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How Amaranth Grain Affects Total Body Weight and Major Organ Weight in the Mongolian Gerbil

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The Edwin P. McCabe Honors Program

Senior Thesis

"How Amaranth Grain Affects Total Body Weight and Major Organ Weight in the Mongolian Gerbil

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HOW AMARANTH GRAIN AFFECTS TOTAL BODY WEIGHT AND MAJOR ORGAN WEIGHT IN THE MONGOLIAN GERBIL

By

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May 1994

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IN THE MONGOLIAN GERBIL

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CHAPTER I

INTRODUCTION

Background of the Study

The amaranth plant has been long recognized in North America and other parts of the world for its nutritional value. As far back as 5000 B.C., archeological records provided evidence of the cultivation and the use of amaranth grain and leaves as a food source (Cole, 1979). The word amaranth is derived from two Greek words meaning "immortal," referring to the long-lasting colors of the flowers even after they are picked. These plants are annuals (Heywood and Chant, 1982) and are commonly found in tropical, subtropical, and temperate regions around the world (Singhal and Kulkarni, 1988).

Extensive research has shown that the level of protein, essential amino acids (lysine, methionine, and cysteine), dietary fiber, calcium, magnesium, and iron exceed the levels found in more common grains such as corn, rye, wheat, and rice. For example, three and one-half ounces of amaranth grain contain more calcium than a glass of milk (Cole, 1979).

Purpose and Objectives

The purpose of this research is to determine effects of amaranth grain of the body weight and organ weight of the Mongolian gerbil. The literature research indicates that amaranth results in physiological changes that appear to be species-specific rather than general.

Since the gerbil is a granivorous animal, it is hypothesized that amaranth will be completely digested and that its nutritional quality will maintain normal to increased growth. While the results of this study cannot be applied to other animals, information has been gained with regard to the influence of amaranth grain on the Mongolian gerbil.

CHAPTER II

Review of Literature

The broadleaf amaranth plant is used worldwide for human and animal consumption. While fifty to eighty percent of the plant is eaten (Singhal and Kulkarni, 1988), its seeds have had varied uses. The use of black seeds for medicinal purposes, as garden ornaments and ritual accessories, as well as sources of dye dates as far back as the Middle Ages (Singhal and Kulkarni, 1988).

White seeds are commonly planted for grain in South America, but are also used to make products such as alegria (a candy), atole (a beverage) (Singhal and Kulkarni, 1988), baked goods, soups, pasta, salad condiments, and snack foods (American Amaranth, Inc). In some parts of Africa, amaranth is eaten as a leafy vegetable, while in China it is commonly used as forage.

Over the past fifteen years, much of the information about amaranth resulted from experiments carried out at the Rodale Research Center in Pennsylvania, where more than three hundred varieties were maintained. While earlier studies focused largely on enhancing grain yield and improving resistance to insects and disease, more recent studies are concerned with producing new varieties with earlier maturity, larger seeds, shorter statue (height), and an even greater grain yield Cole, 1979). Because of the varied uses of the more than sixty known species. both the economic value and the research focus of amaranth have increased.

In the United States, Amaranthus cruentus is believed to be one of the oldest domesticated crops. Another species, Amaranthus hypochondriacus, which is known for its high grain yield, was grown by Arizona cliff dwellers in prehistoric times. These two species are currently grown in the United States (Cole, 1979).

Experimental studies in process are aimed at producing new varieties with higher yields, shorter statues (heights), earlier maturity, and larger seeds. The versatility of amaranth grain has influenced its research and economic value. It is suitable for use in confectionary products, baked products, soups, pasta, salad condiments, and snack foods - to name a few. The grain has similar uses when popped (American Amaranth Inc., 1984).

Amaranth grain has high nutritional value. The protein quality of the biological value of the protein is higher than in most true cereals such as corn, rye and wheat. The biological value of a protein refers to its effect on body maintenance and growth (Swenson, 1977).

According to the protein value score chart established by FAO (Food and Agriculture Organization) and WHO (World Health Organization), amaranth protein has a score of 75 out of 100. On the same scale, corn, soybeans, wheat, and cow's milk have scores of 44, 68, 60, and 72 respectively.

The protein composition in Amaranth is 16%, which exceeds that of more common grains such as corn, rye, and wheat with protein percent compositions of 9%, 13%, and 10% respectively (American Amaranth, Inc., 1984).

Among the essential amino acids that contribute to the higher quality of amaranth protein are lysine, cysteine, and methionine. These amino acids are essential for the synthesis of life-sustaining molecules such as glucose. In humans and higher animals, glucose is essential for normal physiological processes within cells and tissues (Brobeck, 1981). Because amaranth is low in the amino acid leucine (Singhal and Kulkarni, 1988), it should be used in combination with other grains.

Amaranth in Research

Richard Harwood, an agronomist at the Rodale Research Center, has devoted much of his time researching amaranth and initiating comprehensive programs to bring amaranth to the point of becoming an economic crop (Cole, 1979). Specifically, his work focused on nutritional analyses, genetics, and the development of new amaranth species.

Additional contemporary studies have been conducted at other laboratories. These include information pertinent to nutritional analyses and development of new varieties.

Some of the following include protein and amino acid analysis at USDA's Western Regional Research Laboratory, oxalate levels in some grain amaranths, and carbohydrate composition (Cole, 1979).

At least one study has shown that some species of amaranth may be a source of nitrite (Singhal and Kulkarni, 1988, cited in Okiei and Adamson, 1979). If nitrites are converted to nitroamines, they are potential carcinogens. There is speculation, however, that vegetables and fruits rich in vitamin C inhibit the conversion of nitrites into nitrosamines (Hamilton, et al, 1988).

Some people with allergies to certain grains use amaranth as a substitute; however, scientific studies have not revealed that amaranth has anti-allergic properties. Still, many individuals are depending more on grains and vegetables for their protein source since plant protein contains little fat. Animal protein usually consist of saturated fat.

Protein efficiency can be measured by the levels of nitrogen stored in the body when the protein is fed alone. The amount of amino acids within the body system determines how much nitrogen will be stored. Therefore, higher nitrogen level means better protein quality (Hamilton, Whitney, and Sizer, 1988).

As stated earlier, amaranth has high levels of fiber, magnesium, and calcium. Glucose synthesis is assisted by the presence of fiber, while magnesium aids in the synthesis of proteins (Nutrition Foundation, 1984). Magnesium also helps blood vessels maintain their elasticity level (Hamilton, et al, 1988, cited in Joffres, Reed, and Yano, 1987). However, both calcium and fiber affect the circulatory system (Hamilton, Whitney, and Sizer, 1988) such as blood pressure is influenced by calcium and fiber and lowers blood cholesterol.

Animals such as rats, chickens, and quail, have been used in amaranth research. One animal study has shown that amaranth grain depresses growth of broiler chicks and rats but not of Japanese quail. The antinutrient component is not known (Vohra, Bersch, and Acar, 1989).

In one experiment, Sprague-Dawley male rats were used to show the nutritional sufficiency of maize and grain amaranth cultivars for growth (Pond, Lehmann, and Clark, May 1989).

It was documented by Pond, Lehmann, and Clark (May 1989) that Amaranthus hypochondriacus-1024, 1046, and K188 produced weight gain which was higher than maize, and Amaranthus cruentus 1011 allowed normal growth at first, but weight loss occurred after two weeks. The unknown toxic factor in Amaranthus cruentus did not seem to have any relationship to permanent organ damage after two weeks of feeding. It was concluded that three of the four cultivars enhanced the growth of rats more than that obtained with maize (Pond, Lehmann, and Clark, May 1989).

The Mongolian Gerbil as an Experimental Subject

The Mongolian gerbil, Meriones unguiculatus, is a small rodent and mammal that has gained popularity as a laboratory model in the western world. Its favorable characteristics include cleanliness, high resistance to disease, and relatively inexpensive maintenance. These gerbils are alert, usually active, and exhibit interesting behavorial patterns. These characteristics of the gerbil provide the experimenter with an ideal model for experimental purposes.

Gerbils are herbivorous mammals that originated in the desert areas of China and Mongolia. Because their natural diet includes mostly seeds or grains, they are often termed granivorous.

Gerbils have been used in the following researches: lead nephropathology, cerebral ischemia/stroke, auditory phenomena parasite infections, epilepsy, histocompatibility, dental disease, behavior, endocrinology, radiobiology, water conservation, and lipid metabolism (Harkness and Wagner, 1989).

CHAPTER III

MATERIALS AND METHODS

Maintenance of the Gerbils

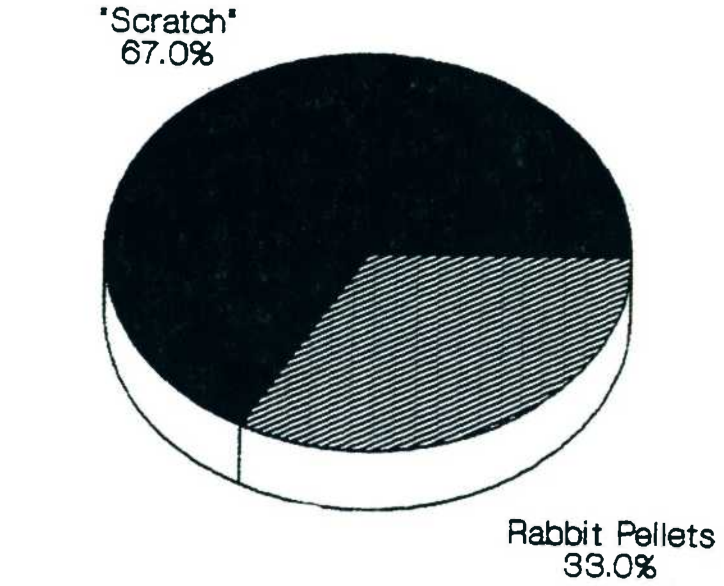
Mongolian gerbils for this study were obtained from the gerbil colony maintained in the Department of Biology at Langston University. These gerbils were housed in metal cages in a well-ventilated room on approximately 12-hour light/dark cycles. Room temperature was relatively constant at 25 degrees Celsius, which is within the range in which gerbils are usually housed (between 18 and 29 degrees). While gerbils tolerate a wide temperature range, they become uncomfortable and irritable at temperatures above 35 degrees Celsius (Harkness and Wagner, 1989). Environmental humidity was not recorded. If humidity exceeds 50%, studies have shown that the coat takes on a rough and matted appearance (Harkness and Wagner, 1989). There was no indication, based on physical appearance, that humidity was unfavorable.

Red cedar shavings were placed in the bottom of the cages for bedding and to help control odor and pests. The depth of the bedding was approximately 2 cm to facilitate nest building, which is typical behavior of gerbils. Cages were changed weekly. Food and water were provided _ad libitum_ in plastic bowls and glass bottles (12 milliliter capacity), respectively.

Experimental Design

Eighteen adult gerbils (14 females and 4 males) were fed two different diets with and without amaranth grain. Diet I contained 67% "scratch"- a mixture of corn and other grains and 33% rabbit pellets, while diet II contained 57% "scratch," 29% rabbit pellets, and 14% amaranth grain (Figure 1). Gerbils feeding on each diet were subdivided into three groups. Each subgroup, containing three gerbils, was housed together and fed the respective diet _ad libitum_. Total body weights were taken on Day 1 of the experiment and again at the end of the eight-week feeding period (Figure 2 and 3). At this time all gerbils were sacrificed by placing them in a covered container with cotton saturated with chloroform. Animals were autopsied and weights of major organs (heart, liver, lungs, and kidneys) were taken (Figure 4). Immediately following weighing, the organs were discarded.

Figure 1. Percentage composition of diet 1 and 2. This figure shows the percentage composition of the ingredients in diet 1 and diet 2. Diet 2 contained 14% amaranth grain.


"Scratch"
67.0%
Rabbit Pellets
33.0%

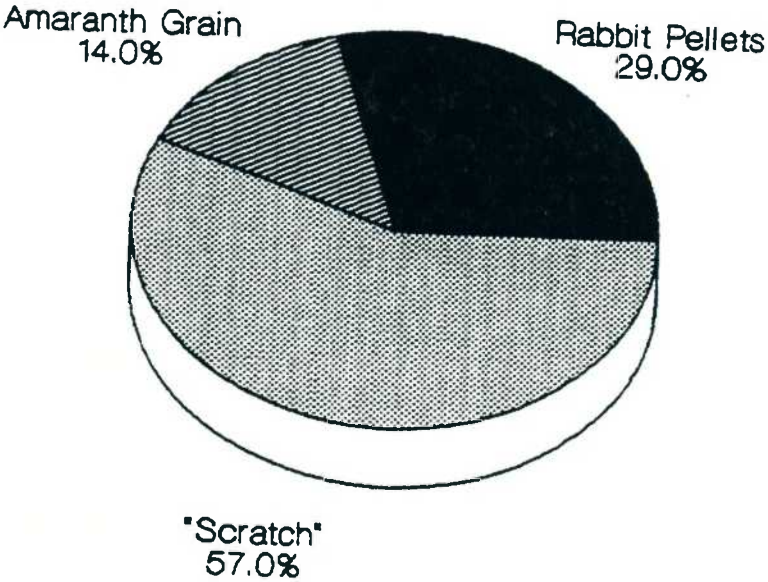
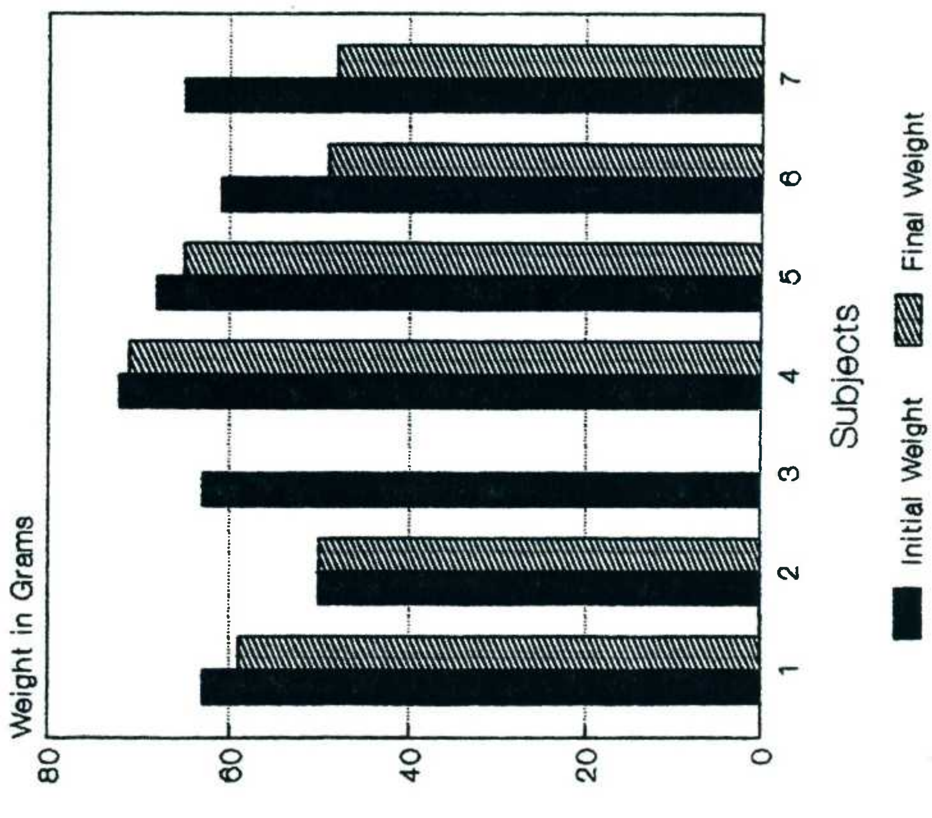

Amaranth Grain
14.0%
Rabbit Pellets
29.0%
"Scratch"
57.0%

Figure 2. Initial and final body weights for the gerbils fed diet 1. The initial weights were taken before the gerbils were subjected to their respective diet. This figure shows the difference between the initial and final weights after the eight week feeding period. The weights decreased during the eight week period.



Weight in Grams
80
60
40
20
0
1
2
3
4
5
6
7
Subjects
Initial Weight
Final Weight

Figure 3. Initial and final body weights for the gerbils fed diet 2 that contained 14% amaranth grain. The initial weights were taken before the gerbils were subjected to their respective diet. This figure shows the difference between the two weights after the eight week feeding period. The body weights changed significantly during the eight week period.

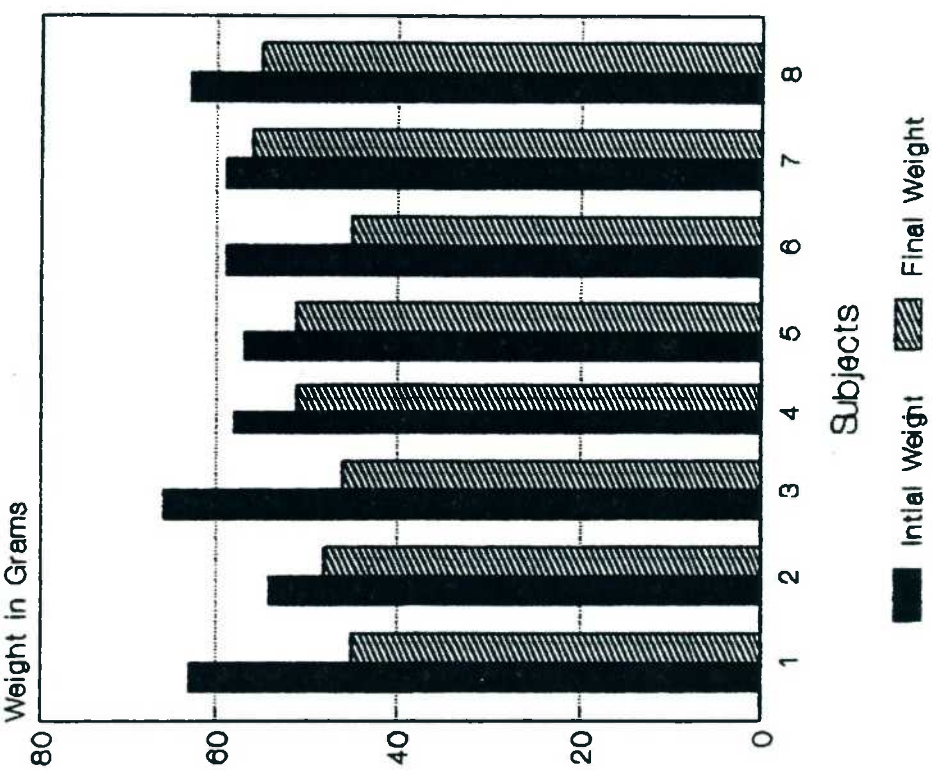
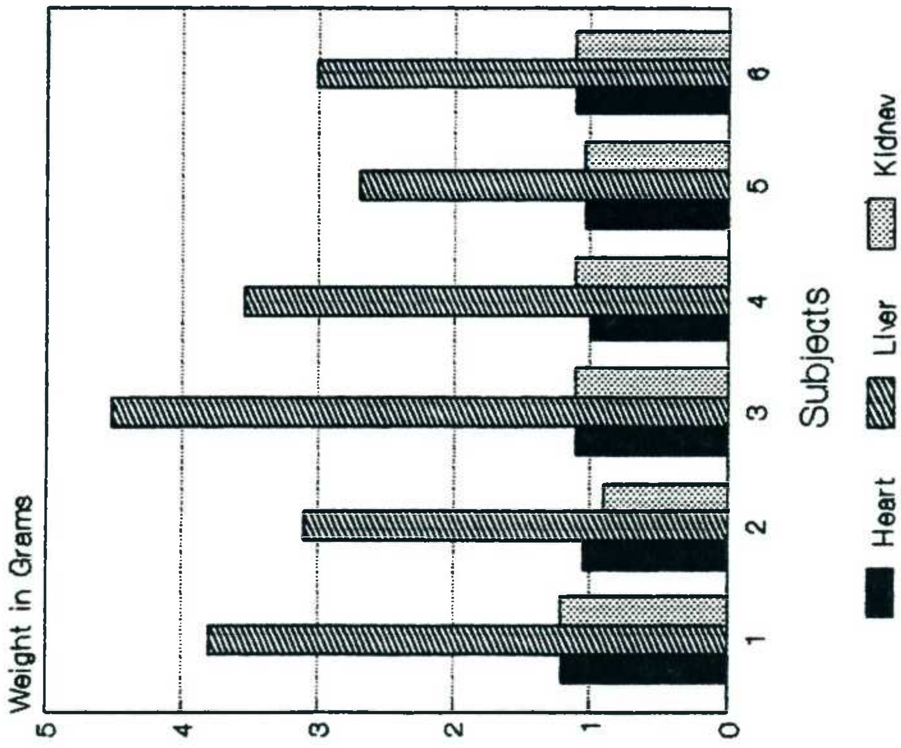
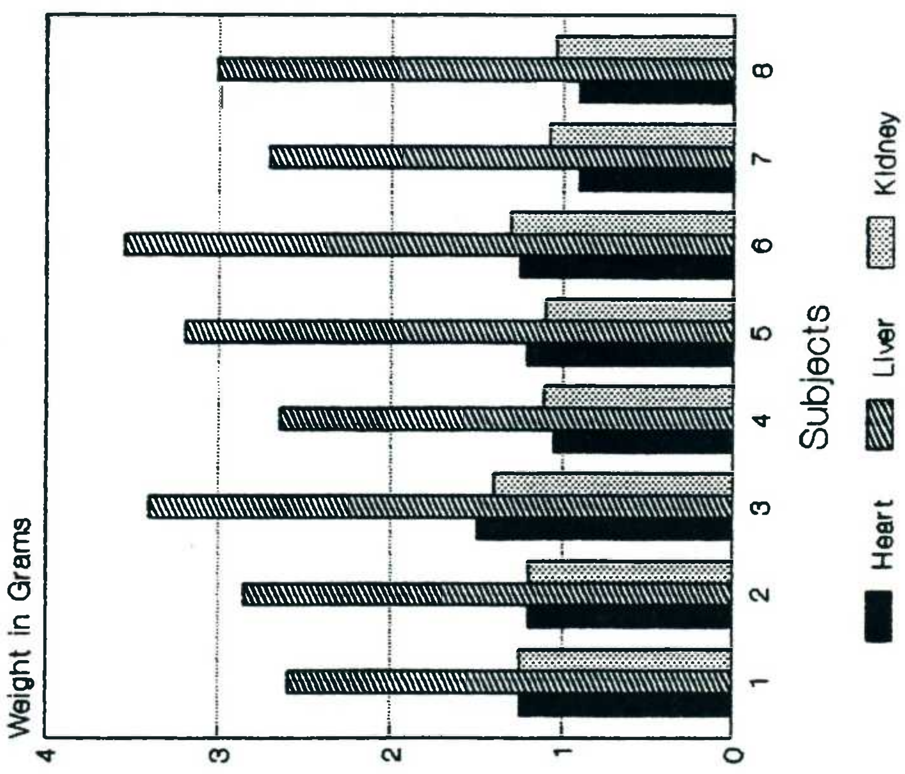

Weight in Grams
80
60
40
20
0
1
2
3
4
5
6
7
8
Subjects
Intial Weight
Final Weight

Figure 4. This figure shows the measurements of the organs (heart, liver, and kidney) of the gerbils that consumed diet 1. The heart and kidney were about in the same range, but the liver showed greater variations.



Weight in Grams
5
4
3
2
1
0
1
2
3
4
5
6
Subjects
Heart
Liver
Kidney

Figure 5. This figure shows the organ measurements of the gerbils subjected to diet 2. The weight of the heart and the kidney in each gerbil varied slightly. The liver weight of all the gerbils varied greatly.


Weight in Grams
4
3
2
1
0
1
2
3
4
5
6
7
8
Subjects
Heart
Liver
Kidney

CHAPTER IV

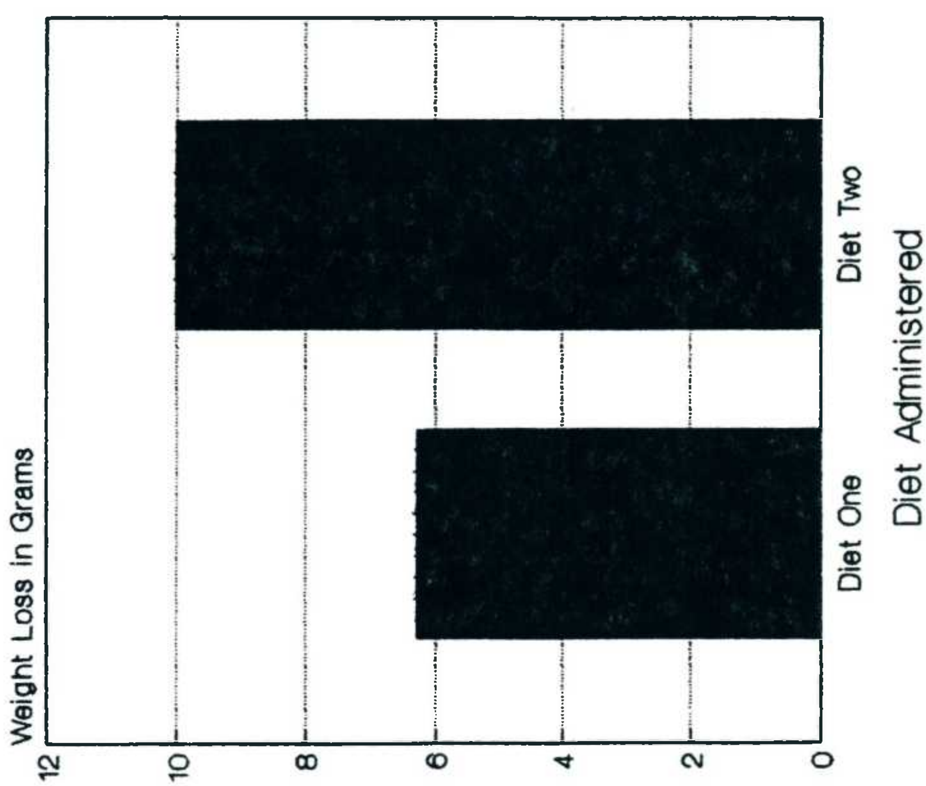
Results

During the eight week feeding period, one gerbil feeding on diet 1 died. No anatomical abnormalities sufficient to cause death were determined at autopsy. A second gerbil was not included in the results because weight change was due to pregnancy. At the end of the feeding period when the gerbils were sacrificed, they were active and alert with all appearances of being healthy.

Changes in initial and final total body weight are summarized in Figure 6. The initial weight of the seven gerbils fed diet 1 ranged from 49.6 grams to 71.8 grams. The mean initial weight for the group was 63.1 grams with a standard deviation of 6.4. At the end of the dietary treatment, individual weights ranged from 48 to 71 grams, with a mean weight of 57 grams and with a standard deviation of 8.7. Gerbils feeding on diet 1 lost more than 6 grams over the eight week period.

Among the gerbils on diet 2, individual weights ranged between 54.1 grams and 66.2 grams with an average weight of 59.8 grams and a standard deviation of 3.6. At the end of the assigned diet, individual weights ranged from 44.9 grams to 56.0 grams. The mean weight for this group was

Figure 6. Comparison of weight loss between diet 1 and diet 2 gerbils. This figure shows the mean weight loss of each group. Diet 2 caused greater weight loss (10.0 grams) than diet 1 (6.27 grams).


Weight Loss in Grams
12
10
8
6
4
2
0
Diet One
Diet Two
Diet Administered

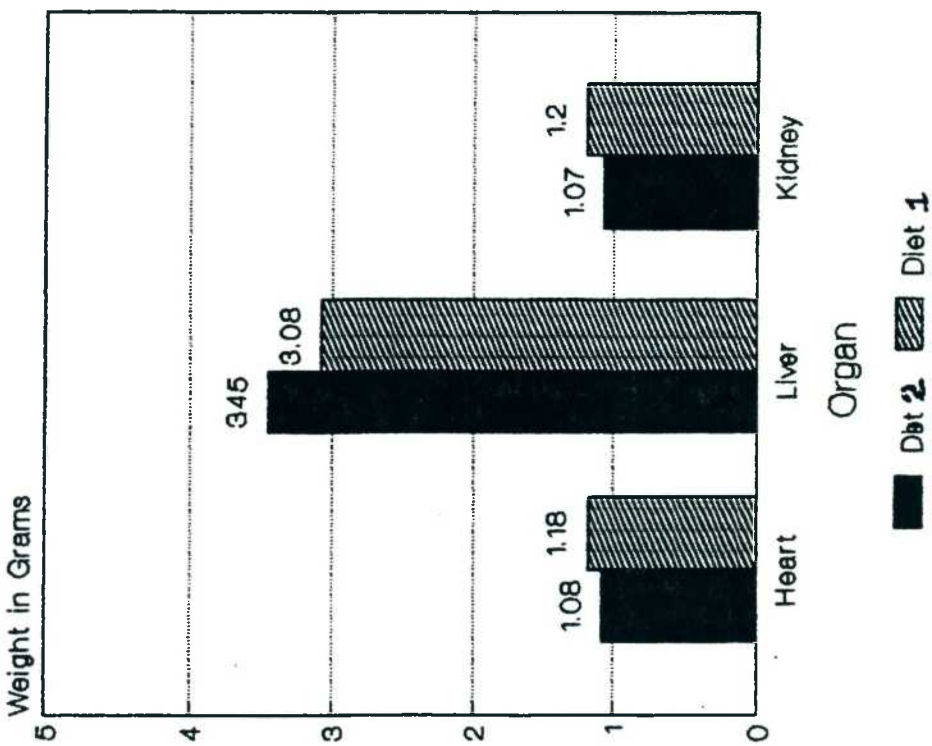
50.0 grams and the standard deviation of 3.7 indicated less variation from the mean body weight of this group than in group 1 at the end of the feeding period. Weight loss, however, was greater than in group 1.

Figure 7 summarizes the differences in the weights of the heart, liver, and kidneys between the two groups. The mean heart weights of gerbils feeding on diet 1 and 2 at the time of sacrifice were 1.08 grams and 1.18 grams, respectively. The standard deviation was greater in the diet 2 group (2.3) than in the diet 1 group (1.0).

Gerbils receiving diet 1 showed a greater liver weight than gerbils feeding on diet 2. These weights, 3.45 and 3.08, respectively, were expected to be greater than weights of other internal organs since the liver is known to be the largest organ in the mammalian body. Gerbils on diet 2 showed less variation in liver weights (3.7 standard deviation) than gerbils on diet 1 (standard deviation of 6.0).

Both kidneys were isolated from each gerbil, weighed and averaged to give one weight. The mean of these six weights for the diet 1 animals was 1.07 grams. Standard deviations were 1.1 and 1.4, respectively.

Figure 7. Comparison of organ weights between gerbils fed diet 1 and diet 2 This figure shows the average weight of the gerbils fed each diet. The heart and kidney of diet 1 weighed less than the kidney in diet 2. The liver in diet 2 weighed less than the liver in diet 1.


Weight in Grams
5
4
3
2
1
0
1.08
1.18
345
3.08
1.07
1.2
Heart
Liver
Kidney
Organ
Diet 2
Diet 1

CHAPTER V

DISCUSSION

Both diets 1 and 2 were a mixture of "scratch" and rabbit pellets. "Scratch," a mixture of grains commonly fed to chickens, contains cracked corn, sorghum grains, and whole wheat. Since the natural diet of gerbils contains mostly seeds and grains, it was hypothesized that they would be able to digest the various grains contained in the feed, including the amaranth. In the gerbil colony at Langston University, scratch is generally a part of the regular maintenance diet, with no adverse effects having been determined. Studies have shown that gerbils exhibit normal growth and reproduction patterns when fed other rodent diets. Therefore, the rabbit pellets were incorporated to add variation and possibly enhance the taste of the diet. No studies were found regarding physical or physiological responses of gerbils to amaranth grain. Since the grain is high in fiber, protein, essential amino acids, and vitamins and minerals and is the subject of ongoing research to enhance its value as a human and animal food source, this study was designed to determine the influence of amaranth on mammalian body and organ weight.

Weight loss was observed in gerbils feeding on diets

1 and 2, with a greater loss in gerbils feeding on diet 2 that included 14% amaranth grain. Reasons for the increased weight loss may be attributed to low intake of amaranth. The grains are very small (about the size of mustard seeds), and therefore much smaller than rabbit pellets and the grains contained in the "scratch." It is possible that these seeds were wasted in the bedding (since gerbils scratch almost constantly) and were not ingested to any significant degree. Although gerbils are granivorous, the possibility exists that certain grains, such as amaranth, with a high food value may be indigestible. The question of aging and body weight must also be considered. Gerbils have a short life-span of three to four years (Harkness and Wagner, 1989), which suggests that aging is a rapid process. It is possible that some weight loss can be expected with aging due to alterations in metabolism. The average weight of the gerbils on diet 2 were not in the normal range (55 to 100) of the ideal weight.

The heart and kidney are possibly at a body-weight ratio aspect. This would explain why the heart and the kidney in diet 2 are smaller in size than those in diet 1. The liver, however, in diet 2 weighed significantly more than the liver in diet 1. The liver functions in synthesizing cholesterol as a product of fat metabolism. On normal diets, gerbils have a high cholesterol level (Holmes, 1984). Amaranth grain has been recorded to have cholesterol-lowering properties (American Amaranth, Inc., 1984). Therefore, the increased liver size, of the gerbils in diet 2 may relate to increased synthesis of cholesterol or other lipids by the liver. More research is needed to determine the accurate data.

From the reported data, it can be concluded that dietary factors influence body weight and organ weight in certain organisms. These findings were not in agreement with the hypothesis that the gerbils could completely digest the amaranth grain and that the high nutritional quality would maintain normal to increased growth.

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VITA

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Bachelor of Science

and

Completion of

E. P. McCabe Honors Program

Thesis: HOW AMARANTH GRAIN AFFECTS TOTAL BODY WEIGHT AND MAJOR ORGAN WEIGHT IN THE MONGOLIAN GERBIL

Major: Biology

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