NUTRITIONAL EVALUATION OF BERMUDA GRASS

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CHAPTER I

INTRODUCTION

The Research Problem

Bermuda grass is native to the Bengal region of India. It is known as sacred grass and is believed to have been the gift of benevolent deity for the support of the cows which are also held as sacred in that country. It was introduced to the United States around 1807 and grows on almost all types of soil, but succeeds best on rich, heavy clay or clay loam (Hansen, 1918). At present, Bermuda grass grows well in most tropical countries and is used intensively for animal feed (Belesky, Perry, Windham, Mathias, and Fedders, 1991).

In many parts of the world, especially in the developing countries, humans and animals equally need protein for growth. Imbalances from deficiencies of animal protein and energy foods are prevalent. In the 1980's, about 87 million cases of protein-energy malnutrition were reported in the third world countries (Young and Peelet, 1984), and the situation is still largely unchanged (Benfice and Simondon, 1993; Uzogara, Morton, Daniel, and Emery, 1991). The awareness of protein shortage stimulated scientists to look for new, inexpensive protein sources to replace expensive animal protein with good quality plant protein.

Most plants especially of the grass family are used as animal feed. Almost all the amino acids consumed by man are initially in the leaves of green plants. Some efficiency is lost when plants are concentrated into protein in seeds or tubers, but there is a considerable waste when plant protein is converted into animal protein for human consumption (Gerloff, 1963). The increasing population and general shortage of animal protein and other foods in some countries have created a need to seek new methods of producing and processing foods (Uzogara, et al 1991).

Carbohydrate based baked products are consumed worldwide. Such staple food products are economical, easy to prepare, shelf stable and can be served in many different ways. Therefore, fortification of flour with high protein plants and protein concentrates provides a good opportunity to improve the nutritional quality of protein consumed by many people.

The staple carbohydrates most commonly used are cereals grains (corn, rice, wheat, sorghum, millet), cassava and plantain. These sources usually do not supply adequate total protein and are deficient in one or more of the essential amino acids which are necessary in specific amounts and proportions to provide synthesis of protein (Lockhart and Nesheim, 1978). Cereal grains, however, play an important role in attempting to meet the nutritional requirements of people in the developing countries by providing 80-90% of the calories, two-thirds of the protein, and furnishing several of the vitamins and minerals as well.

Large segments of the world's population live under conditions where the availability and intake of protein is inadequate. The high cost and shortage of animal protein have created dependence on plant sources. In order to alleviate the protein-energy malnutrition, more attention is being focused on plant sources for high quality protein. Plant foods are being investigated which are high in protein content. Bermuda grass is moderate in protein (12%), has many of the essential amino acids, and average lysine and methionine content of the three cultivars are 3.82 and 1.69 compared to wheat which has 2.8 and 1.83, and sorghum 2.72 and 1.73 (Deshpande, Singh, and Singh, 1991; Yensen and Weber, 1987). Bermuda grass is available worldwide. Due to these merits, it could be incorporated with other cereal grains to improve the protein quality of certain ethnic and staple foods in the developing countries.

Purpose and Objectives

The purpose of these studies was to evaluate the protein efficiency ratio (PER) and nutritional adequacy of Bermuda grass, with or without lysine supplement, in growing rats; and to develop acceptable Bermuda grass mixture breads and evaluate their sensory qualities and acceptability. The specific objectives of these studies were:

1. To compare two experimental and three standard Bermuda grass with wheat flour in amino acids, crude protein, and ash content

2. To compare in Experiment I, the PER of rats fed a control diet with those fed a diet that was 10% Bermuda grass.

3. To evaluate, in Experiment II, the nutritional adequacy of three Bermuda grass cultivars at 10%, 25%, and 40% levels of substitution with and without lysine supplementation, and, to compare the fiber sources of Bermuda grass and cellulose for

growing rats by:

A. Comparing the serum albumin and creatinine levels of rats fed different levels of Bermuda grass.

B Comparing organ weights (heart, liver, kidney, spleen, lung, testicle) of rats fed different levels of Bermuda grass.

C. Comparing the dry matter digestibility (fecal protein) of the rat diets at different levels of Bermuda grass.

D. Comparing the total fecal output and the dry matter of rat diets at different levels of Bermuda grass.

4. To evaluate, in Experiment III, the sensory characteristics of breads made with 5% and 10% Bermuda grass substitutions.

Hypotheses

The following hypotheses were postulated in these studies:

1. There will be no significant differences in protein efficiency ratio of control diet and those diets that contain 10% Bermuda grass substituted for casein.

2. There will be no significant differences in weights of rats fed the 10%, 25%, and 40% substitution Bermuda grass for casein with or without lysine supplementation and the control diet.

3. There will be no significant differences in serum albumin and creatinine in rats fed the control diet and the Bermuda grass substituted diet at levels, 10%, 25%, and 40%.

4. There will be no significant differences in organ weights of rats fed a control

diet or a Bermuda grass, substituted for casein at 10%, 25%, and 40% diet with or without lysine supplementation.

5. There will be no significant differences in total fecal output and dry matter digestibility of rat fed diets with 10%, 25%, or 40% Bermuda grass substitutions for casein.

6. There will be no significant differences in color, flavor, off-flavor, texture, appearance, and overall acceptability between breads made with 5% and 10% Bermuda grass flour substituted for all purpose flour and bread made with all purpose flour.

Assumptions

Seven assumptions were made in this study:

1. All nutrient needs for the rats were met by the diets provided.

2. Nutrients in the powdered form of Bermuda grass when fed the rat would not have been altered by food processing procedures.

3. Fecal excretion (values obtained during the collection period the last seven days) per unit of feed consumed were representative of values for the total feeding period.

4. Chemical analyses of fecal excretion, and blood samples were accurate and precise.

5. The trained panelists understood the research protocol and the research design and evaluated the Bermuda grass flour mixture bread critically and objectively as instructed.

6. The controlled conditions and sensory procedures followed were such as to

ensure that any difference noted in the breads were attributable to manipulated variables.

7. The sensory evaluation panel represented the general population, and the panel selection procedures were valid.

Limitations

Seven rats per treatment were used for the second study as opposed to the first study which had 10 rats per treatment. Only two levels of Bermuda grass substitutions, 5% and 10%, were tested for bread.

Definition of Terms

The following definitions were used in this study:

Limiting amino acid: The amino acid present in the shortest supply relative to the amount needed for protein synthesis in the body.

Protein Efficiency Ratio (PER): The ratio between the weight gain of the growing animal (new tissues formed) and the amount of protein consumed (Chen & Peppler, 1978).

Apparent digestibility: Apparent digestibility measures both the digestibility of amino acids in the feed and those contributed from endogenous sources from the animal. Apparent digestibility is affected by the level of crude protein in the test diet (Cole and Van Lunen, 1994, p. 116).

Color: A combination of hue, purity and lightness which correlates well with the human perception of color (Noble, 1975).

Dietary Fiber: The portion of plant cells that can not be digested by human alimentary enzymes, therefore cannot be absorbed from human small intestine (Slavin, 1987).

Dried distiller's grain: major by-product resulting from the fermentation of cereals in the production of alcohol for beverages or fuel (Cromwell, Herkelman, and Stahly, 1993).

Flavor: A complex sensation with taste, aroma and mouth feel as three categories or components (Amerine, Pangborn, and Roessler, 1965).

Sensory Evaluation: A scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the sense of sight, taste, touch, and hearing (Prell, 1976).

Texture: In foods, the characteristic consistency and overall structure, which includes viscosity, cohesiveness and hardness (Paul and Palmer, 1972).

Format of Dissertation

This dissertation follows the format of the Graduate College Thesis/Dissertation Handbook for Oklahoma State University except for Chapters IV and V. Chapter IV is written following the guideline for authors of the Journal of Food Quality (Appendix A). Chapter V is written following the guideline for authors of the Journal of Ecology of Food and Nutrition (Appendix B). Chapter IV will include Experiments I and III, while Experiment II will be discussed in Chapter V.

CHAPTER II

REVIEW OF LITERATURE

This chapter covers a brief review of protein-energy malnutrition, protein efficiency ratio, quality of plant/Bermuda grass protein and pigmentation, effect of plant fiber on growth and digestibility, and food application of plant proteins. Selected literature on sensory evaluation of breads and other products is reviewed.

Protein-Calorie Malnutrition

The growing population of the third world countries and their domestic animals need protein. The cheapest proteins are those derived from plant materials which, although in abundance, are underutilized in many countries, (Oliveira, Vasconcelos, Gondim, Cavada, Moreira, Santos, and Moreira, 1994). Hunger and malnutrition in different segments of the world's people are serious problems, even though world food supplies are adequate to feed all people. A deficient intake of protein and calories results in protein-calorie malnutrition such as stunted growth, wasting and edema. The Food and Agriculture Organization (FAO) of the United Nation (1987) estimated that Protein-Energy Malnutrition (PEM) affects 500 million people in the world, two-thirds of whom are in the developing countries.

Mild to moderately undernourished children from different parts of the developing countries are less active than adequately nourished children of the same age. In addition, survivors of severe protein-calorie malnutrition have poorer levels of mental development than their better nourished peers or siblings several years later. In studies by Grantham-McGregor, Schofield, and Haggard (1988) in Jamaica and Thailand, severely malnourished children were less responsive to stimuli than adequately nourished children. Overall, long term, severe protein-calorie malnutrition is likely to result in serious defects in cognition functioning (Grantham-McGregor, 1993).

Protein-energy malnutrition is believed to be the primary nutritional problem in most developing countries of the world because cereals and grains are the major sources of calories and protein for a large proportion of the population. In terms of quality, cereal grains occupy the first place as sources of calories and proteins, and legumes in second place (Singh and Singh, 1992). To combat the protein-energy malnutrition, scientists are continuously looking at innovative ways to provide a suitable combination of raw materials at relatively low cost. Sunflower, soybean flour, peanut flour, amaranth, and other sources of vegetable proteins have been brought to the attention of food chemists, food technologists, nutritionists, food engineers, and health professionals to meet the human nutritional requirement of protein, especially in the developing countries where protein deficiency is highly magnified.

Protein Efficiency Ratio

All new non-conventional crops first need a protein evaluation to help determine their potential use as a new plant for animal or human consumption (Yensen and Weber, 1987). Takyi et al (1992) evaluated the protein quality of alfalfa and some common leafy vegetables used in Ghana and compared the growth rate of rats on diets containing alfalfa and the weaning diets prepared from groundnuts. They weighed heart, liver, kidneys, brain, lungs, spleen, adrenal gland, small intestine, large intestine, stomach uterine complex, ovaries, testes, and thigh muscles. Results indicated that rats fed alfalfa mix had significantly higher protein efficiency ratio (PER) of 1.41 than the groundnut mix which had 0.96. The researchers also found significantly higher organ to body weight ratio for heart, kidneys, lungs, stomach, small intestine and fallopian tubes of rats fed the groundnut mix.

Godinez et al (1992) fed growing rats and evaluated the protein quality of common black beans with four preparations: Cotyledon and seed coats cooked together and evaluated with cooking liquor, cotyledon and seed coat cooked together and evaluated without cooling liquor nor seed coats, cotyledon and seed coat cooked together evaluated without cooking liquor, and cooked cotyledon evaluated without cooking liquor. The protein efficiency ratio of the four preparations were 1.14, 0.24, 0.76, and 1.50. Beans without liquor and/or without coat had higher values.

Balasumbramayas and Sampath (1984) evaluated the protein efficiency ratio of *Algae spirulina*, a single cell protein, (17% protein) using growing rats. They fed albino rats *Algae spirulina,* casein, or concentrated milk, containing 10% crude protein diets for

21 days. They measured the PER and found the values for *algae spirulina* as 2.28, casein 2.45, and concentrated milk, 2.46. They also measured hemoglobin values. The researchers concluded that the PER and blood hemoglobin values of rats fed *algae spirulina* were comparable to that of casein or concentrated milk.

Sangiah (1990) evaluated the protein efficiency ratio of two varieties of amaranth (*A. cruentus and A. hypochondricus*) with growing rats. The she used wheat-soymeal diet as a control. The experimental diets were formulated with 50% of each variety mixed with wheat and supplemented with nonfat dry milk. After feeding rats for 14 days, the results showed that rats fed *A. hypochondriacus* mixture had significantly increased body weight compared to the rats fed the control diet, and the protein efficiency ratios of both the amaranth varieties were higher than the control.

Garcia, Alfaro, and Bressani (1987) studied digestibility and protein quality of three amaranth species. Weanling rats were fed diets containing 10% protein either non-defatted, or defatted amaranth flour, cooked, raw, or mixed with cold water and drum dried. After four weeks of animal feeding, the apparent protein digestibility, hemoglobin, hematocrit, and serum protein were measured . The researchers also compared weights of heart, liver, kidneys, lungs, spleen, pancreas and testicles, and examined whether or not feeding the grains induced pathological damage to rats' organs. Feeding the cooked, whole amaranth and the defatted amaranth diets resulted in higher feed intake, weight gain, and protein efficiency ratio of 2.4, and 2.2 respectively, than feeding the non-heat treated diets for the three species. Results indicated that cooking the amaranth flour improved protein digestibility. Slightly damaged lungs and kidneys were observed which was attributed to oxalate and antinutritional factors in the raw, defatted or non-defatted amaranth species.

The nutritive value of the protein of Jack fruit seed meal, indigenous crop of India, with methionine and tryptophan supplementation was studied by Begum, Umapathy, Daniel, and Swaminathan (1989). In Experiment I, they fed weanling female albino rats with Jack fruit seed meal supplemented with methionine and/or tryptophan and used skim milk powder as a control diet. The PER values were 0.43, 0.73, 0.73, and 2.8 respectively. In Experiment II the rats were fed Jack fruit supplemented with skim milk powder or skimmilk protein. The protein efficiency ratio of rats fed Jack fruit with skimmilk protein was 2.88 which was higher than the Jack fruit seed meal alone or supplemented with either methionine or tryptophan. The researchers suggested that the Jack fruit seed meal be supplemented with the deficient amino acids methionine and tryptophan by combining the meal with milk protein, if it is to be used for human consumption.

Delisle, Amiot, Goulet, Brisson, and Jones (1985) evaluated the protein efficiency ratio and apparent digestibility of soybean flour, rapeseed protein concentrate, whole wheat flour , soybean extract and autoclaved soybean using growing rats, and compared them with casein. The PER values were for soybean flour 1.97, autoclaved soybean 2.49, soy extract 1.36, rapeseed 3.29, whole wheat flour 1.7, and casein 3.47. The result of the study showed that rapeseed protein concentrate was comparable to casein and gave the highest weight gain and PER values compared to other protein sources tested. In addition, the apparent digestibility of soybean flour was significantly lower than the other proteins , and the digestibility of all vegetable proteins was lower than that of casein. The researchers concluded that the nutritive value of soybean flour or soybean extract could be improved by autoclaving and by blending with wheat albumin-globulin plus glutinin.

Yensen, and Weber (1987) evaluated *Distichlis palmeri*, a salt grass that produces a wheat-like grain using mice, in two experiments. In the first experiment, they used *Distichlis palmeri* as a sole source of protein and carbohydrates and compared it with whole egg and lactalbumin, as control diets. The PER values were 1.29, 2.5, and 2.02 respectively. In the second experiment, the mice were fed *Distichlis palmeri* supplemented with the limiting amino acids, methionine, lysine and threonine. The PER value of *Distichlis palmeri* improved from 1.29 to 1.9 when supplemented with the limiting amino acids, and significant improvement in body weight was shown. In addition, the protein digestion was found to be similar to other cereals.

Owaegbute, and Nnanyelugo (1987) evaluated the nutritive value of three diets based on cowpea-cereal or cowpea yam tuber mixture using rats. Two other weaning foods, cerelac and corn pap were also evaluated and casein was used as a control diet. Protein efficiency ratio of corn pap was 0.77 which was lower than any other groups, whereas the other mixtures ranged from 2.23 to 2.49. They also weighed liver, kidneys, brain, and muscle The kidneys, brain, and muscle of corn pap fed rats were significantly lighter than those fed the other diets and the nitrogen excretion was also higher than the other groups. The researchers concluded that corn pap as a weaning food demonstrated inferior quality in all characteristics examined.

Nutritional and Pigmentation Quality of Bermuda Grass

The presence of a substantial quantity of β-carotene and xanthophyll in turf and coastal Bermuda grasses have been reported by many investigators (Fishman, and Evans, 1978; Wheeler and Turk, 1963; Barnett, and Morgan, 1959). Coastal Bermuda grass has been evaluated as a potential xanthophyll source for commercial poultry feed. Fletcher, Papa, Halloran, and Burdick (1985) fed chicks with corn gluten meal, three alfalfa feeds and two coastal Bermuda grass feeds and evaluated their effects on xanthophyll utilization and coloring capability in egg yolks. Chicks fed coastal Bermuda grass showed significant improvement on yolk color compared to chicks fed the control feed. Though the corn gluten meal and alfalfa feed showed some similarity on xanthophyll utilization, the egg yokes were more pigmented when Bermuda grass was used in the chick ration. Fishman and Evans (1985) analyzed the amino acid content of coastal Bermuda grass by ion exchange chromatography. They found the coastal Bermuda grass high in lysine which is deficient in cereal grains. The coastal Bermuda grass was also found high in sulfur-containing amino acid, cystine, which is deficient in legumes.

A study undertaken by Wheeler and Turk (1963) compared Bermuda grass meal and alfalfa meal for chicks as a pigment source for egg yolk. The study was also intended to determine the interchangeability of Bermuda grass and alfalfa in chick rations. Chicks were fed rations supplemented with either Bermuda grass or alfalfa at 2%, 5%, and 10%. The researcher found no difference in the growth performance of chicks fed the two meals at 2%, 5%, or 10%. Wheeler and Turk (1963) also suggested that the optimum supplementation of either feeds was at the 5% substitution level and concluded that

Bermuda grass could replace alfalfa for egg yolk pigmentation in chick ration.

Barnett and Morgan (1959) fed one group of laying hens a standard laying ration containing 50% yellow corn and 5% or 10% alfalfa as a source of xanthophyll pigments and the second group, a similar diet with dehydrated coastal Bermuda grass substituting for alfalfa. After the feeding period, all eggs were broken and scored by comparing colors using an Heunan-Carver Color Rotar. The eggs were more highly pigmented when Bermuda grass replaced alfalfa except at the 10% level where the alfalfa resulted in higher pigmentation.

Food Application of Plant Fiber

The physiological effects of fiber are relevant not only to the gastrointestinal functions and pathology, but also to lipid and glucose metabolism and trace mineral homeostasis (McPherson, 1993). Dietary fiber has been implicated in increasing fecal bulk, binding bile acids and excretion, and lowering cholesterol, (Schrijver, 1990; Read, 1986). Some studies also indicated that dietary fiber plays a role in lowering heart disease risks, and in fighting colorectal cancer and short chain fatty acid concentration (Folino, et al, 1994; Anderson and Gustafson, 1988). Bermuda grass is about 30% fiber, and it has been reported by Fishman and Evans (1985) that Bermuda grass is high in the amino acids lysine and cystine which are deficient in wheat and legumes. It is the belief of the researchers that Bermuda grass could be used as a protein and a fiber source. The importance of fiber both in the etiology and treatment of diseases will be reviewed.

It is often suggested that an effective way to increase stool weight is to increase the fiber content of the diet by eating whole meal bread. Eastwood, Elton, and Smith (1986) studied the effects of whole meal bread on stool weight, fecal constituents, bile acid, fat, neutral sterols, and intestinal transit time and found a linear relationship between stool weight and intestinal transit time when there was an increase of 20% whole meal bread in the diet. Wyman Heaton, Manning, and Wicks (1976) studied the effect on intestinal transit time and the feces of rats fed raw and cooked bran in different doses, and found that both raw and cooked bran decreased transit time and fecal weight.

Smith, Drummond, and Eastwood (1981) studied the effect of coarse and fine Canadian red spring wheat and French soft wheat bran. Results confirmed that the coarse type of wheat, whether Canadian or French, had more significant effects on the stool weight, speeded intestinal transit time, and reduced intraluminal pressure in the colon more than did the fine types of wheat from the same source in patients with diverticular diseases.

Physiological effects of fiber-rich types of breads were studied by Van-Dokkum (1983) in young adult males. The researcher found an increase in fecal weight and defecation frequency, and a decrease in intestinal transit time when dietary fiber in the diet was increased from 9 to 22 g per day. When diet containing 35 g neutral detergent fiber was fed, further changes were noted for all factors measured, and Van-Dokkum (1983) concluded that a whole meal bread produced changes similar to feeding 22 g of neutral detergent fiber.

Effects of three cereal bran, wheat, rice, and oat were studied by Kestin, Moss, Clifton, and Nestel (1990). They studied the effects of cereal bran on plasma lipids, blood pressure, and glucose metabolism in mildly hypercholestrolemic men. The research compared the effects of adding 11.8 g dietary fiber per day from each of the three cereal bran to a low fiber diet. Plasma total and low density lipoprotein cholesterol concentration were significantly lowered by oat bran. Oat and rice bran according to Kestin et al (1990) exerted a small but potentially useful effect on plasma lipoprotein risk factors for cardiovascular diseases.

The effects of methylcellulose, coarse wheat bran, fine wheat bran and parboiled rice bran were studied by Folino, McIntyre, and Young (1996). Rats were fed diets containing either no added fiber or 10% fiber from the aforementioned fiber sources. In their findings, the researchers concluded that fecal output increased with methycellulose and coarse wheat bran, and only coarse bran increased short chain fatty acid concentration in the feces. Low fecal pH was also recorded with coarse wheat bran and parboiled rice bran, and there was no effect on colonic epithelial proliferation from all four fiber sources.

Schrijver (1990) fed animal protein (casein) and plant proteins, wheat gluten, soy protein, and potato to mature and immature rats for four weeks,. After four weeks, he found negative correlation between fecal total sterol excretion and plasma total cholesterol and high density lipoprotein cholesterol. The researcher concluded that consumption of plant proteins resulted in a faster migration of sterol than in animal proteins, and further suggested that plant proteins lowered the cholesterol level of the rats because of their fiber content

Effect of Plant Food on Growth and Digestibility

Buchannon (1969) studied the digestibility of wheat leaf protein concentrate using papaya enzyme solubilization procedure in vitro which correlated well with the true digestibility obtained by rat feeding in vitro. Byers (1967) used papaya enzyme to study the digestibility of maize leaf protein of increasing maturity and protein fractions from 14 different species, and reported that papaya enzyme increased the digestibility of the leaf protein, although species differences in digestibility were noticed.

Ershoff (1959) added alfalfa and other succulent plants as oat grass, rye grass, wheat grass, and fescue grass to a mineralized milk ration and evaluated the growth and survival of guinea pigs, by making 5-20% substitutions. Dehydrated rye grass, orchard grass, wheat grass, fescue grass, and oat grass, when fed at 20% level in the diet resulted in significant growth. When the substitution levels increased, the guinea pigs growth was affected negatively. The researcher recommended that the 20% substitution is the maximum to promote growth without any adverse effect.

Woldegiorgis (1976) studied alfalfa protein concentrates using growing rats. He employed three methods of protein preparation: heat coagulation, extraction, and freeze-drying. Rats were fed diets containing 10% alfalfa leaf proteins using three preparation methods and were comparable with rats fed 10% casein (87% protein) control diet. The results of the research showed that freeze-dried concentrates of alfalfa supported rat growth and suggested that freeze-drying protect sulfur-containing amino acid than heat treated alfalfa and concluded that alfalfa proteins are richer and better utilized if freeze drying is used as a method of preparation.

Oliveira et al (1994) studied *Canavalia brasiliensis* seeds by feeding mature seeds, that grow wild in the state of Ceara in Northeastern Brazil, to rats. The raw seeds were not a good protein source and did not support growth. The seeds resulted in weight loss, high nitrogen excretion, and macroscopic alterations of key internal organs such as the stomach, small intestine, cecum, colon, thymus, kidneys, heart, lungs and muscles.

Corn gluten meal, a by-product of the corn wet-milling industry, was studied by Buck, Walker, and Watson (1987) both alone and in combination with soy flour, in sugar cookies, white pan bread, pasta, and extruded snacks. Functional, sensory properties, and protein qualities were analyzed. The four food products possessed in vitro digestibility greater than 80%. Computed PER values of the cookies, breads and extruded products were higher than those of the initial flours and corn gluten meal individually.

Sensory Evaluation

Sensory evaluation is a tool used in food Industry as a guide in the selection of new product targets, product development, and quality control. Jellink (1964) described two classifications of sensory evaluation testing: analytical (objective) tests and affective (subjective) tests. Analytical (objective) tests involve discrimination and descriptive evaluations. These methods are used to evaluate products in terms of differences or similarities and identification or quantification of sensory characteristics. Affective tests include tests to determine consumer acceptance of a product and tests to determine consumer preference for a product (Campbell et. al., 1979). Descriptive tests compare characteristics of samples and show magnitude of differences. They include ranking tests, scoring tests, flavor, and texture properties, quantitative descriptive analysis, and magnitude estimation.

Baked products are consumed worldwide. Therefore, fortification with high-protein sources provides a good opportunity to improve the nutritional quality of protein consumed by many people (Hoover, 1979). Raidl and Klein (1983) studied the effect of substituting 5%, 10%, and 15% field peas or defatted soy flour for wheat flour in a chemically leavened bread on physical characteristics of batters and bread. A sensory evaluation panel found significant differences in crust color, color uniformity, and flavor. Results indicated that defatted soy flour can be successfully substituted for wheat flour in quick breads at levels up to 15% whereas at the same substitution levels, field pea flour had adverse effects on both physical and sensory characteristics.

Ayoub and Knight (1990) evaluated pita 'Arabic bread' with sensory evaluation using an ordinance scale. They added single cell protein at 2%, 4%, 6%, 8%, and 10% substitution for wheat flour. The semi-trained panelists evaluated color, aroma, texture, off flavor, and overall acceptability of the product and found that the 6% substitution was best accepted by their taste panel.

Tsen, Weber, and Eyestone (1983) studied the potential use of distillers dried grain flour as an ingredient for preparing grain-type breads. When wheat flour was supplemented with distiller's grain at 10% and 20% substitution levels, the substitution reduced dough development time and stability. Breads supplemented with 10% distillers' dried grain were superior to whole wheat bread in loaf volume, crumb grain, and color. The substituted breads contained less ash and fiber than whole wheat bread but more than white bread. The breads supplemented with 10% distillers' grain had the same amount of protein and fat as whole wheat bread and retained softness much better than whole wheat bread.

Collins, Kalantari, and Post (1982) studied peanut hull flour. They substituted the peanut flour at 4% and 8% to wheat bread to increase the dietary fiber content. The bread was tested for textural properties, proximate composition, dietary fiber content, specific volume, color, and other sensory attributes. While addition of peanut hull flour caused some changes in the bread, all samples were evaluated by a taste panel as being acceptable. From the standpoint of bread quality, peanut hull flour was an acceptable material which could be added to increase the level of dietary fiber in breads.

Becker, Wagoner, Hanner, and Saunders (1991) studied "Oahe" an intermediate wheatgrass seed. In the study, they found that the seed had a higher level of protein (20.8%) and ash (2.64%) than wheat. The wheatgrass seed was nutritionally limiting in lysine as is wheat, but had higher levels of all other essential amino acids than wheat. Breads, cookies, and cakes containing various levels of the flour were evaluated by a sensory panel to have favorable appearance, texture, flavor, and overall characteristics.

Distillers' dried grain (DDG) was chemically and physically characterized and the effects of varying levels of the DDG on the sensory characteristics of bakery products were studied by Brochetti and Penfield (1989). Total dietary fiber (51.22%) and protein (26.59%) were the principal constituents of the DDG. Compared with all-purpose flour, the DDG was more acidic, absorbed more water and oil, and was darker in color.

Consumer panels evaluated the acceptability and intensities of sensory characteristics of corn muffins, hush puppies, spiced doughnuts and molasses-raisin cookies containing 10, 20, and 30% DDG. As the replacement of DDG increased, the appearance, flavor, and texture of the products were adversely affected, however, acceptable products were prepared with either 10 or 20% DDG.

CHAPTER III

METHODOLOGY

This chapter includes the procedures used to evaluate the nutritional quality of Bermuda grass and the sensory quality of bread with Bermuda grass substitutions. Included are Experiment I, which is the PER study, and Experiment II, which evaluates different levels of substitutions (0%, 10%, 25%, and 40%). Experiment III evaluates bread sensory quality with 5% and 10% substitutions of Bermuda for all purpose flour. The 15% substitution, which was initially included in the proposal, was dropped because of offending odor and very coarse texture.

The Animal Use Protocol was approved by The Oklahoma State University Institutional Animal Care Committee (Appendix C) and Human Subject Research was approved by the University Institutional Review Board (IRB) (Appendix D). Each article (Chapters IV and V) includes references cited, but all references are included in the Bibliography at the end of Chapter VI.

Amino Acid analysis is shown in Appendix E. Based on preliminary experiments and comparisons of protein content, the researcher and graduate committee decided to only use one standard cultivar, Tifton, as the control cultivar. Gordon's Gift and World Feeder were the experimental cultivars.

Experiment I

Diets and Animal Feeding: Fresh Bermuda grass from the three cultivars (Gordon's Gift, World Feeder, and Tifton), four weeks old cut, was obtained from the Agronomy Department of Oklahoma State University. The grasses were sun-dried until they were dry enough for grinding in the field, and ground with a Wiley Mill equipped with a mesh size 2mm sieve. Then the powder was milled again using a Cyclone Sample Mill (UD Corporation, Boulder, CO) to form a fine, uniform powder, and stored under refrigeration until the feeding period. Other diet ingredients, casein, cornstarch, mineral mix, vitamin mix, DL-methionine, choline bitartrate and cellulose were purchased from United States Biochemical (Cleveland, OH). Sucrose, and corn oil were purchased from a local grocery store. Forty weanling Sprague Dawley rats (SASCO, Omaha, NE) initially weighing between 43 and 58 grams were used. In this study, after a three-day adaptation period, the rats were randomly divided into groups of 10 and housed individually in stainless steel cages in a room temperature of 24 ± 1.5° C with 12 hour light 12 hour dark cycle. Diets containing the Bermuda grass cultivars were formulated such that 10% of the protein required was from the grasses Table 1). All diets were supplemented with casein, cornstarch, corn oil, sucrose, minerals, vitamins, DL-methionine, choline bitartrate, and cellulose to adjust to the rat's dietary requirements (AIN, 1976). The rats were given feed and water *ad libitum* for 14 days. Weekly weight gains and feed consumption were recorded. PER values were calculated as average weight gained per day per gram of ingested protein.

Table 1. Composition of Experimental Rat Diet(g/Kg)

Ingredient	Diet1	Diet2	Diet3	Diet4
Gordon's Gift	0	100	0	0
World Feeder	0	0	100	0
Tifton	0	0	0	100
Casein	104	92	92	92
Cornstarch	666	608	608	608
Corn oil	50	50	50	50
Sucrose	80	80	80	80
Mineral mix [1]	35	35	35	35
Vitamin mix [2]	10	10	10	10
Cellulose [3]	50	20	20	20
DL-Methionine	3	3	3	3
Choline Bitartrate	2	2	2	2
Total	1000	1000	1000	1000

[1] AIN 76 mineral mix
[2] AIN 76 vitamin mix
[3] Non nutritive bulk

Amino Acid Analysis: The amino acid for the three Bermuda grass cultivars were analyzed by hydrolysis. Samples were hydrolyzed in Teflon digestion vessel (CEM, Mathews, NC) with 6M hydrochloric acid. AminoQuant (HP, Waldbronn, Germany) was used for amino acids estimation. Amino acids were derivatized with o-phthaldialdehyde and a-fluorenylmethyl chloroformate and separated using a reverse phase column. A diode array detector was used for amino acid detection (Appendix E)

Weight of Organs: After 14 days the rats were anesthetized with Ketamine (0.3 ml/animal) and Xylazine (0.2 ml/animal) and sacrificed, their heart, liver, kidneys, spleen, lungs, and testicles were excised, rinsed, and weighed.

Experiment II

Diets and Animal Feeding: As in Experiment I, fresh Bermuda grasses (Gordon's Gift, World Feeder & Tifton) were obtained from the Agronomy Department of Oklahoma State University, and the same procedure as the first experiment were followed for drying and grinding the grasses. Other ingredients, casein, cornstarch, mineral mix, vitamin mix, DL-methionine, choline bitartrate, cellulose and lysine were purchased from United States Biochemical (Cleveland, OH). Sucrose and corn oil were purchased from a local grocery.

TABLE 2

Composition of basal diet (Diet 1) g/Kg diet	
Casein	104.0
Cornstarch	665.5
Corn oil	50.0
Sugar	80.0
Mineral mix[1]	35.0
Vitamin mix[2]	10.0
Cellulose	50.0
DL- methionine	3.0
Choline bitartrate	2.0
Lysine	0.5
Total	1000

[1] AIN 76 mineral mix
[2] AIN 76 vitamin mix
[3] Non nutritive bulk

Twelve Diets were Prepared. Diet 1 was a control diet (Table 2). Diets 2-10 were, one diet each containing 10%, 25%, and 40%, of the three cultivars. In addition, Diet 11 had 40% Bermuda grass but no lysine. Diet 12 had cellulose as a fiber source at an amount comparable to the fiber at the 40% Bermuda grass level in order to compare the effects of the two fiber sources (Table 3). Eighty four weanling Sprague Dawley rats (SASCO, Omaha, NE) initially weighing between 25 and 45 g were used. After a three-day adaptation period, the rats were randomly divided into groups of seven and housed individually in stainless steel cages in room temperature of 24 ± 1.5° C and a 12 hour light 12 hour dark cycle. Diets containing 10%, 25%, and 40% of the Bermuda grass flour were formulated to contain 10% crude protein (N x 6.25) as a combination of Bermuda grass protein and casein. All diets were balanced for lysine except for Diet 11, which had no lysine. The diets were randomly assigned to sets of animals (7/group). The diet formulae included casein, cornstarch, corn oil, sucrose, mineral mix, vitamin mix, DL-methionine, choline bitartrate, and cellulose to adjust to 100 g. Rats were given feed and distilled water *ad libitum* for 28 days, and weekly weight gains, feed consumption and weight of wet and dry feces were recorded.

Table 3. Composition of experimental diets (g/kg)

Ingredient	10%	25%	40%	40% no lysine	12.5% fiber
Bermuda grass	100	250	400	400	0
Casein	92	73	55	55	104
Cornstarch	613	495.15	362.4	365	590.5
Corn oil	50	50	50	50	50
Sucrose	80	80	80	80	80
Mineral mix[1]	35	35	35	35	35
Vitamin mix[2]	10	10	10	10	10
Cellulose	14	0	0	0	125
DL-methionine	3	3	3	3	3
Choline bitartrate	2	2	2	2	2
Lysine	1	1.85	2.6	0	0.5
Total	1000	1000	1000	1000	1000

[1] AIN 76 mineral mix
[2] AIN 76 vitamin mix
[3] Non nutritive bulk

Organ, and Blood Sample Collection: On the 27th day, the rats were made to fast for 24 hours for blood sampling. On the 28th day the rats were anesthetized and sacrificed, and blood was collected from the heart of each rat with a 3mm heparinized syringe and centrifuged using a Beckman TJ-6R centrifuge (Beckman Instruments Palo Alto, Ca) for 30 minutes at 4^0 C. The plasma were separated and stored at -20^0C for analysis. Plasma albumin and creatinine were analyzed using COBAS FARA chemistry analyzer (Roche Diagnostic Systems, Somerville, NJ). Organs (liver, heart, kidney, lung, spleen, and testicles) were excised, rinsed and weighed.

Fecal Samples Collection. All wet feces collected every morning throughout the trial period were weighed and oven-dried for 48 hours at 100° C and reweighed to determine the fecal dry weight. The fecal samples collected during the last 7 days of the experiment for each animal were sealed in sample bags and frozen at –20° C for analysis.

Kjeldahl Analysis and Protein Determination Prior to the Kjeldahl analyses, the frozen samples were oven-dried for five hours at 60° C. Duplicate fecal samples were used to determine dry matter digestibility. Nitrogen and protein content of feed and fecal matter were determined following the macro Kjeldahl method (AOAC, 1990) using a KelTec 1030 Auto Analyzer (Tecator, Sweden) for digestion and distillation, with each sample being run in duplicate. In order to calculate the nitrogen and protein content the following formulae were used:

% Nitrogen = [(1.401g/mole x 0.10mole/litre)/dry sample wt.(grams)] x mls HCL

% Protein = % Nitrogen x 6.25 (standard conversion for plant material)

Experiment III

Bermuda Grass in Bread

Preparation of bread: Breads were made with either 0%, 5% or 10% Bermuda grass flour substituted for equal weight of All Purpose flour (Table 4). The ingredients used for the bread making were active dry yeast, water, sugar, all purpose flour (10.5% protein) and Bermuda grass flour. Breads were prepared one day before sensory evaluation. All formulae assembly steps followed procedures as described in Knight (1995). All doughs were kneaded for exactly 10 minutes, and proofed in a proofing chamber (Proof Hot Cabinet, Cleveland, OH) at a temperature of 85° F (29° C), with controlled humidity, for 15 minutes. Doughs were then removed from the proofing chamber, kneaded for two additional minutes, shaped into loaves, and placed into a 1/2 pound sized loaf pan. Proofing was continued until the loaves were ready to bake as determined by the compression test illustrated by Freeland-Graves and Peckham (1995). The loaves were baked in electronically controlled gas oven at 350° F (177° C), six at a time, for 45 minutes. The loaves were removed from pans within five minutes after baking and cooled to room temperature before packaging. Bread volumes were measured by Volumeter using rape seed displacement (Cathcart and Cole, 1938; Penfield and Campbell, 1990) and refrigerated overnight in preparation for taste panel evaluation.

Table 4. Composition of Breads made with 5% or 10% Bermuda grass substitution

Ingredient	Control Bread	5% Bermuda grass flour (g)	10% Bermuda grass flour (g)
Bermuda grass	0	5	10
All purpose flour	100	95	90
Active Dry Yeast	1.7	1.7	1.7
Sugar	14	14	14
Shortening	6	6	6
Salt	1.5	1.5	1.5
Water	57	57	57

Sensory Evaluation: Ten volunteer panelists from the student population attending Oklahoma State University signed consent forms as required by the Review Board to evaluate the breads. On the day of sensory evaluation, breads were taken out from the refrigerator and rethermalized at room temperature for four hours before presenting to test panelists. Prior to the sensory testing, panelists were given training about procedures in judging the breads. The training developed a common understanding of terminology used in expressing the attributes in the sensory evaluation. The attributes measured were flavor, off-flavor, texture, appearance, and overall acceptability. The evaluations were done in individual booths, with white flourecent lighting and environmental control such that room temperature remained constant over the period of testing, but there was no control over room humidity. Panelists were seated in a room free from external distraction. Paper, pencil and distilled water were provided, and panelists were informed to rinse their mouth between samples. All samples were judged using a 10-point descriptive scale. Samples were presented one at a time on a plate in random order during each session. The randomization schedule was as follows. Samples were assigned 3-digit number code drawn from a random number table. Samples were presented to panelists, 3 at a time, on a single plate. Each plate contained 0%, 5%, and 10% samples of a single cultivar (Gordon's Gift, World Feeder, Tifton). Sample plates were randomly presented to the panelist so that each panelist receive samples in a random order and each cultivar had equal opportunity to be selected first. All three cultivars were rated on each testing day. Panelists were allowed about 15 minutes between sample presentation in order for the palate to clear. Testing sessions were held at approximately the same time each day (3 p.m.) for three consecutive Fridays, and the same procedures were followed for all sessions.

Statistical Analysis

The data for the Protein Efficiency Ratio study (Experiment I) was analyzed by one way ANOVA using the Statistical Analysis System (1985). When p values were significant ($p \leq 0.05$), differences among means were compared with t-test (Steel and Torrie, 1980). The data for Experiment II were analyzed with the General Linear Model (SAS, 1985), and Duncan's Multiple Range Test (Steel and Torrie, 1980) was used to compare the means. The data from Experiment III, sensory attributes (flavor, off-flavor, texture, appearance, and acceptability), were analyzed using Repeated Measures Analysis of Variance, and t-test (LSD) was used to determine the mean differences.

CHAPTER IV

PROTEIN EFFICIENCY RATIO OF RATS FED 10% BERMUDA GRASS RATION AND SENSORY EVALUATION OF BREADS WITH 5% and 10% SUBSTITUTION

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ABSTRACT

A Protein Efficiency Ratio study (PER) was used to evaluate the nutritional adequacy of three Bermuda grass cultivar (Gordon's Gifts, World Feeder, and Tifton) flours. All diets were 10% protein with the Bermuda grass flour in each case furnishing 1.2% of the total protein and casein furnishing 8.8%. The reference diet was 10% protein as casein. The mean weights of rats fed Bermuda grass flour were not different ($p \leq 0.05$) from those fed the reference protein. The PER values of 10% Gordon's Gift, World Feeder, Tifton and the control were 2.71, 2.62, 2.86, and 2.87.

There were no significant differences in organ weights except for the rats fed World Feeder whose hearts and spleens were smaller than the control. For sensory evaluation, breads were made with 0, 5, or 10% substitution of the Bermuda cultivar

flours for all purpose flour. For most characteristics, panelists found the 0% (all purpose flour control) more favorable ($p \leq 0.05$). Overall, Gordon's Gift 5% substitution was rated favorably with the control while the other cultivars were significantly rated lower than the control.

INTRODUCTION

The increased population and general shortage of animal protein and other foods in some countries and the rapid spread of malnutrition in the third world countries have created a need for new methods of producing and processing foods (Uzogara, et al, 1991). Third world countries are predominantly agricultural, and produce only half of their total protein requirements which is mainly vegetable protein. Dependence on an incomplete protein source has resulted in the prevalence of qualitative dietary protein deficiency (Bass and Carl, 1972; Bakashi, et al, 1989). Composite flour technology has been used as a means for extending scarce supplies of protein from wheat or corn used in the preparation of breads or other baked goods (Shurpalekar and Shukla, 1992). Ayoub and Knight working with pita bread (1990) substituted single-cell protein at 2%, 4%, 6%, 8%, and 10% of wheat flour and found that as much as 6% substitution was accepted by their taste panels. Tsen *et al.* (1983) used distiller's grain for preparing grain-type bread. Breads supplemented with 10% distiller's dried grain were superior to whole wheat bread in loaf volume, crumb grain, and color. The supplemented breads had about the same amount of protein and fat as whole wheat bread and retained softness much better than did whole wheat bread.

For third world countries, locally available plant proteins would be an economical approach particularly if they produced a complete protein source. The high cost and shortage of animal protein have created dependence on plant sources. In order to alleviate the protein-energy malnutrition, more attention is being focused on plant sources for high quality protein. Oliveira et al (1994) evaluated the protein quality of Canavalia brasiliensis, a wild grass seed , using growing rats. Yensen and Weber (1987) evaluated *Distichlis palmeri*, a salt grass that produces wheat-like grain using rats. Garcia et al (1987), and Sangiah (1990) evaluated the protein efficiency ratio of amaranth grain while Woldegiorgis (1976), and Takyi, et al (1992) tested alfalfa protein using growing rats. Balasumbramayas and Sampath (1984) evaluated protein efficiency ratio of *Algae spirulina*, (about 17% protein) casein, and concentrated milk, using growing rats and found that the PER of *Algae spirulina* was comparable to that of casein or concentrated milk. Begum et al (1989) evaluated the protein quality of Jack fruit, an indigenous crop of India, using growing rats and suggested that the fruit could be utilized for human consumption if supplemented with methionine and tryptophan. Bermuda grass is moderately high in protein (12%), grows well in a wide variety of climatic conditions, and is under investigation as a substitute for low protein flours in staple foods for some countries. Bermuda grass, like all grasses, however, is high in fiber. Ershoff (1959) found that a diet high in coastal Bermuda grass (50%) decreased growth in guinea pigs. Three Bermuda grass cultivars were reported to be good protein sources, hardy, and grow in conditions common to many third world countries. Thus these cultivars were investigated as substitutes for low protein flour or to improve the protein quality in different staple foods. The goals of this study were to test the protein quality of three cultivars of

Bermuda grass as fortified feed for rats, and to determine their acceptability in human food. The specific objectives of these studies are to:

(1) Determine the protein efficiency ratio (PER) of three Bermuda grass cultivars (Gordon's Gift, World Feeder, and Tifton) at 10% of the diet using growing rats and;

(2) To determine the sensory characteristics of breads fortified with Bermuda grass flour at three levels (0%, 5%, 10%) of substitution.

MATERIALS AND METHODS

Diets and Animal Feeding

Bermuda Grass from three cultivars (Gordon's gift, World Feeder, and Tifton) was obtained from the Agronomy Department of Oklahoma State University. The grasses were planted either in the month of June or July and were harvested after four weeks. First cuts were used for this experiment. The grasses were suspended and sun-dried in the field until they are dry enough for grinding and ground with a Wiley Mill equipped with a mesh size 2mm sieve. Then the powder was milled again using a Cyclone Sample Mill (UD Corporation, Boulder, CO.) to form a fine, uniform powder. Other diet ingredients, casein, cornstarch, mineral mix, vitamin mix, DL-methionine, choline bitartrate and cellulose were purchased from United States Biochemical (Cleveland, OH). Sucrose and corn oil were purchased from a local grocery store. The fiber content of the grasses was obtained from the Proximate Analysis of Cereal grains and Forages (National Research Council 1958). A basal diet was prepared based on the American Institute of Nutrition, (AIN 1976). Forty weanling Sprague Dawley rats (SASCO, Omaha, NE) initially weighing between 43 and 58 grams were used. After three days of adaptation, the rats were randomly divided into groups of 10 and housed individually in stainless steel cages at a temperature of 24 ± 1.5^0 C with a 12 hour light and 12 hour dark cycle. Diets containing the Bermuda grass cultivars were formulated such that 10% of the diets were from the grass and were randomly assigned to each group of rats. All diets were supplemented with casein, cornstarch, corn oil, sucrose, minerals, vitamins DL-methionine, choline bitartrate, and cellulose to adjust to the rat's dietary requirements (Table 1). The rats were given feed and water *ad libitum* for 14 days. Weekly weight gains and feed consumption were recorded. PER values were calculated as average weight gained per day per gram of ingested protein. The Animal Use Protocol and Human Subject Research were approved by The Oklahoma State University Institutional Animal Care Committee and University Review Board for Protection of Human Subjects.

TABLE 1.
COMPOSITION OF EXPERIMENTAL RAT DIET(G/KG)

Ingredient	Diet1	Diet2	Diet3	Diet4
Gordon's Gift	0	100	0	0
World Feeder	0	0	100	0
Tifton	0	0	0	100
Casein	104	92	92	92
Cornstarch	666	608	608	608
Corn oil	50	50	50	50
Sucrose	80	80	80	80
Mineral mix [1]	35	35	35	35
Vitamin mix [2]	10	10	10	10
Cellulose [3]	50	20	20	20
DL-Methionine	3	3	3	3
Choline Bitartrate	2	2	2	2
Total	1000	1000	1000	1000

[1] AIN 76 mineral mix
[2] AIN 76 vitamin mix
[3] Non nutritive bulk

Weight of Organs

After 14 days of trial feeding the rats were anesthetized with ketamine (0.3 ml/animal) and xylazine (0.2 ml/animal) and sacrificed. Their heart, liver, kidneys, spleen, lungs, and testicles were excised, weighed, and compared.

BERMUDA GRASS IN BREAD

Preparation of Bread

Breads were made with either 0, 5% or 10% Bermuda grass flour substitution for equal weight of all purpose flour. The ingredients used for the bread making are shown in Table II.

Table II Composition of Breads made with 5%, and 10% Bermuda grass substitutions

Ingredient	Control Bread	5% Bermuda grass flour (g)	10% Bermuda grass flour (g)
Bermuda grass	0	5	10
All purpose flour	100	95	90
Granulated dry yeast	1.7	1.7	1.7
Sugar	14	14	14
shortening	6	6	6
salt	1.5	1.5	1.5
Water	57	57	57

Breads were prepared one day before sensory evaluation. All doughs were kneaded for exactly 10 minutes, and proofed in a proofing chamber (Proof Hot Cabinet, Cleveland, OH) at a temperature of 85° F (29° C), with controlled humidity, for 15 minutes. Doughs were then removed from the proofing chamber, kneaded for 2 additional minutes, and shaped into loaves and placed into a 1/2 pound sized loaf pan. Proofing continued until the loaves were ready to bake. The loaves were baked in an electronically controlled gas oven at 350° F (177° C) for 45 minutes. The loaves were removed from pans within 5 minutes after baking and cooled to room temperature before packaging. Bread volumes were measured by Volumeter using rape seed displacement (Cathcart and Cole, 1938; Penfield and Campbell, 1990) and refrigerated overnight in preparation for taste panel tasting.

Sensory Evaluation

Ten volunteer panelists from the student population attending Oklahoma State University participated in the sensory evaluation and signed consent forms as required by the Institutional Review Board. On the day of sensory evaluation, breads were taken out from the refrigerator and rethermalized at room temperature for four hours before presenting to test panelists. Prior to sensory testing, panelists were given training about procedures in judging the breads. The attributes measured were flavor, off-flavor, texture, appearance, and overall acceptability. All samples were judged using a 10-point descriptive scale. The evaluations were done in individual booths, with proper lighting and environmental control such that room temperature remained constant over the period of testing. Panelists were seated in a room free from external distraction. Panelists participated in the sensory evaluation at approximately the same time for three consecutive Fridays. Samples were presented at a time on a plate in random order during each session The order of cultivar rating and the order of sample presentation during testing were randomly assigned. The interval between products was controlled and panelists were instructed to rinse their mouths with distilled water between samples.

Statistical Analysis

The data for the protein efficiency ratio study was analyzed by one way ANOVA using the statistical analysis system (SAS, 1985). Significant ($p \leq 0.05$), differences among means were identified using t-test (LSD) (Steel and Torrie, 1980). For sensory evaluation, the attributes (flavor, off-flavor, appearance, texture, acceptability) were analyzed with the General Linear Model using Repeated Measures of Analysis of Variance. Significant differences among means were compared with t-test (LSD) (.Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Experiment I Animal Study

The animal feeding, weight gain, and PER values are summarized in Tables 2. The mean PER values of the control and the three cultivars of Bermuda grass at 10% substitution level were 2.87, 2.71, 2.62, and 2.86 and were not significantly different from each other. The PER values indicated that the growth of rats fed 10% Bermuda grass substituted diets were comparable to the casein control diet. The organ weight values are summarized in Table 3. The mean weights of the heart and spleen of the rats fed World Feeder were significantly less than those fed the other diets. In fact, weights for 5 of the 6 organs tested were lowest for the World Feeder, although these differences were only significant for the hearts and spleens. We do not know if these data are an anomaly or whether a longer feeding trial would show a significant trend in these organ weights for rats fed the World Feeder cultivar.

Many research work had been carried out to evaluate the protein quality, apparent digestibility, PER, biological value and safety of leaf protein in general (Woldegiorgis, 1976; Godinez, et al, 1992; Takyi, et al, 1992; Uwagbute and Nnanyelugo, 1987; Garcia, et al, 1987; Begum, et al, 1989). Results of comparative amino acid analysis of Bermuda grass showed that lysine contents of Gordon's Gift, World Feeder, and Tifton are 3.55, 3.45, and 4.46 mg/g compared to wheat grain which is 2.82 mg/g. The most limiting amino acid in Bermuda grass is the sulfur containing amino acid, methionine. In general, independent of cultivar, the PER values for the 10% Bermuda grass substitution diets gave statistically similar values. The similarity in PER could indicate good utilization of the protein from Bermuda grass.

TABLE 2.
TOTAL WEIGHT GAIN, PROTEIN INTAKE, WEIGHT GAIN PER DAY, AND PROTEIN EFFICIENCY RATIO (PER) OF RATS FED 10% OF DIET AS BERMUDA GRASS (MEAN ± SEM)

Diet	Total Weight gain (g)	Protein Intake (g/day)	Average Daily Weight Gain (g)	PER
Control	66.6 ± 3.85^{a}	1.65 ± 0.03^{a}	4.75 ± 0.03^{a}	2.87 ± 0.17^{a}
Gordon's Gift	63.0 ± 3.85^{a}	1.66 ± 0.03^{a}	4.50 ± 0.03^{a}	2.71 ± 0.17^{a}
World Feeder	60.8 ± 3.85^{a}	1.66 ± 0.03^{a}	4.34 ± 0.03^{a}	2.62 ± 0.17^{a}
Tifton	66.2 ± 3.85^{a}	1.65 ± 0.03^{a}	4.73 ± 0.03^{a}	2.86 ± 0.17^{a}

Values in the same column with different superscript are significant ($p \leq 0.05$).

TABLE 3.
WEIGHT OF ORGANS OF RATS FED 10% BERMUDA GRASS AND CASEIN (REFERENCE) DIET (MEAN ± SEM).

Diet	Heart (g)	Liver (g)	Kidney (g)	Spleen (g)	Lung (g)	Testicle (g)
Control	0.53 ± 0.06^{a}	5.70 ± 0.74^{a}	0.96 ± 0.13^{a}	0.38 ± 0.06^{a}	0.81 ± 0.12^{a}	1.15 ± 0.22^{a}
Gordon's Gift	0.51 ± 0.06^{ab}	5.44 ± 0.74^{a}	0.97 ± 0.13^{a}	0.36 ± 0.06^{a}	0.80 ± 0.12^{a}	1.13 ± 0.22^{a}
World Feeder	0.47 ± 0.06^{b}	5.31 ± 0.74^{a}	0.95 ± 0.13^{a}	0.31 ± 0.06^{b}	0.72 ± 0.12^{a}	1.08 ± 0.22^{a}
Tifton	0.51 ± 0.06^{ab}	5.99 ± 0.74^{a}	0.99 ± 0.13^{a}	0.34 ± 0.06^{ab}	0.78 ± 0.12^{a}	0.90 ± 0.22^{a}

Values in the same column with different superscripts are significantly different ($p \leq 0.05$)

Experiment III Sensory Evaluation

The sensory results are summarized in Table 4. The sensory characteristics were analyzed by day and by the Bermuda Grass flour substitution levels. In order to examine the consistency of panelists over the three days of sensory evaluation, a day by substitution level interaction effects were analyzed.

TABLE 4.

SENSORY EVALUATION OF BREADS MADE WITH THREE CULTIVARS OF BERMUDA GRASS FLOUR AT 0%, 5%, AND 10% SUBSTITUTION FOR WHEAT FLOUR

Mean Flavor Rating

Bermuda Cultivar
World Feeder

	Day 1	Day 2	Day 3
0%	6.90^{a}	7.34^{a}	6.74^{a}
5%	2.73^{b}	2.67^{b}	2.97^{b}
10%	2.50^{b}	2.48^{b}	1.80^{b}

Gordon's Gift

	Day 1	Day 2	Day 3
0%	6.94^{a}	7.06^{a}	6.49^{a}
5%	5.42^{a}	6.48^{a}	6.44^{a}
10%	5.32^{a}	5.96^{a}	4.95^{b}

Tifton

	Day 1	Day 2	Day 3
0%	6.38^{a}	6.74^{a}	6.68^{a}
5%	4.48^{b}	4.80^{b}	5.08^{b}
10%	3.65^{b}	2.64^{c}	2.85^{c}

Flavor ratings were 0=poor bread flavor, 10=good bread flavor. Values in the same column with different superscripts are significantly different ($p \leq 0.05$).

Mean Off-Flavor Rating

Bermuda Cultivar
World Feeder

	Day 1	Day 2	Day 3
0%	2.30^{b}	2.21^{c}	2.25^{b}
5%	5.74^{a}	5.34^{b}	5.32^{a}
10%	6.43^{a}	6.86^{a}	6.55^{a}

Gordon's Gift

	Day 1	Day 2	Day 3
0%	2.38^{b}	2.48^{b}	3.26^{a}
5%	3.55^{ab}	3.77^{b}	4.08^{a}
10%	4.16^{a}	5.98^{a}	4.29^{a}

Tifton

	Day 1	Day 2	Day 3
0%	1.34^{c}	1.51^{c}	1.63^{c}
5%	4.94^{b}	5.12^{b}	5.52^{b}
10%	8.01^{a}	7.57^{a}	7.99^{a}

Off-flavor ratings were 0=no off flavor, 10= strong off flavor
Values in the same column with different superscripts are significantly different ($p \leq 0.05$).

Mean Appearance Rating

Bermuda Cultivar
World Feeder

	Day 1	Day 2	Day 3
0%	6.32^{a}	6.08^{a}	6.10^{a}
5%	5.96^{a}	5.36^{ab}	5.88^{ab}
10%	3.95^{b}	4.19^{b}	3.99^{b}

Gordon's Gift

	Day 1	Day 2	Day 3
0%	5.81^{b}	6.69^{ab}	6.07^{ab}
5%	8.25^{a}	7.70^{a}	7.79^{a}
10%	4.75^{b}	6.19^{b}	5.58^{b}

Tifton

	Day 1	Day 2	Day 3
0%	7.45^{a}	7.27^{a}	6.59^{a}
5%	5.67^{b}	5.86^{b}	5.22^{b}
10%	5.31^{b}	4.99^{b}	2.76^{c}

Appearance ratings were 0= poor appearance, 10= good appearance
Values in the same column with different superscripts are significantly different ($p \leq 0.05$).

Mean Texture Rating

Bermuda Cultivar
World Feeder

	Day 1	Day 2	Day 3
0%	5.77^b	5.92^b	6.16^a
5%	8.17^a	8.25^a	6.93^a
10%	6.62^b	6.49^b	6.48^a

Gordon's Gift

	Day 1	Day 2	Day 3
0%	4.64^b	4.67^b	4.99^b
5%	7.25^a	6.96^a	7.18^a
10%	8.53^a	7.68^a	7.46^a

Tifton

	Day 1	Day 2	Day 3
0%	6.21^c	6.38^b	6.75^b
5%	8.31^a	7.85^a	8.66^a
10%	7.33^b	6.33^b	6.82^b

Texture ratings were 0= poor texture, 10=good texture
Values in the same column with different superscripts are significantly different ($p \leq 0.05$).

Mean Acceptability Rating

Bermuda Cultivar
World Feeder

	Day 1	Day 2	Day 3
0%	6.64^a	7.23^a	7.28^a
5%	5.16^b	5.77^b	5.10^b
10%	1.44^c	1.36^c	1.61^c

Gordon's Gift

	Day 1	Day 2	Day 3
0%	6.90^a	6.99^a	7.22^a
5%	5.80^a	5.34^b	5.27^b
10%	3.36^b	3.90^b	3.17^c

Tifton

	Day 1	Day 2	Day 3
0%	6.33^a	6.69^a	6.95^a
5%	4.39^b	4.51^b	4.33^b
10%	2.02^c	1.65^c	1.93^c

Acceptability ratings were 0=not acceptable, 10= very acceptable
Values in the same column with different superscripts are significantly different ($p \leq 0.05$)

In sensory evaluation, the control bread were consistently rated better than the breads with 5% and 10% Bermuda grass substitution levels for flavor , off-flavor, appearance, and acceptability. For the attribute, texture, however, breads with Bermuda grass were rated more favorably than the control bread. Panelists in general, were not consistent in rating breads during the three days of sensory evaluation. Panelists detected grassy flavor in the 5% and 10% Bermuda grass substitution breads as distinct from the control. The 10% substitution levels of breads were dark green for three of the cultivars than the 5% level. Color, therefore contributed to the differences in appearance ratings. Bermuda grass flour enhanced the texture of wheat breads. With 5% substitution, the Bermuda grass flour breads have uniform cells and appeared lighter than the other breads. With 10% substitution, however, the texture became dense and compact as the liquids in the formula were kept constant.

The overall acceptability determines whether panelists like or dislike the bread. Addition of Bermuda grass resulted in changes in bread quality, and quality generally influences consumers selection of products (Stone, et al, 1991). The 10% substitution level had adverse effects on volume, texture, color, and detectable off-flavor. The results from the sensory evaluation indicated that flavor and color of the Bermuda grass flour breads were not acceptable to the panelists, except for the bread with 5% Gordon's Gift which was judged as similar to the control bread in all sensory attributes. Although substituting Bermuda grass in food products was intended to alleviate the protein quality in third world countries, the population group need to be identified who would be willing to incorporate Bermuda grass in their staple diets.

CONCLUSION

The protein efficiency ratio (PER) study showed that the 10% Bermuda grass diets support growth and were not significantly different from the control diet. The weights of the heart and spleen of the rats fed experimental diets, however, were significantly lower than those from control group. Further study is needed to see if Bermuda grass has a deleterious effect on monogastric animals. Overall, sensory evaluation for the breads indicated that those with 5% Gordon's Gift were rated favorably with the control, whereas the other cultivars were significantly different from the control. Other baked products with 5% substitution level need to be tested and developed to suit local needs and preferences relative to color, flavor, and texture of products if Bermuda grass is to be used as a protein enhancer. Both the 5% and 10% substitution levels of the three Bermuda grass cultivars also need to be incorporated in other baked products such as quick breads with zucchini and carrots, international breads or deserts, and pasta products with vegetables, herbs or spices to discover if acceptable products could be prepared for a select group or general public.

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CHAPTER V

GROWTH PERFORMANCE OF RATS FED DIFFERENT LEVELS OF BERMUDA GRASS SUPPLEMENTED WITH LYSINE

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ABSTRACT

Growth performance of rats fed different levels of Bermuda grass cultivars (World Feeder, Gordon's Gift and Tifton) was evaluated. Twelve diets were prepared with levels of 0%, 10%, 25%, and 40% Bermuda Grass with or without lysine supplement. Eighty-four weanling Sprague Dawley rats, initially weighing between 25 and 48 g, seven assigned to each diet treatment, were used in the study. The rats were provided feed and water ad libitum; and weekly weight gains, feed consumption, and fecal output were recorded. All diets were 10% protein with the Bermuda Grass flour furnishing 1.2% to about 4% of the protein and casein furnishing the rest of the protein. The reference diet was 10% protein, all as casein. After 28 days, the rats were anesthetized, and sacrificed. Blood was collected from the heart and the serum was separated for albumin and

creatinine analyses. Organs such as heart, liver, kidneys, spleen, lungs, and testicles were excised and weighed. The research followed a completely randomized design. Analysis was by General Linear Model, with Duncan's Multiple Range Test to determine differences between means. There were significant differences ($p \leq 0.05$) in body weights, kidneys, lungs, serum albumin, creatinine, total wet fecal output, dry matter digestibility and fecal protein between the control diet and experimental diets for the 25% and 40% level of the Bermuda grass substitutions; whereas, no significant differences were found between the control diet and the 10% level for body weights, organ weights, serum albumin, or creatinine for all cultivars.

INTRODUCTION

In rural farming populations throughout the world, ecological and economic constraints on food availability are frequently reflected in chronic malnutrition, poor growth and reduced adulthood working capacity (Leonard et. al. 1993). Protein-calorie malnutrition is still the cause for worldwide concerns because of increasing human population with little corresponding increase in food resources (Uzogara et. al., 1991). Increased agricultural production is a critical objective for developing countries experiencing rapid demographic growth, in which agriculture is confronted with extremely difficult climatic conditions (Benefice and Simondon, 1993). The situation in the 1990's is little changed from the 1980's, with rapid spread of malnutrition in third world (Young, and Peelet, 1984) and developing countries where the average annual population growth outpaces food production. (Paulino, 1986) and where 95% percent of the future population growth will occur (Moffett, 1994). These countries are predominantly agricultural, and produce only half of their total protein requirements, mainly as vegetable protein. So researchers from all over the world have sought and continue to seek for new protein sources from different indigenous plants in different countries. Straus et al (1994) concluded that rats on protein restricted diets have reduced serum albumin because of reduced albumin synthesis in the liver. Oliveira et al (1994) evaluated the protein quality of *Canavalia brasiliensis*, a wild grass seed, using growing rats. Garcia et al (1987), and Sangiah (1990) evaluated the protein quality of amaranth grain as a food for human. consumption while Woldegiorgis (1976) tested alfalfa protein using growing rats. Balasumbramayas and Sampath (1984) fed growing rats *Algae spirulina* (about 17% protein) and measured the PER, hemoglobin and hematocrit and found that the plant can support growth. Begum et al (1989) evaluated the protein quality of Jack fruit, an indigenous crop of India, using growing rats and suggested that the fruit could be used for human consumption if supplemented with methionine and tryptophan. Bermuda grass is moderately high in protein (12%), grows well in a wide variety of climatic conditions, and is under investigation as a substitute for low protein flours in staple foods for some countries. Bermuda grass, like all grasses, however, is high in fiber. Ershoff (1959) found that a diet high in Bermuda grass (50%) decreased growth in guinea pigs, but it is not clear whether it was Bermuda grass fiber, per se, that caused the decrease. The fiber source in most experimental control diets is refined cellulose, usually at 5%, and it is not known whether the effect of Bermuda grass fiber is similar to that of the refined cellulose fiber. The objective of this study is to evaluate the nutritional adequacy, using growing rats, of three Bermuda grass cultivars, Gordon's Gift, World Feeder, and Tifton, at different levels of substitution in diets for growing rats and to compare Bermuda grass as a fiber source with refined cellulose.

MATERIALS AND METHODS

Preparation of Feed:

Fresh Bermuda grasses (Gordon's Gift, World Feeder, and Tifton) were obtained from the Agronomy Department of Oklahoma State University. The grasses were sun-dried and ground with a Wiley mill equipped with a 2 mm mesh sieve. Then the coarse powder was milled again using a Cyclone Sample mill (UD Corporation, Boulder, Colorado) to form a fine and uniform powder. The other ingredients, casein, cornstarch, mineral mix, vitamin mix, DL-methionine, choline bitartrate, celufil (refined cellulose) and lysine, were purchased from United States Biochemical (Cleveland, OH). Sucrose and corn oil were purchased from a local grocery store.

The protein content of the grasses was determined by Kjeldahl, and the amino acid components were estimated by AminoQuant (HP, Waddbronn, Germany). The fiber content of the grasses was obtained from the Proximate Analysis of Cereal Grains and Forages (National Research Council, 1958). A basal diet was prepared based on the American Institute of Nutrition, (AIN 1976) with casein as the protein source (Table 1). Test diets were calculated that derived part of the required protein from the Bermuda grasses and the balance from casein such that diets were sufficient in protein quality and quantity for the animals' growth. In all, 12 diets were formulated (Table 2). Diet One, which was the control diet, was the basal (AIN) diet with no Bermuda grass added (Table

TABLE 1.

Composition of basal diet (Diet 1) g/Kg diet

Casein	104.0
Cornstarch	665.5
Cornoil	50.0
Sucrose	80.0
Mineral mix[1]	35.0
Vitamin mix[2]	10.0
Cellulose	50.0
DL- methionine	3.0
Choline bitartrate	2.0
Lysine	0.5

[1] AIN 76 mineral mix
[2] AIN 76 vitamin mix
[3] Non nutritive bulk

TABLE 2.
Composition of experimental diets (g/kg)

Ingredient	10%	25%	40%	40% no lysine	12.5% fiber
Bermuda grass	100	250	400	400	0
Casein	92	73	55	55	104
Cornstarch	613	495.15	362.4	365	590.5
Corn oil	50	50	50	50	50
Sucrose	80	80	80	80	80
Mineral mix[1]	35	35	35	35	35
Vitamin mix[2]	10	10	10	10	10
Cellulose	14	0	0	0	125
DL-methionine	3	3	3	3	3
Choline bitartrate	2	2	2	2	2
Lysine	1	1.85	2.6	0	0.5
Total	1000	1000	1000	1000	1000

[1] AIN 76 mineral mix
[2] AIN 76 vitamin mix
[3] Non nutritive bulk

1). Nine diets (Diets 2-10) containing 10%, 25%, and 40% from each cultivar were formulated and were supplemented with lysine. Diet 11, which had 40% World Feeder was formulated with no lysine to test the need for lysine for rat growth. An additional diet, (Diet 12) had 12.5% cellulose but no Bermuda grass. All diets were adjusted with casein to contain 10% crude protein. Amino acid analysis showed all three of the Bermuda grass cultivars to be deficient in lysine. Therefore, control and Diets 2-10 and Diet 12 were supplemented with lysine at the level required for rat growth. Diet 11, 40% World Feeder but no added lysine is included in order to test the need for lysine. The 12.5% cellulose diet is included to see if there is a difference between the Bermuda grass fiber and the refined fiber.

Eighty-four weanling Sprague Dawley rats (SASCO, NE) initially weighing between 25 and 48 g were used. The animal use protocol was approved by the Oklahoma State University Institutional Animal Use and Care Committee. The rats were allowed free access to a semipurified diet for four days of adaptation period, then were randomly divided into groups of seven and housed individually in stainless steel cages in a room temperature of 24 ± 1.5^0 C and 12 hour light 12 hour dark cycle. The rats were given feed and water ad libitum for 28 days; and weekly weight gains, feed consumption, and weight of wet and dry feces were recorded.

Blood Sample and Weight of Organs:

On day 27, rats were deprived of feed for 24 hours to take blood samples at a fasting stage. On the 28th day, the rats were anesthetized and sacrificed. A blood sample was collected from the heart of each animal with a 3mm heparinized syringe and centrifuged using a Beckman TJ-6R Centrifuge (Beckman Instruments, Palo Alto, Ca) for thirty minutes at 4^0C. The serum was separated and stored at -20^0C for analysis. All chemicals for albumin and creatinine analyses were obtained from Sigma Chemicals (St. Louis, MO) and Roche Diagnostics Systems (Roche, Somerville, NJ). Plasma albumin and plasma creatinine were analyzed using a COBAS FARA chemistry analyzer (Roche, Somerville, NJ). The heart, liver, kidneys, lungs, spleen, and testicles were excised, rinsed with distilled water, and weighed.

Fecal Samples:

All feces collected every morning throughout the experiment period were weighed, oven-dried for 48 hours at 100° C and reweighed to determine the fecal dry weight. The fecal samples collected during the last 7 days of the experiment for each animal were sealed in a sample bag and frozen at –20 ° C for analyses.

Kjeldahl Analysis and Protein Determination:

Prior to Kjeldahl analysis, the frozen samples were oven-dried for five hours at 60° C. The nitrogen and protein levels of feeds and fecal samples were determined following the macro Kjeldahl method (AOAC, 1990) using a KelTec 1030 Auto Analyzer (Tecator, Sweden) for digestion and distillation, and each sample was run in duplicate.

STATISTICAL ANALYSIS

Data were analyzed using the General Linear Model in the Statistical Analysis System (SAS, 1985). When p values were significant ($p \leq 0.05$), mean differences were identified using Duncan's Multiple Range Test (Steel and Torrie, 1980). All data are presented as means ± standard error of the mean (SEM).

RESULTS

Protein status was evaluated by body weight, organ weight, plasma albumin, and plasma creatinine concentrations. The apparent digestibility was determined by fecal dry matter protein (N × 6.25). The total wet fecal output, dry fecal matter, and apparent dry matter digestibility were also used as parameters to evaluate the growth performance of the experimental animals.

The albumin values are summarized in Table 3. Rats fed Gordons Gift 25% were significantly different from rats fed World Feeder 25%, Gordon's Gift 40%, Tifton 40% and World Feeder 40% without lysine. The albumin of rats fed 10% substitution level were not significantly different from rats fed the two control diets. The creatinine of Gordon's Gift 25% was significantly different from the control diet. Other substitutions were not different from each other or the 25% Gordon's Gift or the control diet.

TABLE 3.

Effect of different levels of Bermuda grass on serum albumin and creatinine in growing rats

Dietary treatment	Albumin (mg/dl)	Creatinine (mg/dl)
Control # 1 casein diet	4.10 ± 0.14^{ab}	0.75 ± 0.04^{a}
Gordon's Gift (10%)	$3.87 \pm 0.14.^{ab}$	0.63 ± 0.04^{ab}
World Feeder (10%)	3.81 ± 0.14^{ab}	0.71 ± 0.04^{ab}
Tifton (10%)	4.01 ± 0.14^{ab}	0.72 ± 0.04^{ab}
Gordon's Gift (25%)	4.25 ± 0.14^{a}	0.62 ± 0.04^{b}
World Feeder (25%)	3.74 ± 0.14^{b}	0.67 ± 0.04^{ab}
Tifton (25%)	3.90 ± 0.14^{ab}	0.67 ± 0.04^{ab}
Gordon's Gift (40%)	3.71 ± 0.14^{b}	0.66 ± 0.04^{ab}
World Feeder (40%)*	3.88 ± 0.15^{ab}	0.67 ± 0.04^{ab}
Tifton (40%)	3.70 ± 0.14^{b}	0.65 ± 0.04^{ab}
World Feeder no lysine	3.62 ± 0.14^{b}	0.69 ± 0.04^{ab}
Control #2 (12.5%) cellulose	3.99 ± 0.14^{ab}	0.72 ± 0.04^{ab}

Values in the same column which do not share the same superscripts are significant ($p \leq 0.05$)
* (n=6). One rat died

The mean body weights are summarized in Table 4. The mean body weights of rats fed 10% Bermuda grass were not significantly different from the controls. The mean body weights of rats fed World Feeder 40%, Gordon's Gift 40% and Tifton 40% were significantly different from control diet with 5% fiber and control diet with 12.5% cellulose (refined fiber). Mean body weigh of rats fed Gordon's Gift 25% and Tifton 25% were significantly different from the two control diets. In general, low body weight resulted as the substitution level increased for most substitution levels.

The data for organ weights are summarized in Table 4. Weights of organs were expressed as percent of body weight. Weights of heart, liver, spleen, and testicles of rats fed all levels of substitutions were not significantly different from the control. Nevertheless, on the higher Bermuda diets, the kidneys were significantly different from the control. The kidneys of rats fed World Feeder 40% without lysine, 40% Tifton, and 40% Gordon's Gift were significantly larger than the control with 5% fiber or the control with 12.5% fiber (refined cellulose). As reported by Garcia, et al, (1987), rats fed non defatted amaranth flour had damaged kidneys and these researchers attributed the damages to oxalate or antiphysiological factor in the amaranth grain. In this study, enlarged kidneys may have been caused by some factors in the Bermuda grass, however, we do not have any data to support our assumption. The lungs of rats fed Tifton 40% were significantly larger than rats fed Tifton 10% diet and Tifton 25% was different from Tifton 10%. Other substitution levels were not significantly different from each other or from the controls. In general, there was no pattern in response to the higher or lower substitutions diets in the lung data.

TABLE 4.

Final weights, and weights of organs of rats fed different levels of Bermuda grass with or without lysine supplement (total body weight). Mean ± SEM

Diets	Body Weight (g)	Heart % body weight	Liver % body weight	Kidney % body weight
Control	139.7 ± 6.8^{a}	0.50 ± 0.02^{a}	4.14 ± 0.20^{a}	0.88 ± 0.04^{c}
GG (10%)	134.2 ± 6.8^{ab}	0.49 ± 0.02^{a}	4.29 ± 0.20^{a}	0.99 ± 0.04^{bc}
WF (10%)	124.8 ± 6.8^{abc}	0.50 ± 0.02^{a}	4.55 ± 0.20^{a}	0.93 ± 0.04^{bc}
Tifton (10%)	139.4 ± 6.8^{a}	0.48 ± 0.02^{a}	4.26 ± 0.20^{a}	0.94 ± 0.04^{bc}
GG (25%)	111.4 ± 6.8^{cd}	0.53 ± 0.02^{a}	4.43 ± 0.20^{a}	1.03 ± 0.04^{abc}
WF (25%)	120.0 ± 6.8^{abcd}	0.49 ± 0.02^{a}	4.41 ± 0.20^{a}	1.05 ± 0.04^{abc}
Tifton (25%)	117.4 ± 6.8^{bcd}	0.50 ± 0.02^{a}	4.50 ± 0.20^{a}	1.04 ± 0.04^{abc}
GG (40%)	104.0 ± 6.8^{cd}	0.51 ± 0.02^{a}	4.60 ± 0.20^{a}	1.08 ± 0.04^{ab}
WF (40%)*	102.7 ± 7.3^{d}	0.52 ± 0.03^{a}	4.54 ± 0.21^{a}	1.03 ± 0.05^{abc}
Tifton (40%)	109.7 ± 6.8^{cd}	0.53 ± 0.02^{a}	4.62 ± 0.20^{a}	1.16 ± 0.04^{a}
WF 40% no lysine	100.6 ± 6.8^{d}	0.53 ± 0.02^{a}	4.77 ± 0.20^{a}	1.15 ± 0.04^{a}
Control 12.5% cellulose	140.6 ± 6.8^{a}	0.46 ± 0.02^{a}	4.44 ± 0.20^{a}	0.90 ± 0.04^{c}

Values in the same column which do not share superscripts are significant ($p \leq 0.05$)
* (n=6). One rat died

Table 4 Continued

Diets	Spleen % body weight	Lung % body weight	Testicle %body weight
Control	0.34 ± 0.02^{a}	0.67 ± 0.03^{abc}	1.59 ± 0.09^{a}
GG (10%)	0.32 ± 0.02^{a}	0.69 ± 0.03^{abc}	1.58 ± 0.09^{a}
WF (10%)	0.31 ± 0.02^{a}	0.67 ± 0.03^{abc}	1.50 ± 0.09^{a}
Tifton (10%)	0.32 ± 0.02^{a}	0.62 ± 0.03^{c}	1.49 ± 0.09^{a}
GG (25%)	0.32 ± 0.02^{a}	0.71 ± 0.03^{abc}	1.76 ± 0.09^{a}
WF (25%)	0.29 ± 0.02^{a}	0.69 ± 0.03^{abc}	1.59 ± 0.09^{a}
Tifton (25%)	0.33 ± 0.02^{a}	0.77 ± 0.03^{a}	1.46 ± 0.09^{a}
GG (40%)	0.30 ± 0.02^{a}	0.67 ± 0.03^{abc}	1.69 ± 0.09^{a}
WF (40)*	0.31 ± 0.03^{a}	0.67 ± 0.04^{abc}	1.56 ± 0.10^{a}
Tifton (40%)	0.36 ± 0.02^{a}	0.75 ± 0.03^{ab}	1.57 ± 0.09^{a}
WF 40% no lysine	0.30 ± 0.02^{a}	0.68 ± 0.03^{abc}	1.78 ± 0.09^{a}
Control 12.5% cellulose	0.31 ± 0.02^{a}	0.64 ± 0.03^{bc}	1.51 ± 0.09^{a}

Values in the same column which do not share superscripts are significant ($p \leq 0.05$)
* (n=6). One rat died

TABLE 5.

Effect of different level of Bermuda grass and lysine supplement on total fecal output, fecal protein, and dry matter (Mean ± SEM)

Diet	Feed Intake (g)	Wet Fecal Output (g)	Fecal Dry Matter (g)	Fecal Protein (%)	Total fecal protein (g)
Control	308.71± 4.86^{e}	32.29 ± 5.13^{e}	29.71 ± 4.59^{e}	11.89 ± 0.26^{a}	3.53
GG (10%)	311.71 ± 4.86^{e}	45.00 ± 5.03^{ed}	45.00 ± 4.59^{d}	11.76 ± 0.26^{a}	5.29
WF (10%)	314.00 ± 4.86^{e}	50.16 ± 5.13^{d}	42.14 ± 4.59^{ed}	11.29 ± 0.26^{a}	4.75
Tifton (10%)	314.28 ± 4.86^{e}	46.28 ± 5.13^{ed}	44.71 ± 4.59^{d}	11.45 ± 0.26^{a}	5.12
GG (25%)	324.14 ± 4.86^{e}	98.00 ± 5.13^{b}	87.85 ± 4.59^{b}	10.17 ± 0.26^{b}	8.93
WF (25%)	317.29 ± 4.86^{e}	98.00 ± 5.13^{b}	91.00 ± 4.59^{b}	9.22 ± 0.26^{c}	8.39
Tifton (25%)	338.14 ± 4.86^{d}	96.71 ± 5.13^{b}	90.14 ± 4.59^{b}	9.00 ± 0.26^{c}	8.11
GG (40%)	381.57 ± 4.86^{b}	148.42 ± 5.13^{a}	140.85 ± 4.59^{a}	8.17 ± 0.26^{d}	11.50
WF (40)*	366.16 ± 5.25^{c}	150.20 ± 5.54^{a}	132.00 ± 4.96^{a}	9.09 ± 0.28^{c}	11.99
Tifton (40%)	394.29 ± 4.86^{b}	160.00 ± 5.13^{a}	143.71 ± 4.59^{a}	8.17 ± 0.26^{d}	11.74
WF 40% no lysine	397.00 ± 4.86^{a}	152.14 ± 5.13^{a}	142.86 ± 4.59^{a}	8.57 ± 0.26^{cd}	12.24
Control 12.5% cellulose	349.85 ± 4.86^{d}	71.85 ± 5.13^{c}	68.00 ± 4.59^{c}	7.21 ± 0.26^{e}	4.89

Values in the same column with different superscripts are significant ($p \leq 0.05$)
* (n=6). One rat died

The data for the feed intake is summarized in Table 5. Rats fed 10% substitution levels, Gordon's Gift 25%, and World Feeder 25% were not significantly different from the control diet with 5% fiber. The control with 12.5% fiber, however, was significantly different from either the control with 5% fiber or the other substitution levels. Feed intake of rats fed Gordon's Gift 40%, and Tifton 40% were significantly different from those fed World Feeder 40%. Rats fed 40% Bermuda grass substitution from the three cultivars, in general, consumed more feed during the 28 day experimental period.

The wet fecal output data is summarized in Table 5. The wet fecal output of the rats fed 10% Bermuda grass were not significantly different from the control with 5% fiber. The wet fecal output of Gorgon's Gift 25%, World Feeder 25% and Tifton 25% were significantly different from the controls and the 40% Gordon's Gift, World Feeder and Tifton. The control with 12.5% cellulose (refined fiber) was significantly different from the control with 5% fiber. The wet fecal output of rats fed 40% Bermuda grass substitution, however, was significantly higher from both controls, the 25% substitutions, and 10% substitutions. The wet fecal output of rats fed World Feeder 10% was significantly different from the two controls, the 25%, and the 40% substitution levels. The wet fecal output increased as the substitution levels increased for all the cultivars

The fecal dry matter of rats fed Gordon's Gift 10% and Tifton 10% were significantly different from both controls. Fecal dry matter of rats fed 25% of the three cultivars were significantly different from the two controls or the 40% substitutions. The fecal dry matter increased as the substitution level increased, and there were no cultivar differences.

The data for the fecal protein is summarized in Table 5. Percent fecal protein (N x 6.25) of Bermuda grass 10% substitutions was not significantly different from the control diet with 5% fiber. Diets with 25%, and 40% substitution had a low percentage fecal protein. The total fecal protein excreted, however, is proportional to the fecal dry matter, that the higher Bermuda grass levels had high total fecal protein excretions. The highest fecal dry matter output and total fecal protein is the highest for 40% World Feeder without lysine. The low digestibility of protein of Bermuda grass could probably be explained by high dry matter output accompanied by high total fecal protein. Slow weight gain of rats fed the 25% and 40% Bermuda grass substitution diets may be attributed to the dilution factor. The control diets which contained 5% and 12.5% fiber are similar in total fecal protein output.

DISCUSSION

The results of this experiment showed that rats fed high Bermuda grass substitution levels (25%, 40%) had lower body weight. The Bermuda grass cultivars are not poor sources of protein when incorporated into the diets of rats even without supplementing with the limiting amino acid, lysine, but the high fiber has interfered with the protein utilization and resulted in high fecal dry matter output The fecal dry matter of the rats fed 25% and 40% Bermuda grass was high and the total fecal protein was also high compared to the control group which indicates that protein was not utilized. Studies by Oliveira, et al (1994), Cornu and Delpeuch (1981) showed that higher dry fecal matter was associated with high fecal nitrogen and in this study, total fecal protein output was in proportion with fecal dry matter output which agreed with their findings.

The plasma albumin content in the blood is a measure of the assimilation of protein in the diet. The albumin of rats fed all levels of Bermuda grass was not different from the control. In studies by Straus, et al, (1994), decreased concentration of circulating albumin in protein-restricted diets was caused by a decrease in albumin synthesis in the liver. This suggests that the rats did not utilize the amino acids; perhaps, they were eliminated in the feces before they were utilized by the body.

Rats fed on higher Bermuda grass substitutions tended to have large kidneys and variably larger lungs. In general, there was no pattern in response to the high or low substitution diets in the lung data. Some adverse effects on kidneys, lungs, and liver implicated to be a result of the oxalate have also been observed in rats fed amaranth grain which was implicated as a result of the oxalate (Garcia, et al, 1987). Perennial grasses have a high level of oxalate which affects the kidney of monogastric animals, and the greener or the younger the grass the higher the oxalate level (Libert and Franceshi, 1987). In addition, toxins, and mycotoxins in forage grasses and their effects on monogastric animals have been reported by Cheekes (1994) The result of this study suggest that Bermuda grass has antinutritive properties causing inhibition of rat growth due to interference with metabolism which leads to high dry matter in feces accompanied by low retention of nitrogen. There was no clear relationship between nitrogen retention and fecal dry matter output in the higher levels of Bermuda grass substitutions.

This experiment demonstrated that the 10% Bermuda grass substitution diet was not different from the control diet and adequately support growth. The weight of the organs is a reflection of the weight gained, and in this respect a certain tendency can be found. Feeding the 25% and 40% Bermuda grass diet, however, resulted in slow growth. The mean values of organs of the rats fed the higher Bermuda grass substitutions weighed more than those rats fed either 10% Bermuda grass or the control diets. Exception is that rats on 25% and 40% Bermuda grass diet, had large kidneys. Rats fed the 40% substitution levels with or without lysine ate more feed showing that rats self-regulated energy intake despite the variation in energy density of the diets. Rats on the high level of Bermuda grass had also more fecal output and dry matter compared to the control and these animals had poor hair growth and hair color change, and appeared malnourished indicating poor nutrient utilization. Supplementation with the limiting amino acid did not bring improvement of growth performance of rats. Further research is recommended to see if there is negative vitamin or mineral interaction with the higher Bermuda grass substitution.

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CHAPTER VI

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This research evaluated the nutritional adequacy and sensory quality of three Bermuda grass cultivars (Gordon's Gift, World Feeder, and Tifton). We conducted two feeding experiments and one sensory evaluation to examine the nutritional adequacy of the Bermuda grass. The first experiment utilized rats to compare the Protein Efficiency Ratio of a Bermuda grass diet at 10% substitution level to a casein control diet. The second experiment was designed to study the same effects examined in the first experiment but using three levels of substitution: 10%, 25% , and 40%. The third experiment was a human sensory evaluation of breads made with 5% substitutions of all three cultivars. The objectives of these studies were to evaluate the nutritional adequacy of three cultivars of Bermuda grass (Gordon's Gift, World Feeder, and Tifton) using weanling rats, and evaluate the sensory quality of breads made with 5% Bermuda grass flour substitution. Six hypotheses related to the study were made prior to starting the experiments. Each hypothesis will be addressed individually.

Hypothesis One stated that the Protein Efficiency Ratio of the three Bermuda grass cultivars at 10% level of substitution would not significantly differ from the control. There were no significant differences in calculated values of protein efficiency ratio of diets at 10% substitution level and the control diet. Based on these results, the first null hypothesis was not rejected.

Hypothesis Two stated that there will be no differences in the weights of rats fed Bermuda grass at 10%, 25%, and 40% substitution levels and the control. Weights of rats fed the different Bermuda grass substitution did differ significantly for the 25 % and 40% levels (Chapter V). Based on these results, the second null hypothesis was rejected.

Hypothesis Three stated that there will be no significant differences in serum albumin and serum creatinine in rats fed 10%, 25%, and 40% substitution of Bermuda grass and the control diet (Chapter V). At least one difference was observed. Based on these results, the third null hypothesis was rejected.

Hypothesis Four stated that there will be no significant differences in organ weights of rats fed different experimental diets. Organ weights of rats fed different experimental diets did differ significantly for kidneys and lungs (Chapter V). Based on these results, the fourth null hypothesis was rejected.

Hypothesis Five stated that there will be no significant differences in dry matter digestibility between rat diets at 10%, 25% and 40% Bermuda substitution and the control diet. Dry matter digestibility did differ significantly from the control (Chapter V). Based on these results, the null hypothesis was rejected.

Hypothesis Six stated that there will be no significant differences in color, flavor, texture, and overall acceptability of breads made with 5% Bermuda grass flour and bread made with all purpose flour. Color and overall acceptability did differ significantly from the control bread (Chapter IV). Based on these results, the sixth hypothesis was also rejected.

CONCLUSIONS

The Protein Efficiency Ratio study (Experiment I) showed no differences in weight gains or organ weights (liver, kidney, lung and testicle) of rats fed any of the 10% substitution or the control. Heart and spleen of rats fed World Feeder at 10% substitution level were significantly different from the control. The PER values of the three Bermuda grass cultivars were similar to the control.

Experiment II showed differences in body weight, kidneys, lungs, albumin, creatinine, total fecal output, dry matter digestibility and feed consumption of rats at different levels of substitutions. The results of the first experiment and the second experiment confirmed that, at the 10% levels of substitution, the body weight of the rats did not differ from the control diets.

At the higher levels of substitutions (25% and 40%) of all the cultivars, the rats showed significantly reduced body growth. The rats fed high levels of Bermuda grass had greater fecal output and higher total fecal protein excretion, the highest total fecal protein was from the 40% World Feeder without lysine.

The kidneys in the second experiment were significantly different from the control diet. In general, for most organs, the smaller animals had larger organs and were not significantly different from the control when organs were expressed as a percent of body weight. The kidneys of rats fed the 40% were significantly larger than both the control and the control with added fiber. The lungs of rats fed 25% Tifton were significantly larger than the control with 12.5% fiber. In general, there is no pattern in response to the high or low substitution diets in the lung data.

The protein digestibility decreased as the substitution level increased. Bermuda grass contains about 30 percent fiber. Those diets with less Bermuda fiber differed significantly from diets with 25% and 40% substitutions which showed that the increase in fiber decreased the digestibility of protein, which increased the total fecal protein output

The two animal experiments demonstrated that the rats fed 10% Bermuda grass substitution diets were comparable to the rats fed the control diet. Rats fed 25% and 40%, Bermuda grass, however, had slow growth which resulted in reduced weight. It was also observed that those animals with 40% Bermuda grass with or without added lysine had poor hair growth and hair color change and appeared malnourished indicating poor protein, vitamin, or mineral utilization.

As shown from the fecal data, the fecal output with the 25% and 40% substitution levels with or without added lysine, increased as the substitution level increased. There were no cultivar differences for fecal output. Rats fed 40% Bermuda rations, with or without lysine consumed more feed during the experimental period. The percent total fecal protein excreted increased as the Bermuda grass substitution increased. This finding is similar to data reported in the literature, that Bermuda grass fiber reacted similarly to different kinds of fiber on fecal nitrogen excretion and total fecal protein.

Five percent Bermuda grass in breads was the maximum which could be used for human consumption without an offensive grassy odor. The Bermuda grass flour substitution of 10% resulted in reduced volume, strong grassy odor, and very coarse texture for all three cultivars. Bread made with 5% Gordon's Gift was not significantly different from the control bread in flavor, off flavor, texture, appearance, and overall acceptability.

RECOMMENDATIONS

Further studies are needed where Bermuda grass flour is used in various human food preparations, such as quick bread, yeast bread, and pasta to improve the nutritional quality of the products. The effects of other ingredients such as carrots, spinach, zucchini, nuts, fruits, herbs and other natural flavorings, which may enhance the acceptability of products containing the Bermuda grass also need to be evaluated. The development of such products would also be of special benefit to people who need to increase their intake of dietary fiber.

We did preliminary studies on pasta and cakes. In today's market, different products are sold with different health claims. The color and odor of the Bermuda grass could be masked using different types of sauces and spices. The pasta and other noodles could be introduced as gourmet foods for the pasta consuming population though the application may be limited. Some companies incorporate wood pulp in bread for human consumption to reduce energy. Therefore, Bermuda grass as well could be used to reduce energy in breads, cakes or muffins. As soy extracts are used in human food as well as animal feed, it is also a possibility that Bermuda grass protein extract could be produced to be used as protein source for human consumption, and the residue could be used for animal feed. Finally, further studies on animals are required before incorporating the Bermuda grass into human food to provide a clear view regarding any nutrient interactions between the Bermuda grass and other food /nutrient components.

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APPENDICES

APPENDIX A

JOURNAL OF FOOD QUALITY

(GUIDE TO AUTHORS)

GUIDE FOR AUTHORS

Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

Page one should contain: the title which should be concise and informative; the complete name(s) of the author(s); affiliation of the author(s); a running title of 40 characters or less; and the name and mail address to whom the correspondence should be sent.

Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

The main text should begin on page three and will ordinarily have the following arrangement:

Introduction: This should be brief and state the reason for the work in relation to the field. It should indicate what new contribution is made by the work described.

Materials and Methods: Enough information should be provided to allow other investigators to repeat the work. Avoid repeating the details of procedures which have already been published elsewhere.

Results: The results should be presented as concisely as possible. Do not use tables *and* figures for presentation of the same data.

Discussion: The discussion section should be used for the interpretation of results. The results should not be repeated.

In some cases it might be desirable to combine results and discussion sections.

References: References should be given in the text by the surname of the authors and the year. *Et al.* should be used in the text when there are more than two authors. All authors should be given in the References section. In the Reference section the references should be listed alphabetically. See below for style to be used.

DEWALD, B., DULANEY, J.T. and TOUSTER, O. 1974. Solubilization and polyacrylamide gel electrophoresis of membrane enzymes with detergents. In *Methods in Enzymology*, Vol. xxxii, (S. Fleischer and L. Packer, eds.)pp. 82–91, Academic Press, New York.

HASSON, E.P. and LATIES, G.G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57*, 142–147.

ZABORSKY, O. 1973. *Immobilized Enzymes*, pp. 28–46, CRC Press, Cleveland, Ohio

Journal abbreviations should follow those used in *Chemical Abstracts*. Responsibility for the accuracy of citations rests entirely with the author(s). References to papers in press should indicate the name of the journal and should only be used for papers that have been accepted for publication. Submitted papers should be referred to by such terms as "unpublished observations" or "private communication." However, these last should be used only when absolutely necessary.

Tables should be numbered consecutively with Arabic numerals. Table titles should appear as below. Type tables neatly and correctly as tables are considered art and are not typeset. The temperature degree symbol should not be used (4C) in tables or text.

TABLE 1.
ACTIVITY OF POTATO ACYL-HYDROLASES ON NEUTRAL LIPIDS, GALACTOLIPIDS, AND PHOSPHOLIPIDS

Description of experimental work or explanation of symbols should go below the table proper.

Figures should be listed in order in the text using Arabic numbers. Figure legends should be typed on a separate page. Figures and tables should be intelligible without reference to the text. Authors should indicate where the tables and figures should be placed in the text. Photographs must be supplied as glossy black and white prints. Line diagrams should be drawn with black waterproof ink on white paper or board. The lettering should be of such a size that it is easily legible after reduction. Each diagram and photograph should be clearly labeled on the reverse side with the name(s) or author(s), and title of paper. When not obvious, each photograph and diagram should be labeled on the back to show the top of the photograph or diagram.

Acknowledgments: Acknowledgments should be listed on a separate page.

Short notes will be published where the information is deemed sufficiently important to warrant rapid publication. The format for short papers may be similar to that for regular papers but more concisely written. Short notes may be of a less general nature and written principally for specialists in the particular area with which the manuscript is dealing. Manuscripts which do not meet the requirement of importance and necessity for rapid publication will, after notification of the author(s), be treated as regular papers. Regular papers may be very short.

Standard nomenclature as used in the biochemical literature should be followed. Avoid laboratory jargon. If abbreviations or trade names are used, define the material or compound the first time that it is mentioned.

EDITORIAL OFFICE: Dr. J.J. Powers, 205 Glenwood Drive, Athens, Georgia 30606-4617

APPENDIX B

GUIDE TO AUTHORS

(ECOLOGY OF FOOD AND NUTRITION)

Notes for Contributors for ECOLOGY OF FOOD AND NUTRITION

SUBMISSIONS

Papers should be typed on good quality paper with double spacing and wide (3 cm) margins, using one side only, and submitted in triplicate to the appropriate editor.

For food systems, food composition, dietary intake, and nutritional status:
Harriet V. Kuhnlein
School of Dietetics and Human Nutrition
Macdonald Campus of McGill University
21,111 Lakeshore Road
Ste. Anne de Bellevue
PQ, Canada H9X 3V9

For human biology, clinical nutrition, and food science:
Peter L. Pellett
Dept. of Food Science and Nutrition
College of Food and Natural Resources
University of Massachusetts at Amherst
Amherst, Massachusetts 01003, U.S.A.

For social science aspects of food and nutrition:
Christine S. Wilson
P.O. Box 3178
Annapolis, Maryland 21403-0178, U.S.A.

One copy of the typescript must be the original 'top' copy; photocopies or clear carbon copies are acceptable for the others. Pages of each copy must be sequentially numbered. Use underlines only for emphasis and where italics are intended. Papers are accepted only in English, and the maximum preferred length is 7,500 words. Manuscripts do not receive further copyediting by the publisher or typesetter; authors should ensure before submission that papers are correct in style and language.

For anonymity in the review process, names, affiliations for all authors and a complete mailing address for the lead author should appear on a separate title page. This page should also note to which author all reader correspondence should be addressed. Indicate for the typesetter which author will check proofs. Each paper requires an abstract of 150 words or less and a set of no more than ten index terms (key words) suitable for use in a computer database. As a group, these should characterize the paper. Authors should also provide an abbreviation of the paper's title (no more than 35 characters) for use as a running head. Include acknowledgements under a separate head at the end of the paper but before the reference list.

The publisher encourages authors to submit accepted manuscripts on computer disks. Word Perfect 5.1 is the preferred software, but other software and formats are acceptable, as are all sized disks. Authors must enclose a printed copy of the manuscript (in triplicate) along with the disk. All disks should be marked with the name of the software package that was used and the file name. Disks will be returned to the author with page proofs.

Submission of a manuscript is taken to imply that the paper represents original work not previously published, is not being considered elsewhere for publication, and if accepted for publication, will not be published elsewhere in the same form, in any language, without the consent of the publisher. It is also assumed that the author has obtained all necessary permissions to include in the paper items such as quotations, reprinted figures, results of government-sponsored research, etc. It is a condition of acceptance for publication that the publisher acquires copyright of the paper throughout the world.

FIGURES

All figures should be numbered with consecutive Roman numbers, have descriptive captions, and be mentioned in the text. Keep figures separate from the text, but indicate an approximate position for each in the margin. A list of figure captions, with relevant figure numbers, should be typed on a separate sheet and included with the manuscript. Capitalize only the first letter of the first word in figure captions.

All figures must be of high enough standard for direct reproduction. Line drawings should be prepared in black (India) ink on white (or tracing) paper, with all lettering and symbols included. Alternatively, good sharp photoprints (or 'glossies') are acceptable. Photographs must be good glossy original prints of maximum contrast. Using pencils, clearly label each figure with the author's name and figure number: indicate top where this is not obvious. Redrawing or retouching of unusable figures will be charged to authors. Figures should be submitted as close to final size as possible to minimize reduction; figures will be sized to a maximum of 12.5 cm wide or less, according to subject matter. Artwork should be labeled so that after any reduction, lettering does not exceed 4 mm in height.

COLOR PLATES:

Whenever the use of color is an integral part of the research or where the work is generated in color, the journal will publish the color illustrations without charge to the authors. Reprints in color will carry a surcharge. Please write to the Editor for details.

MATHEMATICAL EXPRESSIONS:

Whenever possible, mathematical expressions should be typewritten, with subscripts and superscripts clearly shown. It is helpful to identify unusual or ambiguous symbols in the margin when they first occur. To simplify typesetting, please use the 'exp' form of complex exponential functions, fractional exponents instead of root signs, and the solidus (/) to simplify fractions—e.g. exp × ½. Equations must be displayed exactly as they should appear in print. Underline mathematical symbols to indicate italics; bold symbols should be underlined with a wavy line.

TABLES:
Tables should be typed on separate sheets, numbered consecutively with arabic numerals, and have a short descriptive caption at the top. Capitalize the first letter of the first word in the table captions. Tables may be placed in the typescript immediately following the page of text with which they should be printed, or they may be grouped separately. In the latter case, indicate in the text where the tables are to appear. Avoid the use of vertical rules. Each table should be mentioned in the text. Extensive and/or complex tables will be reproduced as illustrations. Therefore, they should be typed carefully and in the exact format desired. Computer printouts will normally be reproduced as illustrations.

REFERENCES:
References are indicated in the text by Harvard (name and date) system: Either 'Recent work (Smith, 1975) . . .' or 'Recently Smith (1975) has found . . .' The full list of references should be collected at the end of the paper in alphabetical order, and set out in the manner described and illustrated below. Note that in all cases, the initials of the first author (only) are placed after the name.

Papers:
Author's name, year (in parentheses), period. Paper title (no quotation marks), period. Name of journal (underlined), volume number (underlined in a wavy line), inclusive page numbers.

Books:
Author's name, year (in parentheses), period. Book title (underlined), period. Publisher, location, period. Chapter or page numbers.

Edited collections:
Author's name, year (in parentheses), period. Paper title (no quotation marks), period. 'In' followed by editor's name (Ed.), book title (underlined), period. Publisher, location, period. Volume number, chapter number, inclusive page numbers. Examples:

Annegers, J.F. (1974). Protein quality of West African foods. Ecology of Food Nutr., 3, 125–130.

Mohsenin, N.N. (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach, New York, Chapter 7, pp. 309–382.

Ruffner, J.D., and W.W. Steiner, (1973). Evaluations of plants for use in critical sites. In R.J. Hutnik and G. (Eds.), Ecology and Reclamation of Devastated Land. Gordon and Breach, New York, Vol. 2, chapter v-1, pp. 3–12.

TEXT HEADINGS:
Type first-level headings in capital letters over to the left; begin the text on the following line. Second-level headings should be typed in lower case letters but with all main words capitalized; underline the heading and start the text on the next line. For third-level headings, only the first letter should be a capital; underline, then run on the text after three spaces.

PROOFS:
Authors will receive page proofs by airmail. Corrected proofs must be returned to the printer within 48 hours. Authors' alterations in excess of 10% of the original composition cost will be charged to authors. There are no publication page charges to individuals or institutions.

REPRINTS:
The publisher will supply 25 free reprints of each paper to the first named author. Further reprints may be ordered by completing the appropriate form sent with proofs. Free reprints are sent by surface mail, but ordered reprints by air mail. The two sets will not therefore normally arrive together.

BOOK REVIEWS:
Publishers are invited to submit books for review in Ecology of Food and Nutrition. These should be sent to the appropriate editor (see Submissions).

APPENDIX C

PROVISIONAL APPROVAL FORM FOR ANIMAL STUDIES

PROVISIONAL APPROVAL FORM FOR ANIMAL STUDIES
INSTITUTIONAL ASSURANCE NUMBER A3722-1

Principle Investigator Lea Ebro & Subiah Sangiah Protocol # 355

Dept. Nut. Sci. & Physio. Campus Address NSCI 425 HES; CVM 274

Project Title Evaluation of "World Feeder" & "Gordon Gift" Bermuda Grass

Sponsoring Agency OCAST New X Continuing ____

Animal Species Rats Animal Number 54-60

EFFECTIVE PERIOD WILL BE FOR THREE YEARS FROM FINAL APPROVAL DATE. PROJECT IS SUBJECT TO REVIEW BY THE IACUC COMMITTEE AT ANY TIME.

PLEASE REFER TO ABOVE PROTOCOL NUMBER WHEN PLACING ANIMAL ORDERS.

This project has been reviewed in accordance with Federal Regulations and guidelines, specifically considering the following:

✓ a. The type and dosage of anesthetic, analgesic and/or tranquilizing agent(s) to be used on animal subjects during the proposed studies are appropriate to relieve all unnecessary pain and distress, or adequate explanation and justification for not using these agents is provided.

✓ b. Surgery, if performed, will be accomplished following aseptic procedures if animals are allowed to recover from anesthesia or adequate explanation and justification for not following aseptic procedures is provided.

✓ c. Measures employed to alleviate pain and distress following surgery or other procedures are appropriate or adequate explanation and justification for not providing such care is included.

✓ d. The method of euthanasia employed is in accordance with the 1993 Report of the AVMA Panel on Euthanasia.

Action Taken:

✓ Approval is recommended.

____ Disapproval is recommended for reason stated in the attached memorandum.

____ Approval is contingent upon making changes stipulated in the attached memorandum.

[signature] Signature Date 6/15/93

APPENDIX D

INSTITUTION REVIEW BOARD FOR HUMAN RESEARCH

(IRB)

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
FOR HUMAN SUBJECTS RESEARCH

Date: 08-17-93 IRB#: HE-94-007

Proposal Title: EVALUATION OF "WORLD FEEDER" AND "GORDON'S GIFT" BERMUDA GRASS

Principal Investigator(s): Lea L. Ebro, Tideg Debessu

Reviewed and Processed as: Expedited

Approval Status Recommended by Reviewer(s): Approved

APPROVAL STATUS SUBJECT TO REVIEW BY FULL INSTITUTIONAL REVIEW BOARD AT NEXT MEETING.
APPROVAL STATUS PERIOD VALID FOR ONE CALENDAR YEAR AFTER WHICH A CONTINUATION OR RENEWAL REQUEST IS REQUIRED TO BE SUBMITTED FOR BOARD APPROVAL. ANY MODIFICATIONS TO APPROVED PROJECT MUST ALSO BE SUBMITTED FOR APPROVAL.

Comments, Modifications/Conditions for Approval or Reasons for Deferral or Disapproval are as follows:

Signature: John H. Wycoff Date: August 26, 1993

Chair of Institutional Review Board

APPENDIX E

AMINO ACID COMPOSITION OF BERMUDA GRASS

Amino Acid Comparison (m/g)

Amino Acid	Wheat[1]	Gorgon's Gigt[2]	World Feeder[2]	Tifton[2]	Midland[2]	Hardie[2]
ASP	5.4	22.98	22.82	24.12	22.77	24.12
GLU	34.2	12.86	11.88	14.04	13.64	14.30
SER	5.0	4.45	3.95	4.74	4.44	4.89
HIS	2.4	1.63	1.75	1.85	2.07	1.71
GLY	4.5	4.20	3.98	4.58	4.66	4.44
THR	3.1	4.05	3.72	4.33	4.25	4.72
ALA	3.8	6.80	6.08	7.08	7.04	7.12
ARG	5.0	4.93	4.40	5.26	5.34	5.19
TYR	3.2	2.55	2.31	2.72	2.88	2.63
VAL	4.7	5.13	4.44	5.55	6.05	5.40
MET	1.7	1.68	1.50	1.90	1.93	2.01
PHE	5.0	4.93	4.66	5.36	5.48	5.22
ILEU	3.8	3.37	2.96	3.64	4.23	3.62
LEU	7.1	6.62	6.08	7.37	7.80	7.09
LYS	2.8	3.55	3.45	4.46	4.86	4.17
PRO	11.0	1.45	2.02	3.03	2.60	3.39

[1] Nutrition Reports International. 1986, Vol. 35, p 969.
[2] Goat Research Institute, Langston University, Langstone, Oklahoma, 1994.

2

VITA

Debessu Tideg

Candidate for the degree of

Doctor of Philisophy

Thesis: NUTRITIONAL EVALUATION OF BERMUDA GRASS

Major Field: Human Envirnmental Sciences: Food Science

Biographical:

Personal Data: Born in Gonder, Ethiopia, April 13, 1948, the daughter of Mrs. Woleteyesus Gebreyesus and the late Captain Debessu Roma.

Education: Received high school diploma from Harar Teacher Training Institute, Ethiopia, July, 1967; received two-year college diploma from Alemaya College of Agriculture, Ethiopia, July, 1972; received Bachelor of Science degree in Home Economics from Oklahoma State University, December, 1985; Received Master of Science degree in Food, Nutrition and Institution Administration from Oklahoma State University, December, 1986; completed requirements for degree of Doctor of Philosophy at Oklahoma State University in May, 1997.

Professional Experience: Graduate Research Associate, August 1992 to December 1996. Graduate Teaching Assistant, January 1993 to May 1996; Nutritional Sciences Department, Oklahoma State University; Senior Expert, Ministry of Coffee and Tea Development; March 1988 to August 1992; Expert, Ministry of Urban Development from March 1987 to February 1988; Graduate Research Assistant, January 1986 to December 1986; Laboratory Technician, May, 1985 to December 1985, Food, Nutrition and Institution Administration, Oklahoma State University; Instructor, College of Teacher Education, Addis Ababa, Ethiopia, 1976 to 1984; Instructor, Kotebe Teacher Training Institute, Addis Ababa, Ethiopia from 1972 to 1976.