03
706-32	8.70	8.70	8.44	8.44	8.70	8.88	8.44	8.88	8.70	8.70	8.66	0.14	0.04

Table IX.--Amino acid composition of processed navy beans and potatoes

	NAVY BEANS		POTATO STRAINS						
	Atmosphere cooked	Retort cooked	Russet Burbank	58	321-65	322-6	711-3	709	Whole Egg
	g/16 g nitrogen								
Arginine	7.29	7.24	6.34	5.57	4.24	5.89	4.23	5.24	6.4
Histidine	3.00	3.29	2.26	2.06	2.02	2.82	2.41	2.06	2.1
Lysine	7.82	7.62	7.13	6.26	7.48	7.64	5.49	6.12	7.2
Tyrosine	3.71	3.62	4.32	3.96	4.45	4.76	4.34	3.72	4.5
Phenilalanine	6.24	6.32	4.62	3.91	5.50	5.68	4.94	4.07	6.3
Methionine	0.56	0.57	1.13	1.25	0.78	1.01	1.27	1.24	4.1
Threonine	4.92	4.91	3.34	3.76	4.94	5.48	4.72	4.19	4.9
Leucine	8.88	9.06	6.46	5.86	8.70	8.33	7.16	6.28	9.2
Isoleucine	5.22	5.32	3.98	4.04	5.90	4.94	4.48	4.18	8.0
Valine	5.84	6.49	6.46	6.22	6.72	6.01	5.34	5.38	7.3
Aspartic acid	12.24	12.42	24.14	20.94	16.90	17.90	19.85	26.35	---
Serine	6.28	6.21	3.41	3.52	4.56	4.48	4.30	4.03	---
Glutamic acid	14.52	14.75	16.86	23.04	13.38	12.52	20.78	16.08	---
Proline	4.16	3.88	3.24	2.10	3.62	4.40	4.72	3.50	---
Glycine	3.71	2.52	2.86	3.81	3.72	3.72	3.22	3.14	---
Alanine	4.13	3.98	2.61	3.59	4.35	4.02	3.11	3.42	---

Table X.--Nutritional values of the non-supplemented proteins.

Replications/Treatments	I	II	III	IV	V	Total	Mean
				grams			
Control casein (4.77%)	0.5	1.5	2.0	0.5	1.0	5.50	1.10
Atm. cooked bean	1.5	1.0	1.0	1.0	1.0	5.50	1.10
Retort cooked bean	1.0	0.0	0.5	1.0	1.0	3.50	0.70
Potato Russet Burbank	1.0	1.0	0.0	0.0	0.5	2.50	0.50
Potato 58	1.0	0.5	2.0	1.5	1.5	6.50	1.30
Potato 321-65	1.0	0.7[+]	1.0	1.0	1.0	4.70	0.94
Potato 322-6	1.0	1.0	1.5	1.5	1.5	6.50	1.17
Potato 711-3	0.5	0.5	1.0	1.0	1.0	4.0	0.80
Potato 709	0.5	0.0	0.5	1.5	0.5	3.0	0.60

[+]Missing value.

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Observed F	Required F 0.05	0.01
Treatments	8	3.48	0.44	2.24	2.21	3.04
Error	36	6.87	0.19			
Total	44	10.35				

Table XI.--Nutritional Values of the supplemented proteins.

Replications/Treatment	I	II	III	IV	V	Total	Mean
				grams			
Control casein (5.28%)	2.0	1.0	1.0	1.5	2.5	8.0	1.6
Atm. cooked bean	3.0	2.5	2.5	2.0	2.5	12.5	2.5
Potato 58	1.5	2.0	2.0	2.5	1.0	9.0	1.8
Potato 321-65	2.0	1.0	2.5	1.0	3.0	9.5	1.9
Potato 322-6	2.5	2.5	3.0	2.5	2.5	13.0	2.6
Potato 711-3	2.5	1.5	2.0	2.5	2.5	11.0	2.2

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Observed F	Required F 0.05	0.01
Treatment	5	4.0	.80	2.50	2.62	3.90
Error	24	7.70	.32			
Total	29	11.70				

Table XII.--Comparison of the means: non-supplemented and supplemented diets.

	EXPERIMENT I						EXPERIMENT II					
	Control	Aa	Da	Ea	Fa	Ga	Cont b	Ab	Db	Eb	Fb	Gb
							grams					
	0.5	1.5	1.0	1.0	1.0	0.5	2.0	3.0	1.5	2.0	2.5	2.5
	1.5	1.0	0.5	0.70	1.0	0.5	1.0	2.5	2.0	1.0	2.5	1.5
	2.0	1.0	2.0	1.0	1.5	1.0	1.0	2.5	2.0	2.5	3.0	2.0
	0.5	1.0	1.5	1.0	1.5	1.0	1.5	2.0	2.5	1.0	2.5	2.5
	1.0	1.0	1.5	1.0	1.5	1.0	2.5	2.5	1.0	3.0	2.5	2.5
Total	5.50	5.5	6.5	4.7	6.5	4.0	8.0	12.5	9.0	9.5	13.0	11.0
Mean	1.1	1.1	1.3	0.94	1.3	0.8	1.6	2.5	1.8	1.9	2.6	2.2

ANALYSIS OF VARIANCE

Source of variation	Degrees of Freedom	Sum of Square	Mean Square	Observed F	Required F 0.05	0.01
Treatment	11	20.28	1.84	7.67	1.99	2.64
Error	48	11.57	0.24			
Total	59	31.85				

Unsupplemented

Aa Atmosphere cooked bean
Da Potato 58
Ea Potato 321-65
Fa Potato 322-6
Ga Potato 711-3

Supplemented

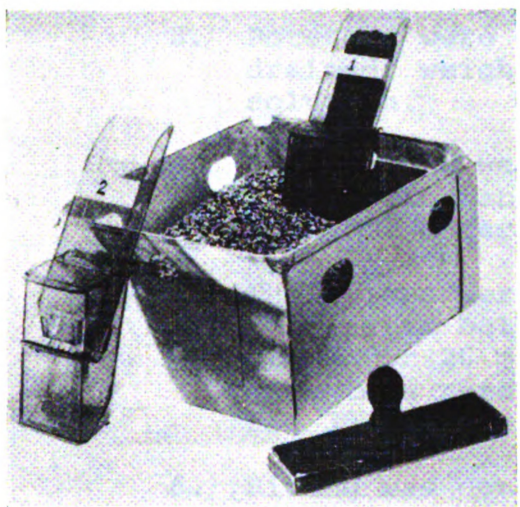
Ab Atmosphere cooked bean
Db Potato 58
Eb Potato 321-65
Fb Potato 322-6
Gb Potato 711-3

Table XIII.--Summary of the comparison between the means of non-supplemented and supplemented diets.

	d.f.	SS	MS	F	F 0.05 (1.48)
All treatments	11	20.28	1.84	7.67	1.99 ++
Aa and Ab treatment	1	4.90	4.90	20.42	4.04 ++
Da and Db treatment	1	0.62	0.62	2.58	4.04
Ea and Eb treatment	1	2.30	2.30	9.58	4.04 ++
Fa and Fb treatment	1	4.22	4.22	17.58	4.04 ++
Ga and Gb treatment	1	4.90	4.90	20.42	4.04 ++
Other contrasts	6	3.34			
Error	48	11.57	0.24		
Total	59				

++ Significance Level.

Unsupplemented		Supplemented	
Aa	Atmosphere cooked beans	Ab	Atmosphere cooked beans
Da	Potato 58	Db	Potato 58
Ea	Potato 321-65	Eb	Potato 321-65
Fa	Potato 322-6	Fb	Potato 322-6
Ga	Potato 711-3	Gb	Potato 711-3



Vole feeder #1 is shown in the plastic nest. The design features are seen in feeder #2. The diet cutter used to make the wafer is in the foreground.

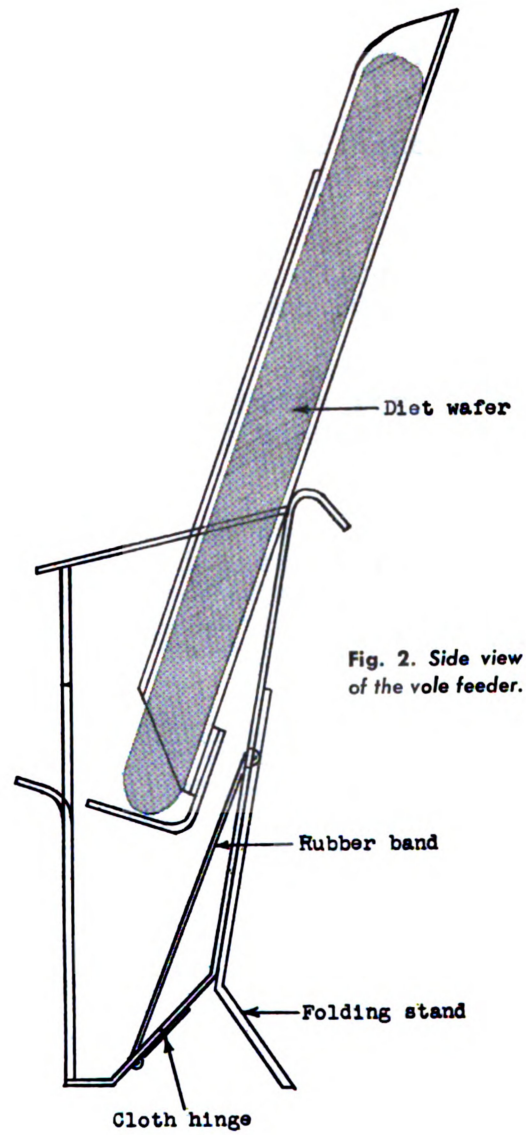


Fig. 2. *Side view of the vole feeder.*

Fig. 4.--A diet feeder for weanling meadow voles (Microtus pennsylvanicus.) John S. Shenk and Fred C. Elliot (1969)

PROCESSING PROCEDURES FOR POTATO FLAKES AND NAVY BEAN POWDERS.

POTATO FLAKE:

1. A 14 to 84 pound sample was taken randomly from storage for processing.

2. The condition of the tubers was noted, they were washed, preheated at 170°F for two minutes in water.

3. Potatoes were sliced into 3/8 inch slices, drained, weighed and returned to the SO_2 solution.

4. Slices were washed with running water for 1-2 minutes and precooked for 20 minutes in 165°F water.

5. The slices were cooled to 70°F - 80°F in a cold water bath, taken out and allowed to stand for 20 minutes before transferring them to a retort for steaming at 212°F, for 30 minutes.

6. Ricing was done using a Kitchen Aid model K-5a mixer with the coarse rotatory grater attachment. Additives or diluting water were added at this point. Samples were removed for solids analysis before additives were incorporated.

7. The mash was dried on an Overton Machine Company model P-36 double drum dryer, 12 inch diameter by 19 1/8 inch length. One drum was used as an applicator roll, the other as a drying roll. Simulation of one to five applicator rolls is possible by a doctor blade control. Conditions normally used were 8 rpm, 4 layers, uncooled applicator roll and 85-90 psi steam pressure.

8. The sheets were placed in a polyethylene bag. At the end of processing the sheets were reduced to flakes using a rotary slicer with 3/8 inch spacing between blades.

BEANS. METHODS OF COOKING.

Two types of cooking process were used:

1. Atmosphere cooked: Beans were soaked and cooked in water at 212°F for 90 minutes.

2. Retort cooked: Beans were soaked in water at 210°F for 45 minutes. Transferred to a wire basket and retorted in steam at 220°F for 30 minutes.

They were dried in a drum drier (described above) under controlled conditions of 85 psi, 23 1/3 rpm and 4 layers. After drying the sheets were powdered with a Fitzpatrick comminuting mill using an 0.125 inch screen. At this stage they were placed into No. 2 cans (307 x 409) and sealed hermetically for storing.