BIOASSAY OF GRAIN SORGHUM FRACTIONS SUPPLEMENTED WITH VITAMINS AND MINERALS

by

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CHAPTER I

INTRODUCTION

Purpose and Scope of the Thesis

One of the most urgent problems facing the world today is the need to increase food production and distribution. The number of people hungry in the world at any time depends on the definition of hunger, but it is well known that great percentages of people are suffering from deficiency of calories and of good quality protein.

Increased production of food to supply calories and good quality protein is essential. Better use of available food and new sources of food will be necessary to solve these problems.

Cereal grain, which provides the calories to sustain many of the world's peoples today, could also provide adequate protein if the efficiency ratio of the protein were increased. Grain sorghums seem particularly promising as a supplier of nutrients because they can be grown efficiently in most areas of the world. As is true for all other cereal grains, the major limiting factor in nutritional quality of protein in grain sorghum is deficiency in lysine, an essential amino acid.

To provide a total food, supplementation with vitamins and minerals, as well as a source of the limiting amino acids, is necessary. This study was to determine the nutritional value of grain sorghum as a source of total protein in the diet, supplemented with vitamins and minerals but not with another source of protein. This study will be the basis for further research in supplementation of grain sorghum in order to produce a more complete food.

Composition of grain sorghum has been found to vary with variety, location, maturity, and fertilization. Therefore, the results of this or any study are not directly applicable to all other grain sorghum. Commercial grain was used because it is currently available not only for research but also in quantities suitable for mass use. This study should provide an indication of the value of grain sorghum as a total food or as a component of vegetable protein mixtures.

Hypotheses

1. Grain sorghum fractions supplemented with vitamins and minerals will produce greater rate of growth in rats with better feed efficiency than unsupplemented grain sorghum.

2. Bran and germ fractions of the grain sorghum kernel, which have approximately the same protein level, will produce comparable rat growth.

3. A difference in protein digestibility occurs in the different fractions of the grain sorghum kernel.

4. The germ fraction of the grain sorghum kernel,

when cooked, will produce greater growth response than the untreated germ.

5. Bran and germ fractions supplemented with vitamins and minerals will not produce a growth response equal to that produced by a diet of adequate protein.

Review of Previous Research

History and Characteristics of Grain Sorghum

The history of grain sorghum goes back to ancient civilizations of Africa and Asia where some investigators have found sorghum dated as early as 2200 B.C. (1,2). Varieties of grain sorghum from all parts of the world were first introduced in the United States by the United States Department of Agriculture in 1857 (3); and, in the same year, sorghum seed was sent to Texas by the Indian Services (4).

Sorghum applies to a group of plants in the grass family, *Sorghum vulgare*. The economically most important type produces large, starchy seeds and is known by various names throughout the world: milo maize, kafir corn or gyp corn in the United States; jowar in India. Most varieties now grown in the United States are about four feet tall, with leaves and stalks resembling corn (1). The grains, flattened spheres approximately 4.0 mm long by 3.5 mm wide by 2.5 mm thick, are borne in clusters on a compact, terminal head (5).

Although grown throughout the world at various elevations and temperatures, grain sorghums are especially important where rainfall is limited or uncertain. The plant is more efficient than corn in absorbing and retaining moisture; it seems to have the ability to remain dormant during dry spells and grow again when watered (1). Drought resistance is promoted by leaves which inroll during moisture privation (6). With high rainfall or under irrigated conditions, grain sorghum thrives and produces much higher yields (7). Use of fertilizers and irrigation commonly produces 6,000 pounds to the acre, but yields of 10,000 pounds are not uncommon (8).

Grain sorghum, the world's third most important food grain, is grown on all continents (6). One-eighth of the farmers in the United States raise grain sorghums. Four-fifths of this acreage is located in three states: Texas, Kansas, and Nebraska (8). In 1967 more than 700 million bushels of sorghum was produced in the United States. Sorghum is the number one grain crop in Texas, with an annual cash value exceeding 300 million dollars (5).

During the past thirty years, an extensive sorghum breeding program has been carried on in the United States to produce greater yields (6). However, quality rather than quantity yield could well be the objective of further research (6,9). Most needed improvements center on those properties affecting the value of grain sorghum as food for humans (5).

Milling of grain sorghum provides fractions of the whole grain which are suitable for various purposes. Wet-milling sorghum grain produces starch, oil, and feed products, while dry-milling products include crude flour, brewers' grits, and dog food grits (5). Harden (10) has suggested that the most nutritious fraction should be used for food or feed, since her results showed that a fraction could produce better animal growth than whole grain. Remaining fractions would be useful for industrial purposes, thereby raising the economic value of the grain. At the present time most of the grain sorghum produced in the United States is used for feeding animals. Only 3 to 5 per cent is used for human food and industrial purposes (5).

Even though the primary use of grain sorghum in the United States is for domestic animal feed, the grain is used in other parts of the world as food for humans. Bantus in South Africa eat kafir mush (6). In northern China millet and sorghum are staples in human diets. Furthermore, the Chinese custom of mixing sorghum with other cereals and legumes improves the nutritive value of their diet (7). But the acceptance of a food is as important in its utilization as is its production. Recipes using grain sorghum were published by the United States government and several state agencies during the first World War (6). During World War II, a booklet of bread recipes substituting two-thirds grain sorghum flour for wheat flour was published by Texas

Technological College's Department of Foods and Nutrition (11). Boren (12) has more recently suggested that grain sorghum might be acceptable on the American market as a major component in a mix for making various hot breads in which grain sorghum is combined with wheat flour in a 3:1 ratio. However, grain sorghum will not become an important part of the American diet as long as more familiar grains are plentiful.

Composition of Grain Sorghum

The composition of grain sorghum has been studied and reported by several workers. In Table 1, Watson's (1) values for whole grain are compared with those reported by other workers for the whole grain and fractions. Hubbard, Hall, and Earle (13) reported the proximate analysis of fractions of five different varieties of sorghum which were analyzed for starch, protein, fat, and ash. Five varieties were analyzed by Stringfellow and Peplinski (14) with analyses of fractions for fiber, protein, moisture, fat, and ash. The fractions varied greatly in composition, as shown in Table 1. The composition of a commercial mill-run whole grain and the most nutritious fraction reported by Harden (10) are also included in Table 1.

Grain sorghum is often compared to corn, but in general it is 2% higher in protein and 1% lower in fat (8). Grain sorghum is slightly higher than corn in niacin and riboflavin content, but lower in vitamin A due to lack of

TABLE 1

COMPARISONS OF COMPOSITIONS OF THE WHOLE GRAIN SORGHUM KERNEL AND ITS MAJOR PARTS

	Whole Grain	Pure Germ	Pure Bran	Pure Endosperm	Concomitant (Bran & Germ)
% of kernel	100.	10.	6.	84.	40-50
Protein					
a.	12.5				
b.	12.3	18.9	6.7	12.3	
c.	10.9	16.6	4.1	10.9	
d.	9.8				10.4
Fat					
a.	3.6				
b.	3.6	28.1	4.9	0.6	
c.	3.5	27.1	5.8	0.6	
d.	2.7				5.7
Starch					
a.	76.0				
b.	73.8	13.4	34.6	82.5	
d.	74.3				66.7
Crude Fiber					
a.	2.7				
c.	1.6	3.3	13.9	0.7	
d.	1.9				4.1
Ash					
a.	1.5				
b.	1.65	10.36	2.02	0.37	
c.	1.36				

a. Watson (1)
b. Hubbard, Hall, and Earle (13)
c. Stringfellow and Peplinski (14)
d. Harden (10)

yellow pigments (5). Bressani and Rios (15) state that grain sorghum could replace corn in a dietary. Oke (16) studied three Nigerian cereals and also concluded that the composition of grain sorghum makes it suitable to replace maize (corn) without changing the nutritive value of the diet.

Lamb, Michie, and Rivers (17) fed grain sorghum and wheat in comparable diets consisting of 80% of each grain to three successive generations of rats and found that wheat was superior to each of three varieties of grain sorghum.

Normand, Hogan, and Deobald (18), studied the protein content of successive peripheral layers of sorghum hybrids (Texas 601, R.S. 610, and a mixed elevator sample from Harvest Queen Mill, Plainview, Texas) milled by tangential abrasion. As scouring progressed from outer to innermost portion of the kernel, the protein content of the successive fractions decreased. They found that in the grain sorghum endosperm, freed of germ and bran, exists a fraction of high-protein-bearing material with an average protein content of more than 18%, which represents about one-fourth the weight of the original kernel. No further information has been published on commercial production of this fraction.

In a study of Kansas grain sorghum, protein levels in the whole kernel varied in 1961 from 6.6% to 12.3% and in 1962 from 5.0% to 12.1%. Significant differences were found to be due to location of production and to hybrid varieties.

Fertilization resulted in both increased yield and increased protein level (19). In 1953 Sauberlich et al. (20) reported that fertilization had its main effect on quantity rather than quality.

Waggle et al. (21) compared the nutritive value of the protein of two sorghum composites containing 7.9% and 11.8% protein on the basis of rat growth and amino acid analysis. The grain with higher protein concentration had higher percentages of all 17 amino acids studied than did the grain with lower protein content, as determined by ion exchange chromatography. Lysine was the most deficient amino acid in both grains, and the sulfur-containing amino acids and threonine were also low. Calculated on an equal nitrogen basis, the levels of dibasic amino acids, especially lysine, the sulfur-containing amino acids, and threonine of the high protein sorghum grains were lower than those of the low protein grain sorghum. The nutritive value of the low protein grain sorghum was superior to that of the high protein grain sorghum as shown by growth of rats and amino acid assay. When lysine, histidine, and arginine were added to the high protein grain sorghum diet to adjust their percentages in the protein to be similar to the content in the low protein grain sorghum diet, an increase in growth resulted.

To determine the relationship of amino acid level to protein content, a total of 77 samples of Kansas grain sorghum were analyzed and evaluated by Waggle and Deyoe (22).

Protein content of grain sorghum affects the amino acid content and its distribution. The higher the protein content, the greater the quantities of amino acids. The concentration in protein of lysine, histidine, arginine, threonine, and glycine tends to decrease as the protein content increases.

Vavich (23), using chick growth as a criterion, found grain sorghum containing 10.5% protein to be superior to sorghum containing 15.3% protein when fed at a constant level of protein in the diet.

Studies involving the amino acid adequacy of grain sorghum for growth have been reported. The work of Hillier et al. (24) and Shelton et al. (25) indicated that lysine is the first limiting amino acid in grain sorghum. Pond et al. (26) confirmed that lysine was the most limiting amino acid for growth and suggested threonine as the second most limiting. Male weanling rats were fed grain sorghum diets supplemented with various levels and combinations of essential amino acids. The most effective supplementation was with 0.5% of L-lysine and 0.2% of DL-threonine. This produced growth approximately equal to that obtained with a purified diet containing 11% casein, but inferior to that obtained with a 21% casein purified diet.

That threonine is the second limiting amino acid of grain sorghum was established by Howe et al. (27) using weanling rats. This study showed that amino acid supplementation involving only lysine, tryptophan, and threonine will increase the quality of the proteins of the staple cereals to make them comparable to a standard milk protein, casein.

Grain Sorghum as a Total Diet

Because grain sorghum can be grown in most areas of the world with good production, it is one possible answer to the food problems in the world. Grain sorghum will provide calories, incomplete protein (which can be supplemented with amino acids known to be limiting), iron, and B vitamins. When comprising the total diet or a large percentage of the diet, grain sorghum must be supplemented with calcium and vitamins A, D, and E (1). Supplementation of any food is necessary when it becomes the sole ingredient of a diet. The nutrients to be added depend on the nutrient requirements of the animal being fed. The laboratory rat, having nutrient requirements similar to man and being a suitable organism for bioassay, has had its requirements established in research reported in the literature.

Nutrient Requirements of the Rat

The requirement of the rat for vitamin A is related to body weight rather than to energy intake, since vitamin A is necessary for the maintenance of body epithelium, which in itself is directly correlated with body mass. The difficulty in establishing the exact requirement stems from the problem of determining a criterion of measurement (28). Lewis et al. (29) found that for maximum growth with some liver storage, 250 I.U. per kilogram of rat per day was adequate. An evaluation of studies suggests that 200 I.U. per kilogram of rat per day or 2,000 I.U. per kilogram of diet should give good weight gains, optimum longevity, and some liver storage of vitamin A (28).

The rat's need for vitamin E has been established, but the exact requirement is difficult to ascertain. Long range studies are necessary to show deficiencies at low levels. Normal growth and reproduction were sustained with a dosage of 0.75 mg of alpha tocopherol six times weekly (30). Selenium and a number of anti-oxidants have been found to have a sparing effect on vitamin E. Dietary fat increases the requirement for vitamin E. Diets reported in the literature include from 50 mg to 200 mg of vitamin E per kilogram (28).

The need of the rat for calcium, phosphorus, and vitamin D must be discussed simultaneously. Absolute amounts as well as relative amounts of these nutrients affect the performance of the rat.

Bethke, Kick, and Wilder (31) studied the interrelation of calcium and phosphorus at different levels or concentrations in the presence and absence of vitamin D. Their experiments confirmed that the calcium/phosphorus ratio affects growth, ash content of bones, and calcium and inorganic phosphorus values in blood serum of rats. The amount of vitamin D required for normal calcification is dependent on the calcium/phosphorus ratio of the ration. The most favorable calcium/phosphorus ratio for growth and bone development was determined to be between 2:1 and 1:1. Vitamin D requirements are at a minimum at these proportions.

Shohl and Wolback (32) studied electrical reactions, calcium and phosphorus blood serum levels, bone ash, X-ray, and bone histology to examine the effect of low-calcium--high-phosphorus diets. These studies reaffirmed the importance of calcium/phosphorus ratios as well as absolute levels.

As dietary calcium was increased from 0.19% to 0.48%, Evans and Ali (33) found retention and deposition of calcium improved. In an early study, Sherman and Campbell (34) added calcium carbonate to a diet already proved adequate for supporting growth of successive generations of rats. Calcium content of the diet was increased from 0.2% to 0.35%. This enrichment resulted in decrease in infant mortality, more efficient utilization of food (energy and protein), better growth, earlier maturity, several indications of higher adult vitality, a longer period between maturity and senility, and increase in average life expectancy. They concluded that the calcium intake most conducive to optimal well-being was significantly higher than that required for normal growth and maintenance.

Requirements of the growing rat for sodium and potassium were established by Grunert, Meyer, and Phillips (35). Minimum requirement was established as the lowest level of sodium or potassium at which the growth rate was not significantly different from the maximum growth rate obtained at any level. The sodium requirement of the rat for growth was established at 0.05%. The requirement for sodium was independent of the requirement for potassium. The potassium requirement was influenced by the sodium content of the ration, with sodium having a sparing effect except at the lowest levels of potassium. In the presence of 0.1% sodium, the potassium requirement was 0.18%. The potassium requirement decreased progressively with increase in age during the six week growing period, while the sodium requirement remained relatively constant.

Bernhart and Tomarelli (36) prepared a salt mixture based on the requirements listed by the National Research Council. Rats receiving adequate protein and 100% of the NRC mineral requirements grew at the maximal rate for rats of the Sprague-Dawley strain fed purified diets (6.15 g/day). Rats receiving 150% of the mineral requirement grew at practically the same rate (6.25 g/day). Less of the salt mixture was necessary when growth was limited by inadequate protein. Although all mineral requirements estimated by the NRC were adequate for maximal growth, 150% of NRC requirements was suggested as a safeguard.

Lack of sufficient thiamine, riboflavin, and niacin inhibits the growth of rats, as well as produces more specific symptoms. Scott and Griffith (37) showed that diets for rats containing 10% fat required at least 0.01 mg of thiamine per day. For diets with less than 20% fat, values between 0.8 and 1.6 mg of thiamine per kilogram of diet have commonly been reported as used (28).

Intakes of 40 mcg of riboflavin per day produced maximum riboflavin concentrations in the liver of rats. Maximum growth occurred with tissue riboflavin concentrations about 75% of maximum levels attainable. A rapidly growing rat may require up to three times as much riboflavin per gram as a non-growing rat to maintain a specific tissue level. Riboflavin destruction was not found to be associated with use. Metabolic mixtures emphasizing fat, carbohydrate, or protein did not influence riboflavin destruction (38). Of all reported values, an approximate average requirement is 25 mcg per day, or 0.25 mg per 100 grams of diet (28).

Because the amino acid tryptophan is a precursor of niacin, the requirement for niacin is dependent on the amount of tryptophan in the diet. When rats are on a diet with tryptophan as the limiting amino acid, little niacin synthesis occurs. With an abundance of tryptophan in the diet, niacin is formed in excess even in the presence of adequate dietary niacin (39). When a minimum amount of tryptophan is supplied in the diet, the niacin requirement of the rat is between 13 and 30 mg per kilogram of diet. From 20 to 100 mg of niacin per kilogram of diet are often used in purified diets (28).

In establishing protein requirements for the rat, three factors must be considered: (1) energy concentration of the diet, (2) amino acid composition of the protein, and (3) digestibility of the protein. Since the feed intake of the rat will be related to the net energy level of the diet, the protein requirement is best expressed as a protein-to-calorie ratio (28).

Protein nutrition is amino acid nutrition. The amount of each essential amino acid present in the protein and liberated in the digestive process becomes available for tissue synthesis. Not all available amino acids may be utilized if essential amino acids are not present in the ratio which conforms to the body's requirements. Thus some of the amino acids present in protein of low quality may be wasted because all of the essential amino acids are not present in sufficient quantity (40).

Means for Improving Grain Sorghum

Several possibilities exist for improving the nutritive value of cereals, including grain sorghum. Changes in the structure of grains are being made through selective plant breeding. Scientists at Purdue University have developed a maize (corn) strain containing the opaque-2 mutant gene. The opaque-2 endosperms have a different amino acid pattern and 69 per cent more lysine than the normal seeds. The major reason for these changes is the synthesis of proteins with a greater content of basic amino acids in the acid-soluble fraction of the mutant endosperm. This is accompanied by a reduction in the ratio of zein to glutelin (41).

The superior quality of the proteins in opaque-2 mutant endosperm was confirmed by rat growth. Weanling rats fed a diet containing 90 per cent opaque-2 maize for 28 days gained an average of 97 grams. In control rats fed a standard hybrid maize the average gain was 27 grams (42). The opaque-2 corn was milled to produce edible fractions of germ, grits, and meal for evaluation with human subjects. The fractions were fed in the same proportions as in the corn to simulate the use of the whole grain. A daily intake of 300 grams of opaque-2 corn was suggested to be adequate for men weighing less than 70 kilograms and for all women. Supplements of lysine, tryptophan, and methionine did not consistently improve nitrogen retention, indicating that the amino acid content of opaque-2 corn is well balanced for human adult subjects. When opaque-2 corn was fed as corn masa to healthy children, two to six years of age, weight gain and nitrogen retention proved the corn to have a protein value nearly 90% as high as that of milk (43).

Another mutant gene affecting the amino acid pattern of maize endosperm proteins is the mutant floury-2 which results in the production of maize endosperm proteins with an altered amino acid pattern (44). The lysine concentration is high, approximately equal to that in mutant opaque-2, and the methionine concentration is higher than in any other stock tested. The biochemical basis of elevated lysine content in floury-2 is apparently different from that in opaque-2, and this suggests the possibility that in the double mutant stocks now being developed there may be higher amounts of lysine than in either mutant alone (44).

The World Sorghum Collection provides the necessary genetic diversity to permit meaningful improvements (4). A rat growth study of ten lines of Sorghum vulgare recently showed two to be superior to the others and one to be definitely inferior (43). Preliminary studies have shown that sorghum responded better to protein shifting than corn, soybean, or rice (14).

The nutritive value of grain sorghum can be improved by adding other foods. Torula yeast is a good source of lysine, but all this lysine is not completely available (45). Dehydrated fish products have proved superior to casein as a supplementation for cereals (46).

Skinner (47) compared rat growth on unsupplemented grain sorghum with that on grain sorghum supplemented with nonfat dried milk, with dried whole egg, and with a combination of the two. Superior rat growth was obtained with a supplementation of both milk and egg.

In some cases purified nutrients may be added rather than supplementing cereals with other foods. Purified vitamins and minerals can be more practical additions than foods to supply these nutrients. With the advent of the fermentation method of production of amino acids, these substances can be produced from low grade materials not suitable for human consumption at a sufficiently low cost to make feasible their use in upgrading cereal proteins (27). In the past, protein has been supplemented with arbitrary amounts of essential amino acids in the diet. The approach now suggested is to supplement the protein with its first limiting amino acid in such an amount as to obtain balance of the first limiting amino acid with the second (48).

The use of heat or cooking to improve grain sorghum is debatable. Moist heat treatments and flaking of the grain showed improvements in feed efficiencies in cattle feeding (49). The extent of moist heat needed for improvement has not been established, and some researchers suggest that since lysine is destroyed by heat the quality of the protein may be lowered (10). Jansen et al. (50) found a progressive loss in nutritive value of bread with supplemental lysine as baking time increased and suggested that baking time be as short as consistent with good product characteristics.

The presence of moderate amounts of nonfat dried milk was found to have a deleterious effect on the nutritive value of protein-bound and free lysine in bread (51). The destruction of amino acids during cooking is a result of many factors including temperature, time, moisture, and type of carbohydrate present (52).

Because the composition of grain sorghum is not homogeneous for all parts of the kernel, some fractions are more nutritious than others. Using the fractions that are most nutritious as food or feed would be a simple way to improve the quality of the grain sorghum product. Parts of the kernel not used for feed could then be utilized for industrial purposes. Harden (10) found that the fraction of the grain sorghum kernel considered offal by the dry-milling industry was more nutritious than the whole grain or any other fraction.

Grain sorghum can be used in some vegetable protein mixtures, such as the ones developed by INCAP (Institute of Nutrition of Central America and Panama). Grain sorghum was used as 28% of Incaparina Vegetable Mixture Nine. In these mixtures, the various vegetable proteins complement one another so that the protein value of the mixture is improved (53).

Through the use of these methods of supplementation to provide for the nutrient requirements of the hungry people of the world, cereal grains, especially grain sorghum, can contribute to the solution of some of the world's food problems.

As President Johnson said in his State of the Union message on January 10, 1967, "Next to the pursuit of peace, the really great challenge to the human family is the race between food supply and population increase. That race tonight is being lost. The time for rhetoric has clearly passed. The time for concerted action is here, and we must get on with the job (54)."

CHAPTER II

METHODS AND PROCEDURES

Three experimental diets of supplemented grain sorghum fractions were tested. All of the grain was from the same lot of commercial mill-run grains, supplied by Harvest Queen Mill, Plainview, Texas. The current milling procedure at Harvest Queen Mill yields the following fractions: brewers' grits, industrial flour, bran, and germ. The bran and germ fractions, having no major industrial uses, are available for feed. Previous research and chemical analysis[1] have shown these two fractions should be supplemented with vitamins A, D, and E, sodium chloride, and calcium to meet the requirements of the growing rat.

In Table 2 the composition of the fractions is compared with the requirements of the growing rat as stated by the National Research Council (28). The formulas for the supplemented diets are shown in Table 3. The vitamins were incorporated into a pre-mix before mixing with the remainder of the grain to insure uniform distribution of these micronutrients. The vitamin pre-mix consisted of 4 g vitamin A, 2 g vitamin D, 10 g vitamin E, and 84 g of the grain sorghum bran or germ. For Diet I the bran fraction was supplemented with the vitamin pre-mix made with bran, NaCl, and $CaCO_3$,

[1]Chemical analyses of sorghum fractions were made by James K. Sikes, Plains Laboratory, Lubbock, Texas.

and for Diet II the germ fraction was supplemented in the same way, with the vitamin pre-mix made with germ rather than with bran. Diet III, a cooked version of the supplemented germ diet (II), was made by adding distilled water and tartrate baking powder to the ground grain. This mixture in wafer form was baked at 425° for 15 minutes and then allowed to remain in the oven with no additional heat until dry. The wafers were ground to a fine meal, similar in particle size to the other diets. Diet IV, an established adequate diet, was used as a control. This diet consisted of 2% cod-liver oil, 4% salt mix, 5% B-complex mix, 8% vegetable oil, 18% casein, and 63% cornstarch. All diets were fed at normal room humidity. In Table 4 the amino acid composition of the diets is compared with the nutrient requirements of the rat.

Twenty female albino rats of the Sprague-Dawley strain, 24 days old and weighing 30.8 to 43.0 grams were assigned to the four diets. The animals were caged individually in raised screen-bottomed metabolic cages. Distilled water and feed were supplied ad libitum. Measurements of weight and feed consumption were made weekly on the same day. Feed consumption was determined by the difference in the weight of feed supplied and that left at the end of the week. Growth of rats and consumption of feed were criteria for assessing quality of the three grain sorghum diets. In addition, the rats were compared with animals on the casein-based adequate diet. Data for rats fed unsupplemented grain sorghum diets in the same laboratory were also available for comparison. Urine and feces were collected the third and fourth weeks of the six-week experimental period for nitrogen determinations.[2]

Factorial analysis was used to analyze the effects of diets, weeks of experiment, and any interaction between the two. Analysis of variance was used to identify differences among the four diets.

[2]Analyses for nitrogen were made by James K. Sikes, Plains Laboratory, Lubbock, Texas.

TABLE 2

COMPARISON OF COMPOSITION OF GRAIN SORGHUM FRACTIONS WITH NRC REQUIREMENTS FOR THE RAT

Nutrient	Bran	Germ	NRC Requirement
Digestible energy (kcal/kg)	3,220.	3,350.	3,600.
Protein (%)	9.71	11.0	20.
Fat (%)	6.53	6.49	5.
Starches (%)	57.37	61.86	
Sugars (%)	4.03	4.38	
Fiber (%)	8.87	3.04	
	Amounts Per 100 g of Diet		
Calcium, mg	66.	20.	600.
Phosphorus, mg	670.	1,120.	500.
Iron, mg	12.3	13.5	2.5
Zinc, mg	19.72	6.5	1.2
Copper, mg	5.43	3.3	0.5
Manganese, mg	6.23	3.3	5.
Magnesium, mg	750.	340.	40.
Potassium, mg	640.	632.	180.
Sodium, mg	7.2	7.1	50.
Vitamin A, I.U.	8.15	12.07	200.
Vitamin D			?
Vitamin E, mg of alpha tocopherol			6.

TABLE 3

COMPOSITION OF GRAIN SORGHUM DIETS

	Grams Per 1000 g of Diet		
	Diet I	Diet II	Diet III
Bran fraction	969.1		
Germ fraction		969.1	969.1
$CaCO_3$	15.8	15.8	15.8
Vitamin pre-mix	10.0	10.0	10.0
NaCl	5.1	5.1	5.1
Distilled water			746.3[3]
Tartrate baking powder			22.4

[3]This water was evaporated during the baking process, and Diet III was fed at room moisture as were the other diets.

TABLE 4

AMINO ACID COMPOSITION OF GRAIN SORGHUM DIETS[4]
COMPARED WITH NRC REQUIREMENTS

Amino Acid	Per Cent of Amino Acid in Diet			
	Bran	Germ	Germ Wafer	NRC Requirements
Lysine	0.33	0.30	0.37	0.90
Histidine	0.18	0.21	0.29	0.30
Arginine	0.40	0.40	0.55	0.20
Threonine	0.30	0.29	0.43	0.50
Valine	0.39	0.43	0.82	0.70
Methionine	0.09	0.13	0.17	0.60
Isoleucine	0.28	0.33	1.43	0.50
Leucine	0.81	1.11	1.50	0.80
Phenylalanine	0.36	0.45	0.67	0.90
Tryptophan	(not analyzed for tryptophan)			0.15
Aspartic Acid	0.72	0.74	1.15	
Serine	0.40	0.42	0.59	
Glutamic Acid	1.33	1.83	2.53	
Proline	0.49	0.73	0.96	
Glycine	0.39	0.35	0.45	
Alanine	0.55	0.84	1.19	
Cystine	0.14	0.11	0.24	
Tyrosine	0.18	0.19	0.31	
Non-essential Amino Acids	4.30	5.21	7.42	6.45

[4]Amino acid analyses were made by Dr. Wendell A. Landman, Professor of Animal Science, Texas A & M University.

CHAPTER III

FINDINGS AND INTERPRETATIONS

The average body weights for rats on all four diets throughout the study are shown in Table 5. Although none of the rats on grain sorghum diets attained weights equal to those of control animals, the rats on Diet I (supplemented bran) were more nearly normal size. Figure 1 shows the average weights of animals on the four diets for the six weeks.

TABLE 5

AVERAGE BODY WEIGHTS OF RATS IN GRAMS

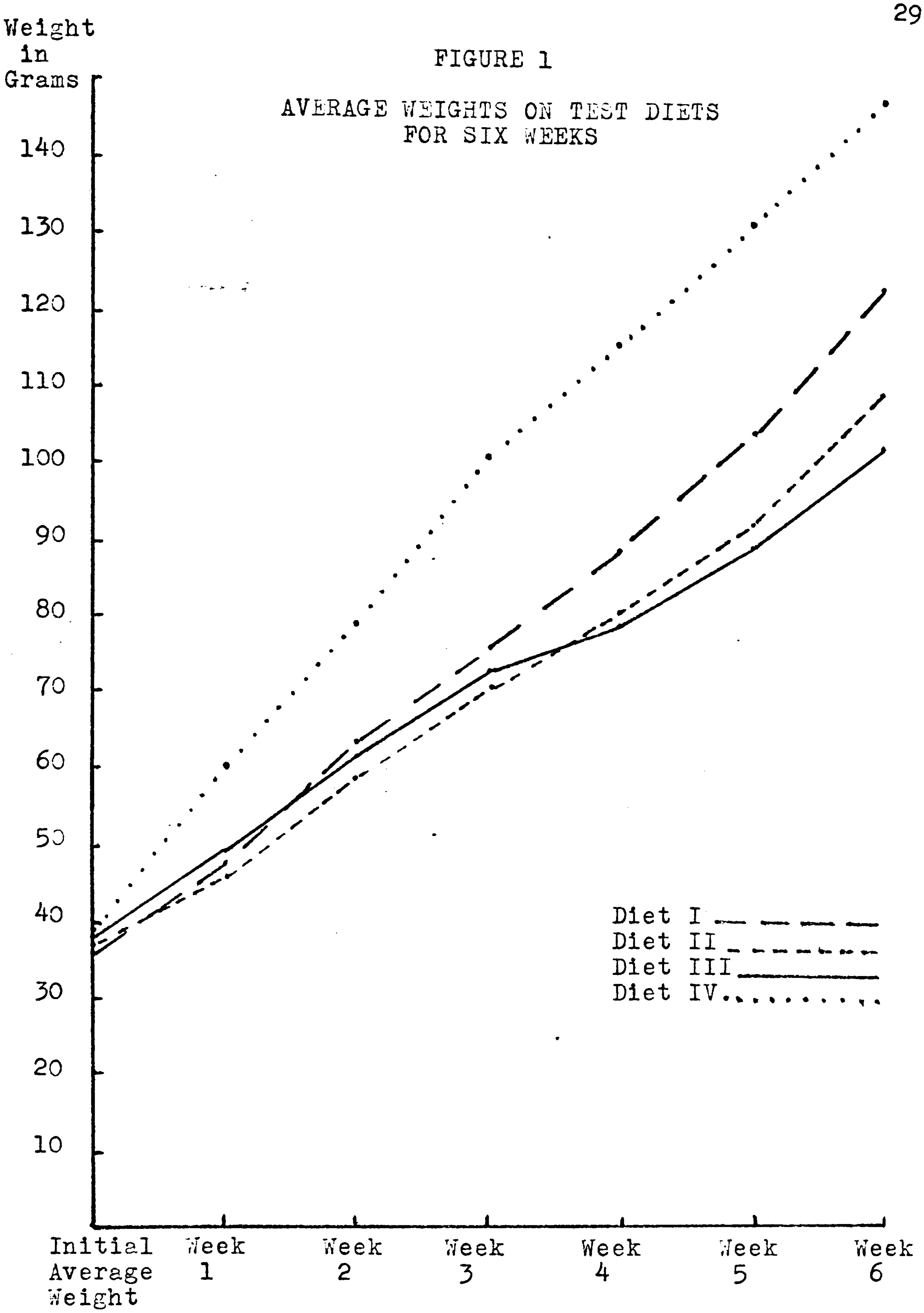
Diets	Initial Weight	Average Weight Per Week 1	2	3	4	5	6
I. Bran*	36.7	47.7	63.8	75.9	88.5	104.6	123.5
II. Germ*	37.3	45.9	59.1	71.2	80.6	92.6	109.6
III. Germ* Wafer	37.6	49.2	62.5	72.8	79.3	89.5	103.1
IV. Control	38.4	60.2	79.2	100.8	115.3	131.6	147.8

*Supplemented with vitamins A, D, and E, NaCl, and $CaCO_3$

The data in Table 6 show the average weight gain per week of rats on each of the diets and the average weekly weight gain. Factorial analysis indicated that the

FIGURE 1

AVERAGE WEIGHTS ON TEST DIETS
FOR SIX WEEKS



variation in weight gain among the five rats on a diet was significant. Variation in weight gain among weeks and among diets was highly significant. Animals on the control diet (Diet IV) gained significantly more weight than those on either supplemented germ (Diet II) or cooked supplemented germ (Diet III). No significant differences in weight gain were found between animals on any two of the grain sorghum diets, even though the total weight gain and average weekly weight gain was higher for the rats on Diet I (supplemented bran).

TABLE 6

AVERAGE WEIGHT GAIN IN GRAMS OF RATS FED TEST DIETS

Diets	Grams Weight Gain Per Week on Diets 1	2	3	4	5	6	Ave.
I. Bran*	11.0	16.1	12.1	12.6	16.1	18.9	14.5
II. Germ*	8.6	13.2	12.1	9.4	12.0	14.8	11.7
III. Germ* Wafer	11.6	13.3	10.3	6.5	10.2	13.6	10.9
IV. Control	21.8	19.0	20.5	14.5	16.3	16.2	18.1

*Supplemented with vitamins A, D, and E, NaCl, and $CaCO_3$

The weight gain of these animals with vitamin and mineral supplementation was better than that reported by Harden (10) for animals on various unsupplemented grain sorghum diets. On a diet consisting of ground whole grain Harden reported an average weight gain of 16.5 grams for four weeks or an average weekly weight gain of 4.2 grams. For the best diet in Harden's study, composed solely of a bran-germ fraction of grain sorghum (concomitant, Diet D-1), 33.5 grams for four weeks or 8.3 grams average weekly weight gain were reported. Average gains in weight of rats on all diets in this study surpassed those reported by Harden for the best gains made on unsupplemented grain sorghum, shown in Table 7.

TABLE 7

AVERAGE WEEKLY WEIGHT GAIN IN GRAMS[5]
ON UNSUPPLEMENTED GRAIN SORGHUM

	Grams Weight Gain Per Week			
Diet	1	2	3	4
A-1 Whole Grain	3.9	5.5	5.6	1.9
D-1 Concomitant	8.8	11.9	6.0	6.4

[5]Reported by Harden (10)

Data on average feed consumption of rats on each diet can be seen in Table 8. Factorial analysis showed a highly significant interaction between diets and weeks of the experiment, and a highly significant difference among rats on a diet, among weeks, and among diets. Considering the entire six weeks as a whole, analysis of variance showed a highly significant difference between the diets. Tukey's D-test revealed that animals on Diet I (supplemented bran) consumed significantly more feed than those on the other diets. This was apparent in the weekly averages as well as in the average of all weeks.

TABLE 8

AVERAGE FEED CONSUMPTION IN GRAMS

Diet	Grams Feed Consumed Per Week 1	2	3	4	5	6	Average
I. Bran*	56.1	81.1	83.6	97.3	114.7	113.7	91.1
II. Germ*	40.0	62.8	66.7	66.4	87.0	68.8	65.3
III. Germ* Wafer	54.3	65.0	69.4	65.1	74.7	70.5	66.5
IV. Control	49.9	70.7	74.0	72.4	87.1	76.2	71.7

*Supplemented with vitamins A, D, and E, NaCl, and $CaCO_3$

In Table 9 data on the efficiency of the diets in promoting growth are compared. Animals on the diet containing an established adequate protein (Diet IV) used less feed per gram of gain than did animals on the other diets; however, the variation was significant only when compared to animals on the cooked supplemented germ (Diet III). Factorial analysis indicated a significant difference between weeks and a significant variation in feed efficiency of individual rats on a diet. Feed efficiency of rats on the four diets is compared in Figure 2. Harden's (10) diets of unsupplemented grain sorghum were not nearly as efficient in promoting growth. A gram of gain in weight required 14.78 grams of unsupplemented whole grain. Feed efficiency of the best fraction in that study was 8.21 grams for a gram of weight gain. Thus, the vitamin and mineral supplementation not only promoted better weight gains but also made the diets more efficient.

TABLE 9

AVERAGE EFFICIENCY OF DIETS IN PROMOTING GROWTH (GRAMS FEED PER GRAM GAINED)

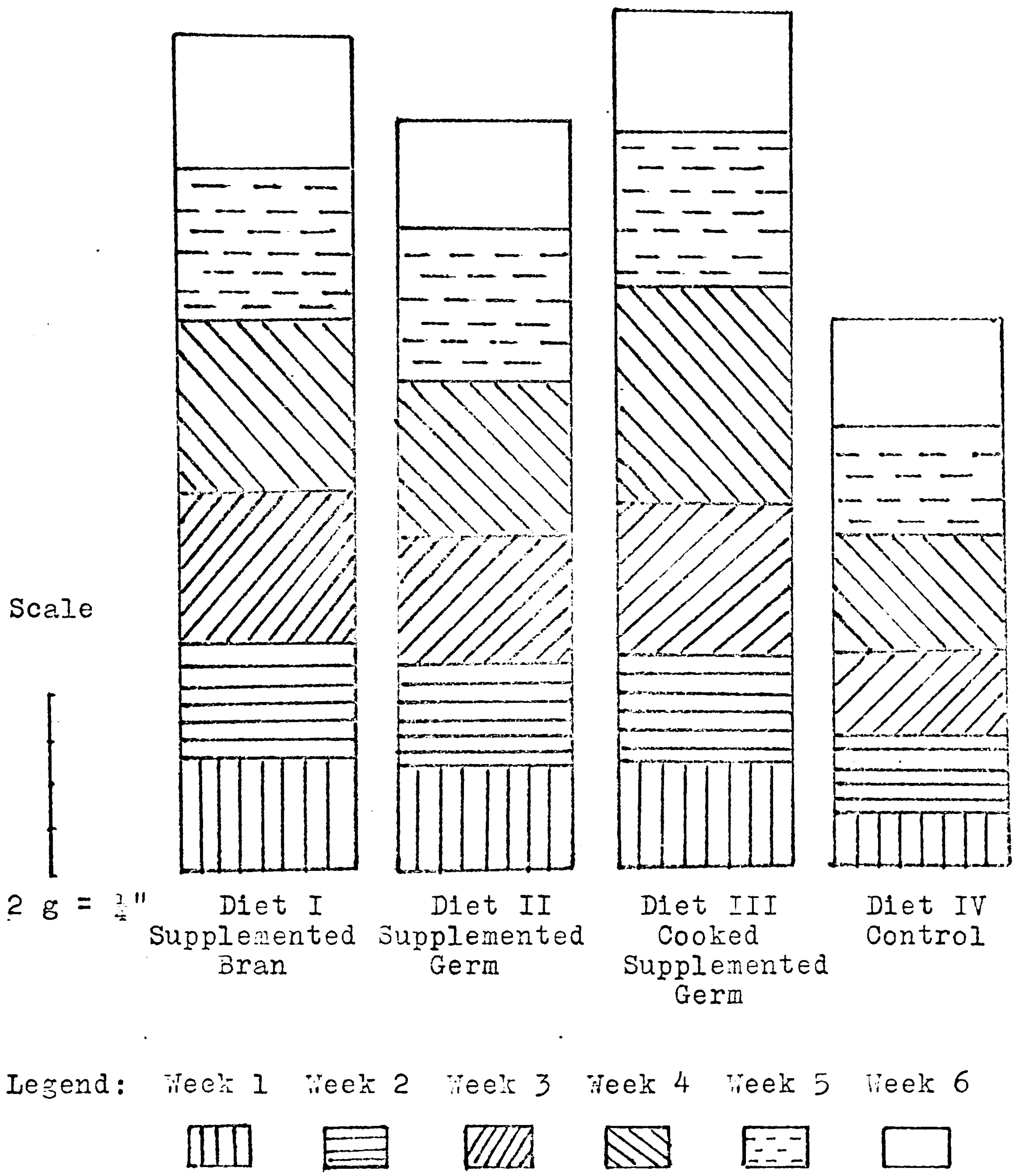
Diet	Efficiency Per Week of Experiment						Average
	1	2	3	4	5	6	
I. Bran*	5.1	5.0	6.9	7.7	7.1	6.0	6.3
II. Germ*	4.7	4.8	5.5	7.1	7.3	4.6	5.7
III. Germ* Wafer	4.7	4.9	6.7	10.0	7.3	5.2	6.5
IV. Control	2.3	3.7	3.6	5.0	5.3	4.7	4.1

*Supplemented with vitamins A, D, and E, NaCl, and $CaCO_3$

Table 10 shows the average nitrogen balance of rats on all diets for the third and fourth weeks of the six-week experimental period. When the amount of nitrogen in the feces was compared with the amount of nitrogen consumed,

FIGURE 2

AVERAGE WEEKLY FEED EFFICIENCY OF TEST DIETS
(GRAMS FEED PER GRAM GAINED)



Tukey's D-test indicated that the nitrogen balance for rats on Diet III (cooked supplemented germ) was significantly different from those on Diet IV and that the balance for rats on Diet I (supplemented bran) was significantly different from those on all other diets. The large amount of fecal nitrogen for rats on Diet I can be related to the greater amount of feces, which resulted from the high percentage of indigestible fiber in the bran. Table 11 shows the average amounts of feces and urine collected for the two-week period.

TABLE 10

AVERAGE NITROGEN BALANCE

Diet	Average Grams of Nitrogen for Two Weeks Consumed	Fecal	Urinary	Total	Balance
I. Bran*	2.79	1.50	0.43	1.93	+0.86
II. Germ*	2.36	0.56	0.50[6]	1.06	+1.30
III. Germ* Wafer	2.46	0.65	0.68	1.33	+1.13
IV. Control	4.11	0.49	0.46[7]	0.95	+3.16

*Supplemented with vitamins A, D, and E, NaCl, and $CaCO_3$
[6]Average of three rats
[7]Average of four rats

In comparing grams of nitrogen in urine with grams of nitrogen consumed, no significant differences were found between averages for rats on the four diets. This would indicate that all these growing rats were in positive nitrogen balance, retaining dietary nitrogen to support growth. The highest balance corresponded to the greatest growth.

TABLE 11

AVERAGE AMOUNTS OF EXCRETA COLLECTED FOR TWO WEEKS

Diet	Feces Grams	Urine Ml
I. Bran*	92.8	41
II. Germ*	17.2	34
III. Germ* Wafer	18.0	55
IV. Control	8.7	29

*Supplemented with vitamins A, D, and E, NaCl, and $CaCO_3$

The apparent digestibility of the protein for the test diets is shown in Table 12. The data were obtained by comparing nitrogen intake with fecal nitrogen, using the formula (I - F) ÷ I. The low digestibility of protein in the bran fraction may be attributed to high fiber content. Table 12 also shows the amount of digestible protein in 100 g of each diet. No grain sorghum fraction studied by Harden (10) had a protein digestibility level as low as the supplemented bran (Diet I) in this study (47%). The protein digestibility of the germ diets (75% for Diet II and 74% for Diet III) was similar to that reported by Harden for whole grain (80%) and for the most nutritious fraction (71%).

TABLE 12

APPARENT DIGESTIBILITY OF PROTEIN

Diet	% Protein In Diet	% Digestible Protein	Grams Digestible Protein/100 g Diet
I. Bran*	9.71	47.	4.56
II. Germ*	11.00	75.	8.25
III. Germ* Wafer	10.90	74.	8.07
IV. Control	18.00	87.	15.56

*Supplemented with vitamins A, D, and E, NaCl, and $CaCO_3$

The control diet (Diet IV) was significantly different from the grain sorghum diets in weight gain for the amount of protein retained. This is to be expected because the control diet contained an adequate protein source in both quantity and quality. Pond (26) found that even by supplementing grain sorghum with the first and second limiting amino acids, lysine and threonine, that growth was inferior to that shown on a 21% casein purified diet. Growth was approximately equal to that of an 11% casein diet.

Weight gains of animals for the initial three weeks on these grain sorghum diets supplemented with vitamins and minerals were three to four times the weight gains of the animals fed unsupplemented whole grain sorghum by Skinner (47), but slightly less than half as great as gains made by rats receiving grain sorghum with protein supplementation in that study.

The bran and germ fractions used in this study were essentially former components of the fraction shown by Harden (10) to be nutritionally superior to the whole grain and to other fractions. Milling procedures at Harvest Queen Mill were changed to produce two separate fractions, bran and germ, from the concomitant fraction studied by Harden. This study has shown that both bran and germ fractions supplemented with vitamins and minerals produce better growth response than the unsupplemented whole grain or fractions.

Animals receiving the supplemented bran diet consumed significantly more feed than those on the other diets. This may be related to the high fiber content of the bran, but possibly the bran also contained more natural B vitamins which stimulated the appetite. Although the rats fed bran did not attain a size comparable to the animals on an adequate diet, they were larger than animals on both of the germ diets.

No significant difference was found in weight gain, in feed consumed, or in feed efficiency between animals on Diets II and III (supplemented germ and supplemented germ wafer). The uncooked germ diet produced a slightly greater average weekly weight gain, but the animals on the cooked diet ate somewhat more feed and had a slightly better feed efficiency. Thus, in this study, cooking the grain was neither beneficial nor detrimental.

This study has shown that vitamin and mineral supplementation raises the nutritional value of grain sorghum fractions as total diets, but it is apparent that protein supplementation is necessary to produce optimum growth.

CHAPTER IV

SUMMARY AND CONCLUSIONS

A bioassay of two grain sorghum fractions, which have limited current use, compared rat growth on these fractions with that on an established adequate diet. These bran and germ fractions were supplemented with vitamins A, D, and E, sodium chloride, and calcium but with no source of additional protein. The germ fraction was cooked to form a third experimental diet. Rat growth on these supplemented grain sorghum fraction diets was also compared with data for animals fed unsupplemented grain sorghum diets.

Twenty female weanling rats were fed these four diets for six weeks, with weekly measurements of weight and feed consumption. Urine and feces were collected the third and fourth weeks of the experiment for nitrogen analyses.

Animals on all supplemented grain sorghum diets gained more weight than has been reported for animals fed unsupplemented grain sorghum diets, but less than animals fed an adequate diet. Rats that received the supplemented bran diet attained a size more nearly comparable to the control animals than did those on either germ diet. Cooking the germ diet was neither beneficial nor detrimental.

The high fiber content of the bran fraction makes it more acceptable as feed for animals rather than as food for humans. This fraction would provide a nutritious feed and leave remaining fractions for other purposes. The germ fraction, however, has possibilities for the United States market as a base for a quick bread mix or as a flour to be used in quick bread products. It would also be an economically feasible component of vegetable protein mixtures for countries where people are hungry.

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