EFFECT OF CALCIUM DEPRIVATION IN

THE DIET OF WHITE RATS

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CHAPTER I

INTRODUCTION

In many parts of the world, including the United
States, osteoporosis is an important problem. There are
approximately four million cases of severe osteoporosis
in the United States today (24). This clinical disorder
is characterized by a decrease in total bone mass without
any known change in its chemical composition (2). This
disease is widespread, particularly during middle and old
age in both men and women. However, osteoporosis usually
appears earlier in women than in men (47), and according
to Lutwak and Whedon (24), the incidence is 1.5 times
higher in women than in men.

Researchers have followed many theories in an attempt
to outline the etiology of osteoporosis. Although it is
recognized that the causes are complex, beliefs exist
that lack of calcium in the diet and duration of negative
calcium balance play vital roles in determining the onset
of the clinical symptoms of the disease. If the body loses
calcium at the rate of 100 mg. per day, then it would take
approximately ten years to develop osteoporosis. In view
of these circumstances, one may possess a tool for assess-
ing calcium nutrition throughout life (12).

Statement of the Problem

Interest in osteoporosis and in calcium nutrition resulted from the author's concern for the health of many of the people throughout the entire world who suffer from this metabolic disorder. This concern extends to those people who are allergic to milk and milk products, which are the main sources of calcium in the diet. Many doctors support the erroneous view that after the stressful periods of life, such as growth, pregnancy and lactation, people no longer require large intakes of calcium. However, there are over 45 references which support the theory that calcium deficiency can cause osteoporosis (23).

In this research the author will study the effects of inadequate calcium nutrition in relation to bone density changes in the weanling albino rat. One group of randomly selected albino rats will serve as the control group, receiving ad libitum distilled water and a dietary ration containing all known nutrients in amounts needed for optimum nutrition in the white rat. The experimental group, also randomly selected, will receive ad libitum distilled water and a diet severely deficient only in calcium.

Assumptions

The following assumptions are accepted as true:

1. The albino rat is a suitable laboratory animal for demonstration of the effect of calcium deprivation on bone density.

2. A dietary ration can be composed which is adequate in all nutrients except calcium.

3. Calcium deficiency is a primary cause of osteoporosis.

4. Lowered amounts of calcium or phosphorus and vitamin D in the diet lead to rickets in young rats and osteomalacia in adult rats.

Hypotheses

The following hypotheses are postulated:

1. Calcium deprivation in albino rats leads to a diminution in total bone mass (osteoporosis).

2. The femurs of calcium deprived rats break under less weight than femurs of control animals.

3. The overall body length and weight of control rats is greater than that of the calcium deprived rats.

After approximately one month of feeding, each rat will be evaluated according to: weight, animal length, visual evidences of health, femur densities, and femur fragilities. Collected data will be analyzed statistically.

CHAPTER II

REVIEW OF LITERATURE

In this chapter some of the literature which pertains
to the study of bone loss from animal skeletons is reviewed.
A short discussion of normal bone conditions is followed by
a discussion of the functions of calcium and phosphorus in
the skeletal system. The relationship between vitamin D,
calcium, and phosphorus also is discussed. The condition
of osteoporosis as it relates to bone composition is examined
as is the relationship of osteoporosis to osteomalacia.
Finally, a comprehensive discussion of the use of the rat
as an experimental animal is covered.

Normal Bone Development

The metabolism of bone is an extremely complicated and
continuous process. Throughout the life process, an ade-
quate supply of nutrients is necessary for the normal growth
and maintenance of the osseous system.

Normal bone is a dense, highly specialized form of
connective tissue. The hardness, strength, and rigidity
of the bone result from the process of calcification which
is the deposition of a combination of calcium, phosphate,
carbonate, and citrate within a soft organic matrix (6).

The organic matrix is composed of approximately 95 per cent collagen fibers and 5 per cent ground substance containing mucopolysaccharides (17). The normal bone is 30 per cent organic matter and 45 per cent mineral matter. The water content of the long bones is 18 per cent as compared to that of the vertebrae, which are 40 per cent (16). The nitrogen content varies from 3.5 to 5 per cent, according to age.

The structure of the long bone is of two types: the shafts (diaphyses), composed of compact bone; and the expanded ends (epiphyses), composed of spongious bone covered with a thin layer of compact bone. The compact portion accounts for the mechanical stability of the bone and the cancellous bone serves as a buffer to maintain chemical homeostasis of the body fluids. Compact bone is that in which marrow spaces are replaced by cylindrical, concentrically laminated Haversian systems which are arranged around a central vascular channel, the Haversian canal. The cancellous bone is a form in which the organic matrix is arranged in a network of rods, plates or tubes, such as the trabeculae, between which are spaces filled with bone marrow (6).

The cellular components of bone are the osteoblasts, the osteocytes, and the osteoclasts. The osteoblasts are located on bone surfaces and are concerned with the formation of the bone. The osteocytes, which are located in the lacunae, are responsible for the maintenance of the bone as living tissue. Finally, the osteoclasts, which are found

on the bone surfaces, are concerned with the destruction
or the resorption of the bone (6).

Except during periods of stress or disease, the rate
of bone deposition and bone absorption are normally equal
to one another so that the total mass of bone remains
constant (18). This continual deposition and absorption
of bone has several physiologically important functions.
Bone usually adjusts its strength in proportion to the
degree of stress on the bone. Whenever bones are subjected
to carrying heavy loads, they become thickened. In addi-
tion, the actual shape of the bone can be rearranged by
this process in accordance with the stress applied to it.
As old bone becomes weak and brittle, new organic matrix
is formed as the old organic matrix degenerates. Thus,
the normal toughness of the bone is maintained. However,
the rate of deposition and absorption decreases with age(17).

The Function of Calcium in the Skeletal System

Calcium is present in the human body to a far greater
extent than any other mineral. According to Sherman (39),
approximately 99 per cent of the body's calcium is present
in the skeletal system, whereas the other one per cent is
contained by the soft tissue. The mineral constituents of
the bone account for the rigidity of it, giving support
and shape to the body.

In the soft tissues, calcium regulates the body
processes. It activates enzyme systems, helps in the

coagulation of the blood, accounts for the contraction and relaxation of muscle cells, maintains and controls neuro-muscular excitability, and keeps the walls of the soft tissues permeable so that substances necessary for the life of each cell can enter and leave (49).

The major role of calcium, however, is in the building and shaping of the body's framework. The bone tissue is composed of hydroxyapatite which is a small, hexagonal, crystalline compound of calcium and phosphorus set in an organic protein matrix (46). Because of the small size of the hydroxyapatite, the bone crystal surfaces offer a large surface area in which compounds are easily absorbed (19). The hydroxyapatite crystals are bathed in intracellular fluid in which an exchange among the mineral constituents, the bone and the body fluids, is favored (5).

The skeletal system, in addition to being a labile tissue, appears to be a firm and inert tissue which main-tains the rigid stability of the body (16). The labile supply of calcium and phosphorus in the bone is present mainly in the trabeculae. These lace-like structures are situated throughout the bones and are in close contact with the blood stream, thus keeping the blood calcium constant (8). Abundant supplies of calcium in the diet are necessary for the development of the bone trabeculae as presented in Bogert, Briggs, and Calloway (5). If calcium is not present in the diet in a supply sufficient to meet the nutrient needs of the body, the trabeculae

POORLY DEVELOPED TRABECULAE   WELL DEVELOPED TRABECULAE

Fig. 1. Diagrammatic representations of bone trabeculae showing poor or good development according to whether the food calcium intake is low or liberal.   (5, p. 150)

are called upon to supply the calcium. In the event that inadequate calcium intake exists for extended periods of time, then the trabeculae will be poorly developed (5). Also, these bones are weakened and break easily.

It has been revealed by dietary surveys that calcium is a nutrient often lacking in the diet. According to Sherman (39), researchers in Britain, England, Scotland, the United States, China, Australia, Africa, and India found that the level of calcium in the diet was far below recommended standards established by the National Research Council. Milk and milk products provide 285 milligrams of calcium or three-fourths of the calcium present in food. Unless these products are consumed daily, it is virtually impossible to attain the Daily Recommended Allowance of 800 milligrams per day unless calcium supplements are provided. Other foods which are considered good sources of the mineral do not contribute as much calcium as might be expected to the diet because such foods as dried fruit,

dark green leafy vegetables, molasses, and dried beans are eaten in minute quantities (5). In a table taken from Bogert, Briggs, and Calloway given on the following page, the figures under the calcium and phosphorus columns give the amounts of each mineral furnished by an average serving of each food in either raw or cooked form. The second column of numbers under each mineral gives the number of milligrams present in 100 grams of food substance (5).

The Function of Phosphorus in the Skeletal System

Phosphorus deficiency is uncommon in humans except during infancy when muscle growth creates high phosphorus requirements. This is essentially attributed to the vast amount of food which contains phosphorus. Phosphorus is abundantly available in meat, and in seed or grain. A detailed list of phosphorus-containing foods taken from Bogert, Briggs, and Calloway (5) are given on the following page.

Phosphorus deficiency is sometimes a limiting factor in crop production, and it can be alleviated by adding phosphorus to fertilizer. In Africa, oftentimes, cattle have developed a fatal disease characterized by the animals eating bones as compensation for the lack of phosphorus (39). The main cause of this aphosphorosis in cattle is attributed to the consumption of plants grown in phosphorus-deficient soil. The findings about the etiology of this disease have given support to the theory that phosphorus is necessary in

TABLE 1   Foods Richest in Calcium and Phosphorus

Calcium (in milligrams)			Phosphorus (in milligrams)		
Food	Per Avg. Svg.	Per 100 gm.	Food	Per Avg. Svg.	Per 100 gm.
Milk, 8 oz. glass, ½ pint	285	118	Liver, fried, 2 sl., 75 gm.	311	358
Cheese, Am. Cheddar, 1 oz.	225	750	Milk, 8 oz. glass, ½ pint	227	93
*Leafy vegetables, avg.			Cod steak or sole, 100 gm.	220	220
½ cup cooked	140	167	Lamb, leg, roast, 2 sl., 100 gm.	208	208
Ice cream, plain, avg.			Beef, rib roast, 1 sl., 100 gm.	186	186
¾ cup	123	123	hamburger, ¼ lb., 85 gm.	165	194
†Molasses, med., 2 tbsp.	116	290	Baked beans, canned, ½ c.	120	92
Artichokes	102	51	Cheese, Am. Cheddar, 1 oz.	140	478
Broccoli, ⅔ cup	88	88	Peanut butter, 2 tbsp. sc.	118	393
Baked beans, canned with			Shredded wheat, 1 biscuit	102	360
molasses, ½ cup	82	56	Whole wheat cereal, ½ c., ck.	113	83
tomato sauce, ½ cup	70	49	Oatmeal, ⅔-¾ c. cooked	105	57
Cream, light, 7 tbsp.	74	97	Cottage cheese, 2 rd. tbsp.	108	189
Orange, 1 medium	62	40	Egg, 1 large	101	210
‡Cottage cheese, 2 rd. tbsp.	52	96	Ice cream, plain, avg., ¾ c.	99	99
String beans, ⅔ c., ck.	50	50	Cream, light, 7 tbsp.	77	77
Parsnips, ½ c. cooked	44	57	Broccoli, ⅔ cup	76	76
Lima beans, ½ c. cooked	38	47	Nuts, mixed, 8–12 nuts, ½ oz.	67	446
Salad greens, raw, avg.			Parsnips, ½ c., cooked	62	80
2 lg. or 4-5 sm. leaves	34	68	Lima beans, ½ c., ck.	97	121
Egg, 1 large	26	54	Peas, canned, ½ cup	62	77
Figs, dried, 1 large	25	125	Corn, canned, ½ cup	43	52
canned, 3, with juice	13	13	Cauliflower, ¾ cup, ck.	42	72
Bread, w.w., 1 slice	23	96	Leafy vegetables, ½ c. ck., avg.	45	45
white, 1 slice (4%			Bread, w.w., 1 slice	60	263
milk solids)	19	79	white, 1 slice		
Peanut butter, 2 tbsp. sc.	22	74	(4% milk solids)	21	92
Peas, canned, ½ c.	20	25	Apricots, dried, ck., 3 halves	34	34
Apricots, dried, ck.,			Figs, dried, 1 large	33	111
4 halves	20	22	canned, 3, w/juice	21	35
Orange juice, 6 oz.	20	25	Prunes, 4-5 medium, ck.	27	40
Dates, 3-4 pitted, 1 oz.	22	72	Dates, 3-4, pitted, 1 oz.	18	60
Prunes, 4-5 medium, ck.	17	25	Orange, 1 small	37	37
Grapefruit, ½ medium	16	16	String beans, ⅔ c., ck.	19	23
Cereal, whole-grain, avg.			Grapefruit, ½ medium	16	16
⅔ - ¾ c., cooked	8-15	9			

*Excluding spinach, beet greens, and chard, in which calcium is in nonutilizable form; including dandelion, mustard, turnip greens, collards, and kale.

†The calcium in molasses is due to addition of lime to neutralize acid in refining sugar; it is in lowest concentration in light molasses and highest in the blackstrap variety.

‡Calcium content of cottage cheese varies according to whether it is made from sour milk or by addition of rennin to sweet milk.   (5, p. 157)

the maintenance of health.

In the human body, there is approximately half as much phosphorus as there is calcium. There is 86 per cent of phosphorus in the skeleton in the form of inorganic phosphate (PO_4) (49). The other 14 per cent is found in body fluids and soft tissues.

Phosphorus exists in many organic combinations important to the physical transport and the chemical transformation of the organic food and body components in the nutritional process (39). In the many-fold functions in the body, phosphorus is essential as follows: high-energy bond formation for anabolic and catabolic reactions; phospholipids in the formation of cell membranes and in cellular permeability; synthesis of genetically significant substances, specifically (DNA) deoxyribonucleic acid; buffering capacity of body fluids and cells; and formation of hard tissues of the body (49). In combination with calcium, phosphorus is also a component of the hydroxyapatite (3 Ca_3 (PO_4)$_2$ Ca (OH)$_2$), an insoluble calcium phosphate salt. Therefore, the formation of the skeletal system is dependent upon the presence of adequate amounts of calcium and phosphorus. Whenever dietary levels of phosphorus are extremely low, the needs of cellular tissues are cared for at the expense of the skeleton (8).

The Calcium-Phosphorus Ratio

Normal calcification of the osseous system in the growing and developing human body clearly requires adequate intakes of both calcium and phosphorus (50). The normal calcium-phosphorus ratio in the infant's blood is ten milligrams of calcium and five milligrams of phosphorus per 100 cc of blood, a ratio of 2:1. Thus, in health throughout the growth period this ratio remains relatively constant.

Sherman (39) reviewed the work of Quinn who found that the gain in phosphorus was twice as great as the gain in other nutrients. The simultaneous gain of calcium is about twice as great as that of phosphorus. Therefore, these retentions of calcium and phosphorus during the growth period are largely devoted to normal mineralization of the skeleton in which the ratio of calcium-to-phosphorus is 2:1. In addition, Sherman showed that if the calcium was suboptimal and calcium retention was slow, the rate of gain of phosphorus in the body was also retarded. Sherman then concluded that in a healthy individual, the calcium-phosphorus ratio of bone mineral does not differ greatly from 2:1.

In his research, Walker (48) found that it is almost universally accepted that a low calcium diet with a distorted calcium-phosphorus ratio is conducive to stuntedness. In tropical and semitropical countries, the ratio

may be as adverse as 1:10. Nevertheless, he felt other factors such as heredity, sex, and seasonal factors had more effect than the calcium-to-phosphorus ratio.

In any event, the minerals calcium and phosphorus are necessary for optimum nutrition. According to the results of recent dietary surveys by the National Research Council, calcium is found to be the most frequent nutrient lacking in the diet (9). If a diet contains one pint of milk, usually it will provide about 0.8 to 1.5 grams of calcium and 1.5 to 2.0 grams of phosphorus. The minimal daily requirement for calcium for the adult is 0.8 grams; for a child, 0.8 grams; for teenagers, 1.1 grams to 1.4 grams; and for pregnant women or nursing mothers, 1.3 grams (9). The minimal daily phosphorus requirement for an adult is approximately 0.8 grams; for a child, about 1.3 grams; for pregnant women, 1.5 grams; and for nursing mothers, 2.5 grams (16).

There has been much controversy over the minimum daily requirement for calcium. In 1963 a symposium was held concerning the human requirement for calcium (11). Six authorities from the Council on Foods and Nutrition of the American Medical Association discussed this contro- versial subject. The 1963 recommended allowances reaffirmed the fact that 0.8 grams of calcium was needed by most healthy people to cover all bodily needs. Furthermore, the calcium requirement in the 1968 revision of the daily recommended allowances remained unchanged.

With deficient amounts of either calcium or phosphorus
in the diet, the desirable calcium-phosphorus ratio would
be altered. Attention has been focused upon calcium de-
ficiency because of its prevalence throughout many parts
of the world (39). If the supply of milk is limited, as in
the case of many poor and underdeveloped countries, then
it is virtually impossible to obtain adequate amounts of
calcium in the diet.

Consequently, most of the world's population thrives
on diets not only deficient in calcium but with badly dis-
torted calcium-phosphorus ratios (26). Because the diet of
these people contains mainly cereals, grains, and vegetables,
it is not surprising that this poor calcium-to-phosphorus
ratio does exist. In the Orient, for example, milk is used
only in the diet of infants and invalids. The main source
of calcium in the diets of these people comes from an acidic
mixture of vinegar, soy sauce, sugar and starch used in the
cooking of fowl, pork, and fish. After extended periods of
cooking these meats, the solution penetrates the bone mar-
row and makes the insoluble calcium phosphate soluble (20).
This method of meat preparation provides the major source
of calcium in the Oriental diet. Nevertheless, the calcium-
phosphorus ratio remains inadequate.

Although the ratio of calcium-to-phosphorus is impor-
tant in assessing dietary adequacy, one should be cognizant
of the actual levels of these minerals in the diet (49).
Diets which contain an unfavorable calcium-phosphorus ratio

or an inadequate content of either or both of these minerals
may allow growth of the soft tissues to proceed while the
skeletal system is inadequately supplied with either mineral.
The result is bone loss.

Vitamin D and the Utilization of Calcium and Phosphorus

Vitamin D is necessary for the normal absorption and
utilization of calcium and phosphorus in the body as well
as for the deposition of lime salts in the formation of
bone (16). Unlike the other vitamins, vitamin D can be
formed within the body by the action of ultraviolet light
on 7-dehydrocholesterol, or other sterols of the skin.
The occurrence of vitamin D in many foods is not abundant.
However, it is provided by eating egg yolk, butter, forti-
fied margarine, and fish liver oils. In addition, many
milk products and cereals have been fortified with
vitamin D.

Usually the requirement of man for vitamin D is ful-
filled from ingestion of natural foods and from the action
of ultraviolet rays of the sun on the precursors of the
vitamin present in the skin. People may live in areas
where they are exposed only to winter sunshine in northern
regions of the world, or sunshine passed through clouds or
certain glasses. Consequently, the supply of ultraviolet
rays from the sun is inadequate for the production of
vitamin D. Likewise, in the Middle East where religious
practices prevent the women from exposing themselves to

sunlight, oftentimes, it is impossible for them to obtain minimum requirement of 400 I.U. of vitamin D. In the foregoing examples, administration of fish liver oils or therapeutic doses of vitamin D are necessary to prevent the loss of calcium and phosphorus from the bone. A deficiency of vitamin D is the direct cause of rickets in infants and osteomalacia in adults (16).

In certain torrid zones exposure to solar radiation is extremely abundant. Researchers (42) observed that in eating subnormal intakes of calcium, the absorption of calcium was to a greater degree as compared with a similar group of women living in the temperate zone. This impaired absorption of calcium, attributed to vitamin D deficiency, has been considered as a factor in negative calcium balance in some women with skeletal atrophy. Nonetheless, the extent to which climate and geography are responsible for the levels of this vitamin in man has not been reported.

Vitamin D favors the utilization of calcium and phosphorus by improving the absorption from the intestine into the blood. The acid medium of the ingested food mass from the stomach then enters the small intestine. The majority of this process takes place in the upper portion of the small intestine. The acidic nature of this food aids in keeping the insoluble calcium salts soluble. On the other hand, the alkaline condition of the lower intestine does not favor the absorption of these salts (49). Normally, one can expect only about 70 per cent absorption of

phosphorus and 20 to 40 per cent absorption of calcium.
In the absence of vitamin D, however, the fecal loss of
calcium and phosphorus is increased because the transfer
of these minerals through the intestinal membrane is res-
tricted. The intestinal mucosa constitutes a barrier to
the calcium and phosphorus (36).

In addition to vitamin D, many other factors affect
the absorption and utilization of calcium and phosphorus.
Phytic acid, commonly found in cereal products, combines
with calcium to form an insoluble calcium complex. This
can be a significant problem in the milk and cereal diet
of small infants. Although milk contains the sugar lactose,
which promotes the utilization of calcium (36), the phytic
acid can form insoluble calcium salts, which are excreted
(17). Oxalic acid, present in spinach, presents the same
type of problem. However, nutritionists believe that the
other nutrients obtained by eating spinach merit occasional
inclusion in the diet.

During various disease states, the absorption of
calcium, phosphorus, and vitamin D is prevented. In mal-
absorption or sprue, the patient is unable to absorb fatty
acids and fat-soluble vitamins. Since vitamin D is a fat-
soluble vitamin, the absorption of this vitamin is hindered.
Because vitamin D is essential for the absorption of cal-
cium, the unabsorbed fats and calcium ions combine in the
intestine to form insoluble calcium soaps. This inability
to absorb the calcium causes the patient to become quite

irritable and nervous (21).

Ulcer patients, oftentimes, are administered large amounts of milk and antiacids to relieve gastric pain. These alkaline substances combine with the calcium and phosphorus in milk to form insoluble calcium soaps. Therefore, these patients are unable to absorb calcium and phosphorus.

Following gastric resection, the patient often has the symptom of hypermotility or rapid emptying of the contents of the stomach into the intestine. This symptom causes a decrease in the absorption of calcium, phosphorus, and many other nutrients. It has been noted (32) that many post-gastrectomized patients do develop osteomalacia. Certain therapeutic agents have been recommended to increase calcium absorption, such as penicillin, chloramphenicol, and neomycin in order to prevent other diseases which may be attributed to the failure to absorb vitamin D, calcium and phosphorus.

Osteoporotic Bone Loss

Osteoporotic bone loss is a worldwide health problem of major magnitude. It is not just a problem of the fragile, inactive, elderly female because it occurs frequently both in men and women during middle and old age (14).

In osteoporosis there is a decrease in total bone mass with no apparent change in its chemical composition (2). This metabolic disorder is characterized by decalcification,

increased porosity, and weakening of bony structure to such

an extent that fractures occur under relatively minor

mechanical stress (35). In addition, pain resulting from

this disorder may be either localized or generalized, the

latter occurring when the spine is affected (12). However,

the symptoms of the disease are not always apparent until

fractures of the bone occur. Osteoporotic bone loss will

not be apparent radiologically until 25 to 50 per cent of

the calcium has already been lost (24).

The exact cause of osteoporosis in the human has not

been determined. However, there are 45 references support-

ing the theory that a lowered intake of calcium throughout

adult and later years can cause bone loss (23). If there

is not an adequate supply of calcium in the diet, then

calcium is withdrawn from the body's storehouse, the

trabecular and cortical areas of the bone. Gradual bone

resorption proceeds at a rate greater than that of bone

formation. Therefore, calcium deficiency may play an

important role in the etiology of this bone disorder (11).

Another factor to consider in the etiology of this

disorder is that many people may adapt to calcium intakes

below what is recommended while others may not have this

capacity (45). Without the ability to adapt to varying

levels of calcium intake, calcium can be withdrawn from

the skeleton since no other reservoir of calcium exists.

This can also result in osteoporotic bone loss (33). It

is not surprising that this condition often responds to

increased amounts of calcium in the diet.

Numerous animal studies have been conducted in support
of the theory that the calcium-phosphorus ratio in the diet
has a direct effect in determining whether the bones will
be normal or pathological. In 1921 McCollum and associates
(27) demonstrated an interaction between calcium and phos-
phorus in nutrition by producing severe osteoporosis with
accompanying multiple fractures in the second generation
of rats. Since that time, the calcium-to-phosphorus ratio
has been the subject of many investigations. Data collected
by other researchers (3) revealed that, in the absence of
vitamin D in rats, the proportion of calcium-to-phosphorus
in the ration should be maintained in relatively narrow
limits in order to secure maximum growth rates. As demon-
strated by Shohl and Wolbach (40), the interrelationship of
both ratios and absolute amounts of calcium and phosphorus
in the diet of rats has an effect on the development of
the skeletal system.

In 1955 Steenbock and Herting (44) found that when
young rats were fed a diet deficient in vitamin D and phos-
phorus, rickets resulted from this altered calcium-phosphorus
ratio. The bones of these animals were soft and low in
ash content with enlargement at the ends of the long bones.
However, when vitamin D and calcium were deficient, the
bones became brittle and porotic without enlargement at
the ends. The latter rats were suffering from osteoporotic
bone loss.

Nordin (28) reviewed the research of Light and Frey as well as Harrison and Fraser. From their findings, the former concluded that in the presence of Vitamin D, whenever the calcium-to-phosphorus ratio was altered by a subsequent lowering of calcium, rats developed osteoporotic bone loss. The bony structures in this disease were hard, but light and fragile. These thin-walled bones showed no evidence of rickets, such as enlargement of the ends or easy malleability.

Results of a recent study (38) indicated that the cortical thickness and mineral content of femurs of aged mice were depressed by chronic consumption of a dietary ration in which there was a suboptimal amount of calcium. In addition, in the aging process they concluded that the porosity of the bones was not related to the level of calcium intake during adult life, but it was enhanced when calcium and phosphorus were consumed in the ratio of 1:1 as opposed to 2:1.

In contrast to the relatively narrow limits of this ratio in laboratory animals for securing maximum growth rates, researchers (51) found that ruminants are less sensitive to the calcium-phosphorus ratio. The most favorable ratio for the ruminant is 8:1. In England 63 lambs were fed diets with low amounts of phosphorus but with adequate amounts of calcium. The availability of phosphorus was lowered by a diet deficient in phosphorus. Nonetheless, the main conclusion was that ruminants tolerate wider

calcium-phosphorus ratios than do non-ruminants.

Since calcium deficiency or an adverse calcium-phosphorus ratio produces osteoporotic bone loss in animals, it seems reasonable that this bone loss will also occur in humans. However, direct measurement of bone deterioration and detailed analysis of bone composition in humans is impossible. Therefore, calcium balance techniques, dietary survey studies and roentgenological methods have been employed to study this condition (30).

Grollman (16) reviewed the study by Nordin, in which a negative calcium balance was demonstrated in many individuals who had idiopathic, senile or postmenopausal osteoporosis. This condition was corrected, as shown by a positive calcium balance, by providing diets adequate in calcium and all other nutrients. Nevertheless, little or no improvement was noted roentgenologically.

Balance studies have not taken into account the dermal losses of calcium. Consolazio et al (10) noted that, when an individual consuming a low calcium diet is subjected to heavy sweating conditions, it is questionable if he ever attains a positive calcium balance.

Further evidence of the effect of the adverse calcium-phosphorus ratio in which calcium was low is supported by the study of Birge and colleagues (4). There was an association between intestinal lactase deficiency and osteoporosis. This study indicated that osteoporotics developed their disease through long-term dietary restriction of

lactose, a carbohydrate primarily present in milk and milk products. This theory seems probable because milk also is the chief source of calcium.

These foregoing studies support the theory that low calcium intake, resulting in adverse calcium-phosphorus ratios in the diet, may be a limiting factor in the nutritional well-being of the skeletal system. For a vast number of people, calcium deficiency may be a causative factor in the development of osteoporotic bone loss. This substantiates the view that abundant dietary calcium is necessary throughout life (45).

Relationship of Osteoporosis to Osteomalacia

Osteoporosis and osteomalacia are recognized as important chronic diseases of the skeleton. However, they are classified in separate categories of bone disease. Osteoporosis is a condition in which bone matrix formation is inadequate for supporting normal skeletal growth and maintenance (1). The second type of bone disorder, commonly referred to as an adult form of rickets, results from a condition in which matrix formation is unimpaired. Nonetheless, the calcium-phosphorus ion product in the fluid in the intercellular bone spaces is inadequate to promote the formation of new osteoid tissue of the growing skeleton, a process resulting in rickets in young bone. In mature bones, this failure of replacement of the osteoid tissue of normal bone lysis results in the disease osteomalacia (1).

The main distinction between these types of bone disorders is that in osteoporosis there is a decreased amount of bone tissue whereas in osteomalacia there is a failure of mineralization of the bone (41). In both disorders the mechanical functions of the bones are affected.

Ordinarily, osteoporosis manifests itself clinically during old age, but it may begin in the early twenties. This bone loss is believed to be a continuous process in which the rate of bone loss increases progressively with age. This condition may be aggravated by immobilization, certain endocrine disorders, and a negative calcium balance (34).

As the osteoporotic changes progress, the older person is more liable to fractures resulting from minimal trauma than is the younger person (2). Severe backache and shrinking of the skeleton attributed to the collapse of the vertebrae are common symptoms of the disease. This metabolic bone disorder is characterized by a decrease in the bony tissue per unit volume of bone in the affected part of the skeleton (29). In contrast to osteomalacia, no specific biochemical abnormality is present. Most authors agree that the chemical composition of the bone is essentially normal although there is a decrease in bone tissue (13).

There is a reduction in the ash content of the bone with no reduction in bone mass in osteomalacia (16). The bones become soft, flexible and enlarged at the ends with excessive osteoid borders in the vicinity of the epiphyseal

cartilage. In osteoporosis, the bones are fragile, brittle and thin with no excessive osteoid seams.

Osteomalacia is usually attributed to a deficiency of vitamin D. This deficiency disease is frequently encountered in women who avoid sunlight for religious or cultural customs and those women who have an increased requirement for vitamin D because of repeated pregnancies and lactation (16). Since vitamin D is necessary for the utilization of calcium and phosphorus, it is probable that a deficiency of these minerals would also be present. In steatorrhea, the disease in which there is a failure to digest fat, undigested fats, the fat-soluble vitamin D and calcium combine to form insoluble calcium soaps (17). The disease osteomalacia is frequently encountered during steatorrhea. In both osteomalacia and osteoporosis, calcium is poorly utilized.

The Rat as an Experimental Animal

Through experimentation with the albino rat, many new improvements have been instigated and much progress has been made in the field of nutrition. The selectively-bred albino rat serves as a desirable laboratory animal for demonstrating the effect of various nutritional deficiencies, especially bone disorders. Among the many virtues that distinguish this animal as desirable for laboratory research in nutritional experiments are the following:

1. The biological body processes, such as digestion,

assimilation and circulation are comparable to those of the human (22).

2. The life cycle of the rat is approximately three years, or one day is equal to thirty days in the life-time of the human being (22). Therefore, effects of nutritional deficiencies can be observed over a life-time or even generations of the rat.

3. The rat will consume and respond to different foods in a similar manner to the way in which the human does. However, the rat does not require vitamin C as his body will manufacture it. Hence, he is not suitable for observing vitamin C deficiencies.

4. Maintenance for the albino rat is relatively inexpensive mainly because of his size and his minimal requirements for food.

5. Although the rat is quiet, gentle and easily handled, he does not require human affection.

6. Albino rats thrive well on highly purified dietary rations. Therefore, the exact components of the diet can be analyzed for nutrients with greater ease than diets containing natural foods. Many nutritional deficiencies can be imposed upon the animal.

To prepare a dietary ration for the rat, it is of importance to know his requirements for the various

nutrients. According to Griffith and Farris (15), the rat

requires the following:

Nutrient	Amount per Day
Protein	25--30%
Calcium	40--50 mg.
Phosphorus	35--40 mg.
Sodium	0.5%
Chlorine	5 mg.
Vitamin A	4 mcg.
Thiamine	1 mcg.
Riboflavin	40 mcg.
Vitamin D	Not required if Ca:P ratio is between 1:1 and 2:1

(15, p. 98)

In addition, the experimenter must consider that the wean-

ling rat (21 through 25 days of age) will consume an average

of 15 grams per day. Based on this information, one can

estimate the amount of dietary ration to mix for a given

period of time.

In preparing the research diet, it is advisable to

have a dry and thoroughly mixed diet. This will insure

that the animal will eat the homogenous mixture, thus

eliminating the possibility of his selecting special foods.

Any mineral salts to be added to the ration should be

thoroughly ground with the mortar and pestle. Finally, the

ration should be stored in a cool, dry place in a brown

bottle, covered with a lid. In any experiments in which

vitamin D is involved, the food as well as the rats should

be kept away from direct sunlight.

Throughout the years the rat has been used in a vast

number of nutritional studies. Although it is difficult to

extrapolate specific data from the rat to man, this

herbivorious creature is responsible for much progress being made in the field of nutrition (34). In the study of osteoporosis or other bone disorders, it is ethical to sacrifice the animal in order to study the skeletal structure. Human studies on bone loss, however, are limited to the methods of dietary balance studies and roentgenological techniques.

Many researchers have shown through experimentation with the rat that calcium deprivation produces osteoporosis. Nordin (28) reviewed the research of Light and Frey who had fed to young rats a diet adequate with respect to vitamin D and phosphorus, but deficient in calcium. The resulting skeletal structure of these animals was hard, but light and fragile. The bones were thin walled. No evidences of rickets were apparent as the bones did not bend easily, had a nearly normal ash content, and were not enlarged at the ends. Aegerter (1) found that Harrison, Fraser, Jowsey, Heaney, and Frost also had studied the effect of calcium deprivation in young animals. The former concluded from these studies on calcium-deprived animals that long standing calcium deficiency may lead to a condition clinicians call osteoporosis in the human skeleton. These foregoing experiments indicate that the white rat is suitable for demonstrating the effect of calcium deprivation upon bone loss.

CHAPTER III

METHOD OF PROCEDURE

Experimental Units

Male albino rats of the Holtzman strain[1], weighing approximately 55 grams, were chosen for this experiment. When the weanling rats arrived, they were randomly selected, weighed, numbered, and marked. The initial body length, measured from the tip of the nose to the tip of the tail, was recorded.

The rats were marked as follows:

Rat 1 no marking
Rat 2 one notch in right ear
Rat 3 one notch in left ear
Rat 4 one notch in both ears
Rat 5 two notches in right ear
Rat 6 two notches in left ear
Rat 7 two notches in right ear and one notch in left ear
Rat 8 one notch in right ear and two notches in left ear
Rat 9 two notches in both ears
Rat 10 red color on head
Rat 11 red color on head and one notch in right ear
Rat 12 red color on head and one notch in left ear
Rat 13 red color on head and one notch in both ears
Rat 14 red color on head and two notches in right ear
Rat 15 red color on head and two notches in left ear
Rat 16 red color on head and two notches in both ears
Rat 17 blue color on head
Rat 18 blue color on head and one notch in right ear

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[1] The rats were purchased from the Holtzman Company, 421 Holtzman Road, Madison, Wisconsin.

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Rat 19 blue color on head and one notch in left ear
Rat 20 blue color on head and one notch in both ears
Rat 21 blue color on head and two notches in right ear
Rat 22 blue color on head and two notches in left ear
Rat 23 blue color on head and two notches in both ears
Rat 24 green color on head
Rat 25 green color on head and one notch in right ear
Rat 26 green color on head and one notch in left ear
Rat 27 green color on head and one notch in both ears
Rat 28 green color on head and two notches in right ear
```

Randomization Procedure

The 28 albino rats were assigned to blocks or replications according to body weight. Four littermates of approximately the same weight were randomly allocated to be housed individually in the rectangular type (Type I) or the round type (Type II) of wire mesh cages. Within each type of these two cages, the rats were fed the Control and Experimental Diets. This arrangement of the four rats in seven replications made a randomized block design with a 2^2 factorial arrangement of treatments.

The cages were constructed of galvanized wire mesh. The floor of the cages allowed the feces and urine to fall into the pan beneath the cages. Each day, newspapers were used as liners on these pans to catch the waste products. The rectangular type of cage is considered more desirable to use because it is more accessible and easier to clean. This experiment was designed to see if the two different types of cages add any variation to the experiment.

In each block, the rats were placed on the same tier level of the two carriers in order to remove the variation

in the experiment attributed to tier levels. Prior to the
arrival of the rats, the two treatments, control and experi-
mental, and the arrangement of the blocks on the two carriers
were identified by the random procedure of flipping a coin.
Heads represented the Control Diet or Treatment I, and
tails represented the Experimental Diet or Treatment II.
After the rats were weighed, numbered, marked, and blocked,
the numbers of the four rats in each block were placed in
a can. The first two numbers that were drawn indicated the
two rats assigned to cage Type I while the two remaining
numbered rats were assigned to cage Type II. See Figures 1
and 2 for the experimental design.

Experimental Procedure

The animals were fed semi-purified diets and were
given distilled water ad libitum. Random selection of the
rats determined the order in which they were fed each day.
The Control Diet (Treatment I) was taken from the Manual
for Nutrition Courses (22). The Calcium-Free Diet (Treat-
ment II) was a modified version of the Control Diet with
alterations only in the salt mixture. Before the rats
arrived, approximately 19 pounds or 8,821.00 grams of each
diet were prepared and stored in labeled, tightly-covered
brown bottles in a freezer. The amount of diet mixtures was
calculated on the basis that each rat would eat 15 grams
per day for approximately one month. Table 1 contains the
Composition of the Control Diet, and Table 2 contains the

CAGE TYPE I   CAGE TYPE II

Fig. 1. The experimental design used showing treatment combinations T_1 (Control Diet) and T_2 (Calcium-Free Diet).

Fig. 2. View of cage Type 1 and
cage Type II.

TABLE 1   Composition of the Control Diet

INGREDIENTS	PERCENTAGE	GRAMS
Casein, Technical	18	1,587.60
Cornstarch	48	4,233.60
Pure Fat[1]	8	705.60
Cod Liver Oil	2	176.40
Salt Mixture[2]	4	353.80
Yeast, dried Brewer's	20	1,764.00
	100	8,821.00

[1] Cottonseed oil

[2] W_3 Salt Mixture, purchased from Nutritional Biochemicals Corporation—percentage: $CaCO_3$, 21.001; $CuSO_4$.$5H_2O$, .039; $FePO_4$, 1.470; $MnSO_4$, .020; $MgSO_4$, 9.000; $KAl(SO_4)_2$, .009; KCl, 12.000; KH_2PO_4, 31.000; KI, 0.005; NaCl, 10.500; NaF, 0.057; and Ca_3PO_4, 14.900.

TABLE 2   Composition of the Calcium-Free Diet

INGREDIENTS	PERCENTAGE	GRAMS
Casein, Technical	18	1,587.60
Cornstarch	48	4,233.60
Pure Fat [1]	8	705.60
Cod Liver Oil	2	176.40
Salt Mixture[2]	4	353.80
Yeast, dried Brewer's	20	1,764.00
	100	8,821.00

[1] Cottonseed oil

[2] Calcium-Free Salt Mixture, Modified W_3 Salt Mixture—percentage: $CuSO_4$ .5 H_2O, .058; $FePO_4$, 2.184: $MnSO_4$, .0029; $MgSO_4$, 13.374; $KAl(SO_4)_2$, .0134; KCl, 17.800; KH_2PO_4, 50.839; KI, .0074; NaCl, 15.603; and NaF,.0847.

Composition of the Calcium-Free Diet. The ingredients for the salt mixture were weighed on a Mettler Micro analytical balance.

The food intakes of each animal were recorded daily. In order to estimate the amount of food eaten, the weight of the uneaten diet was subtracted from the amount of ration given. The rats were weighed to the nearest gram once weekly. The weight gains of the animals were recorded.

The cages were scrubbed weekly with a disinfectant, rinsed and dried. In addition, both carriers were washed using the same cleaning procedure. The food jars and water bubbles were washed daily and rinsed in distilled water. These housekeeping chores, performed during the entire experiment, prevented the laboratory from developing un-pleasant odors and reduced the possibility of infection of animals.

Bone Density Analysis

Following one month of feeding, the animals in each block were sacrificed simultaneously for bone density analysis. The body length of each rat was measured to the nearest centimeter. The right and left femurs of each rat were excised and all soft tissue was removed carefully to prevent breakage. The femurs were numbered, and the wet weight of each bone was recorded.

Using a fractometer , a preliminary uniformity trial, testing the breaking load of toothpicks, was conducted in

order to become adept in using this piece of equipment. The breaking load of each femur was determined by placing the bone across two blunt knife edges of the fractometer[2] 1.2 centimeters apart. Then a known load of calibrated weights was applied at the mid-point of the femur. The specially designed fractometer is shown in Figure 3. The breaking load of each femur was recorded.

Then the dry weight of the fractured femurs was obtained by placing the bones from each block in the drying oven for 24 hours at 105 degrees Centigrade. The bones were weighed, and the dry weight was recorded.

The total ash content was found after heating the femurs overnight in a muffle furnace at 590 degrees Centigrade (38). The ash weight of each femur was recorded in grams. Using the dry weight of each femur, the percentage composition of ash was calculated. Subsequent to the ashing process, the organic weight of each femur was obtained by subtracting the ash weight in grams from the dry weight before ashing.

Statistical Analysis

All data were recorded on IBM Code Sheets so that they could be analyzed by the use of the computer. The statistical plan for this experiment was a modified randomized

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[2]The fractometer was designed and constructed by Mr. Heinz Hall, Department of Physics-Chemistry, Oklahoma State University.
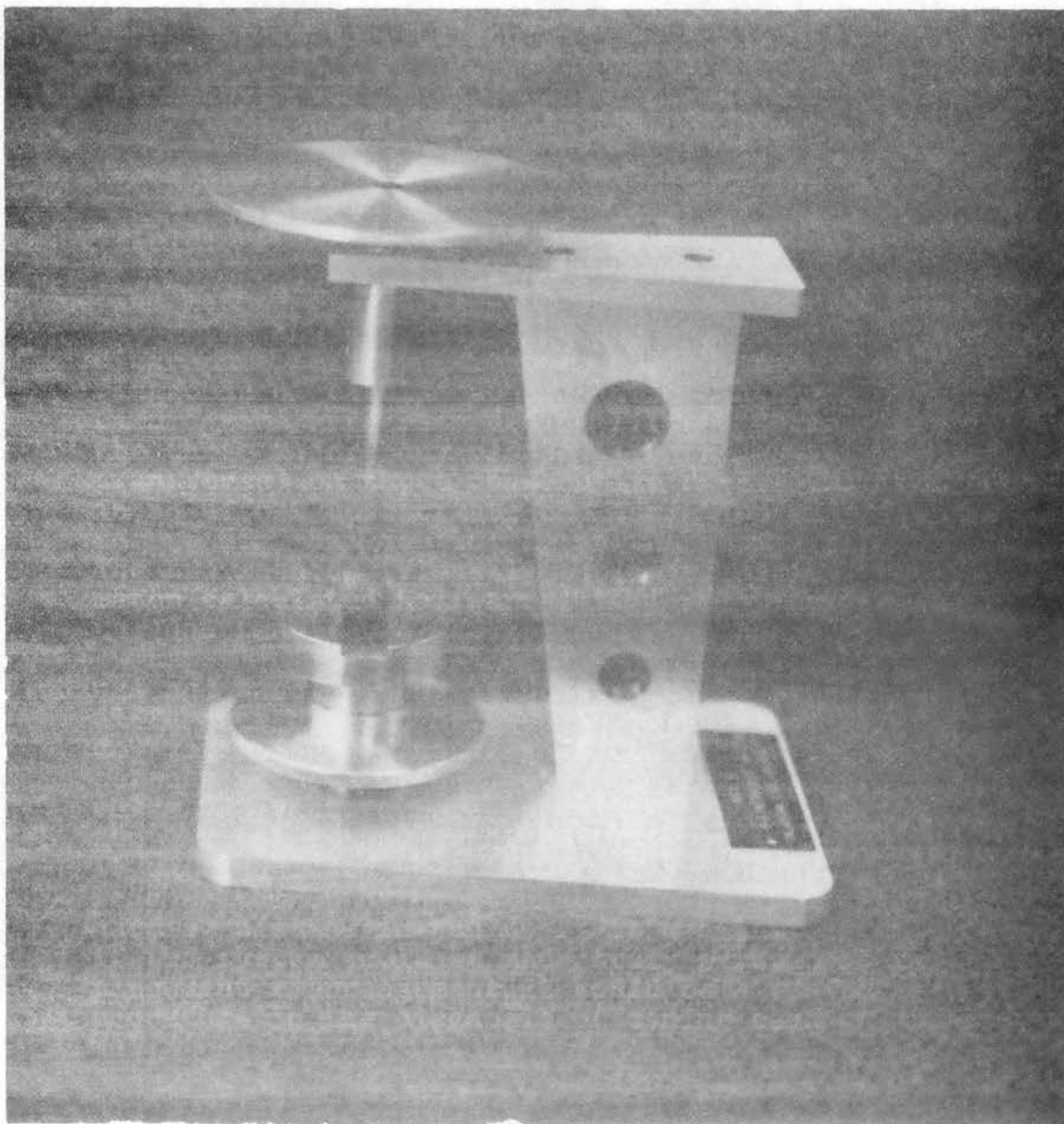
Fig. 3. General view of the fractometer.

block design having a 2^2 factorial arrangement of treatments. There were seven replicates. The factors were the type of cages, one rectangular and the other round, and the diets, one control and the other experimental. The initial weight of the rat was confounded with the shelf differences. The statistical method of analysis used in the evaluation of the results was the analysis of variance. Tests of significance were performed on the estimates of the treatment variances.

CHAPTER IV

RESULTS

Visual Evidences of Health

A daily record of feeding and visual characteristics
of each rat was kept for the entire length of the experi-
ment. The complete record for each rat is included in
Appendix A.

During the first week of the experiment, there was no
obvious difference between the rats on Treatment I and
Treatment II. The majority of the rats appeared to be
healthy, alert, and active with the exception of rat
no. 4 and rat no. 27. Rat no. 4, receiving Treatment I,
had anorexia and was inactive. The right eye of rat
no. 27 in the experimental group was inflammed attributed
to the presence of a minor infection. The average food
consumed for those on Treatment I was ten grams while
those on Treatment II was nine grams.

The rats on Treatment II began to demonstrate the
primary symptoms of the calcium deficiency in the second
week. Their fur became dull, dry, and yellowish in color
while that of the rats receiving Treatment I remained soft,
lustrous, and white. Spots of blood were present on the

food jars of rat no. 10 and rat no 22. This symptom
occurred, as could be expected, in the absence of calcium
in the diet because calcium is of utmost importance in the
coagulation of the blood. The animals on Treatment I
remained active, alert, and curious. The average amount
of food eaten by these rats was 13 grams while 11 grams was
the average for those in the other group.

Beginning with the third week, all of the rats receiv-
ing Treatment II began to display the symptomatology of
calcium deprivation in the diet. The majority of these
rats exhibited bloody noses and denuding of fur. These
animals also appeared to be hyperactive and nervous which
illustrates the importance of calcium in maintaining and
controlling neuromuscular excitability. One rat (no. 12)
continually drank excessive amounts of water and had poly-
uria. The rats receiving Treatment I continued to demon-
strate visual evidences of health. The food consumption
for both groups remained essentially unchanged from the
previous week.

During the final week of the experiment, the teeth
of the experimental animals were badly stained in contrast
to the healthy, white teeth of the control animals. The
former group of rats continued to reveal the aforementioned
symptoms of calcium deprivation in the diet. Rat no. 21
was denuded of fur from the ventral portion of his body.
He was very nervous and hyperactive. The majority of these
rats were eager to eat the ration as soon as it was placed

in the cage, whereas those receiving Treatment I were not as anxious to eat. Nevertheless, the animals on Treatment I ate an average of 17 grams of ration as compared to 13 grams eaten by those on Treatment II.

Body Weight

The rats on Treatment I in cage Type I attained the highest mean body weight, whereas those on Treatment II in cage Type I attained the lowest mean body weight. These growth responses of the experimental units to the various dietary treatments are illustrated graphically in Figure 4 on the following page.

F values were employed for testing the hypotheses of the equality of means. The calculated F value is the ratio of the estimate of the variance of the factor to be tested to an appropriate error mean square. This F value is then compared to the tabulated F value $(F_{m,n})$, where m and n are the degrees of freedom for the estimates of the variance present in the numerator and denominator (43).

An analysis of variance of final body weight data is given in Appendix B. The results indicate that there was a significant difference in dietary treatments exceeding the 0.5 per cent level. The calculated F value for cages was less than the tabulated $F_{(1,18)}$. No differences existed between the responses attributed to cage type. However, statistical evidence revealed that the treatments by cages mean square and the error mean square were different.
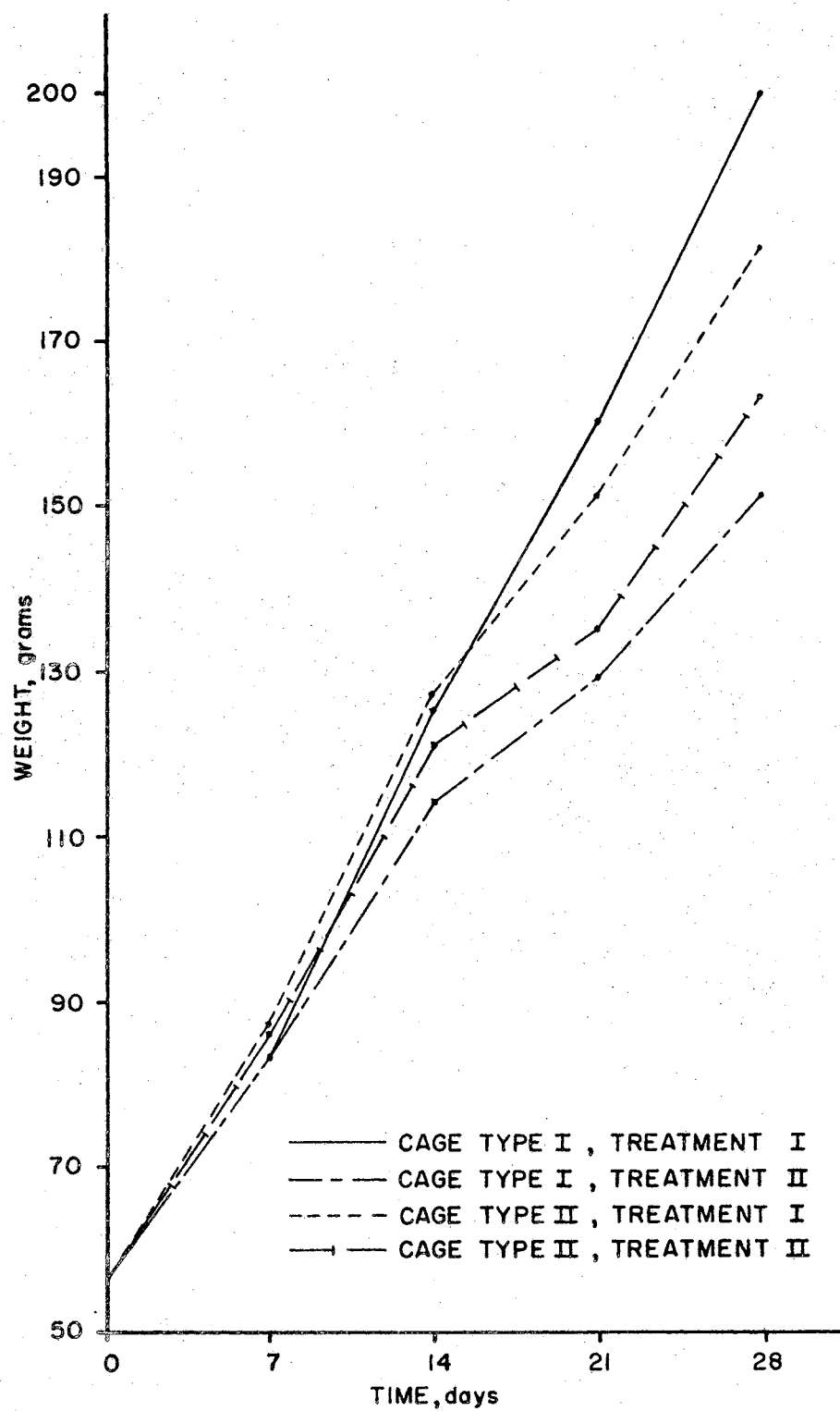
Fig. 4. Body weight means for the different treatments and cage types.

Hence, a treatment by cage interaction was present at the 10 per cent level. This interaction measures the failure of Treatment I and II to be the same for each level of cage type. Therefore, the significant interaction implies that the factors, treatment and cage type, are not independent of one another (43).

The difference in the weight gain of rats on Treatments I and II is demonstrated in Figure 5. This research provided a real difference between dietary treatments on weight gain of the rats at the 0.5 per cent level. However, there was not enough evidence to detect a real difference in final weight gain of rats between the rectangular and round cages. An interaction between the effects of treatments by cage types on final weight gain was present at the 10 per cent level.

Final mean weight gain and length change of the rats within each cage type are listed in Table 3. Throughout the entire experiment, these characteristics of the rats on Treatment II did not compare favorably to those of the rats on Treatment I. An analysis of variance of change in length of the rats is included in Appendix B. A significant difference between the dietary treatments at the 2.5 per cent level, but not at the 1 per cent level, was revealed. Cage types and treatments by cages interaction had no significant effect on change in final length of the animals.
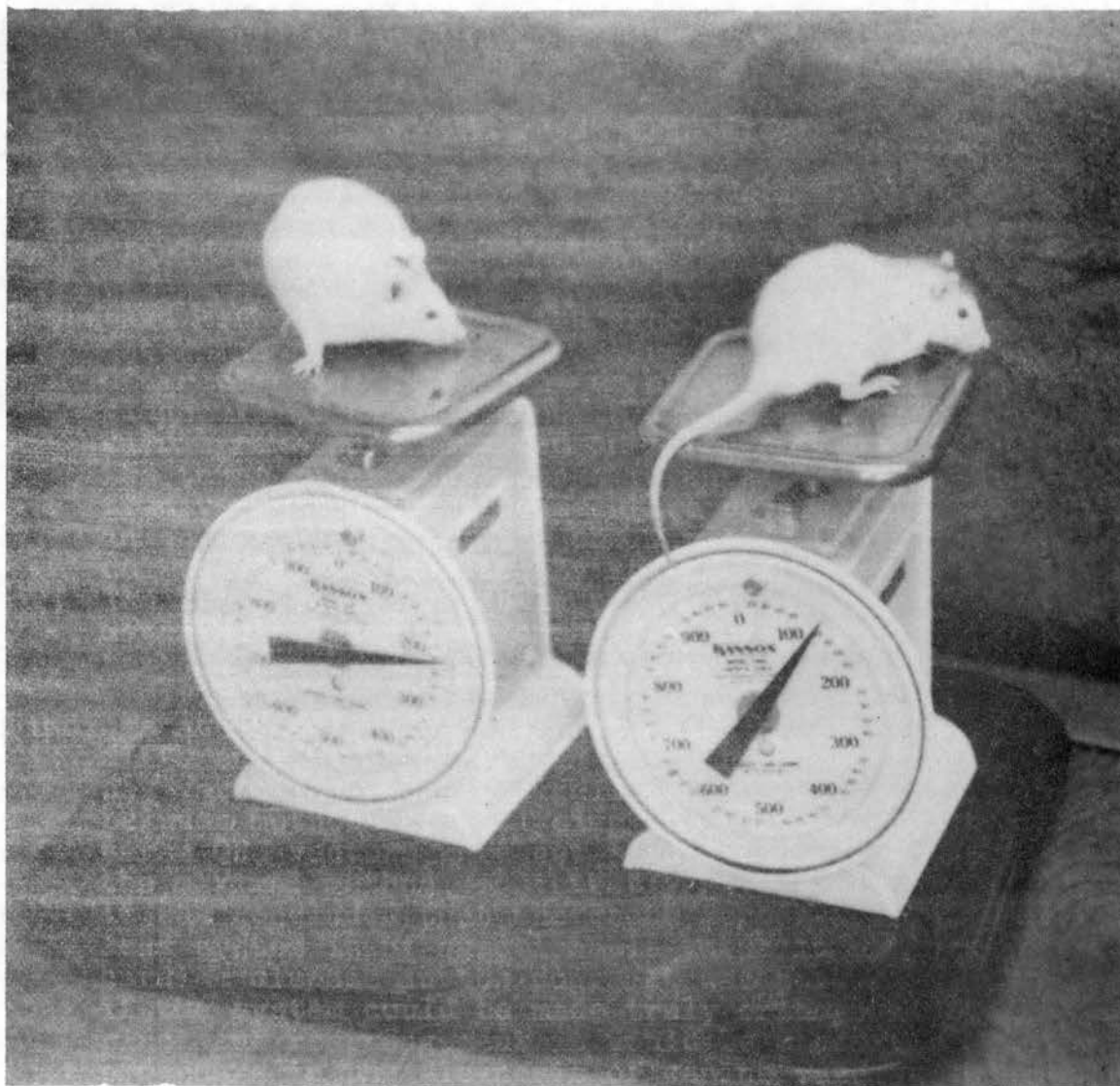
Fig. 5. Difference in weight gain
of rats on Treatment I on the left
and Treatment II on the right.

TABLE 3   Mean weight gain and length change of rats

RATS	WEIGHT GAIN	LENGTH CHANGE
	gm.	cm.
Cage type I, Treatment I	143.4	13.6
Cage type I, Treatment II	95.6	11.3
Cage type II, Treatment I	125.7	13.7
Cage type II, Treatment II	107.3	12.4

Femoral Characteristics

After the animals were sacrificed according to blocks, the left and right femurs were removed for analysis. The femurs of the control animals were healthy and well developed, whereas those of the experimental animals were thin-walled, porous, purple in color, but not enlarged at the ends. The purple color of the femurs of the calcium deprived animals could be attributed to the translucency of the cortex through which the bone marrow was visible. Lack of enlargement at the ends of the bones indicated the rats were suffering from a bone disorder other than rickets. Spontaneous fractures of the right femur of rats no. 24 (cage type I,Treatment II) and no. 27 (cage Type II, Treatment II) occurred. This is common in osteoporosis.

In addition to the two broken femurs attributed to spontaneous fractures, the right femur of rat no. 17 (cage Type I, Treatment I) was abnormal. This femur had grown into the side of the animal. Therefore, a method developed by Yates was used to give an estimate of the three missing

femur values in order to facilitate the statistical analysis of the remaining data. The estimate of the missing data is such that the error sum of squares in each analysis of variance is kept at a minimum (43).

Statistical analysis of the data revealed that there was no real difference between right and left femurs for each experimental unit. In each analysis of variance for the various femoral characteristics, the right and left femurs are represented as subplots in the split plot analysis. (See Appendix B).

Mean wet weight, dry weight, and moisture content of femurs for rats, according to cage type and treatment, are presented in Table 4. The percentage of moisture content of the femurs of the rats on Treatment II exceeded that of the rats on Treatment I.

TABLE 4   Mean wet weight, dry weight, and moisture content
of femurs for rats

RATS	WET WEIGHT	DRY WEIGHT	MOISTURE CONTENT
	gm.	gm.	%
Cage type I, Treatment I	.6899	.2427	61.2
Cage type I, Treatment II	.5056	.1451	70.7
Cage type II, Treatment I	.6560	.2715	58.5
Cage type II, Treatment II	.5492	.1630	70.1

The difference in Treatments I and II had a significant effect on wet weight of the femur at the 0.5 per cent level.

and dry weight of the femur at the 1 per cent level. Not enough evidence existed to indicate a real difference between cage types on wet weight and dry weight of the femurs. An interaction between treatments by cage types was present at the 10 per cent level of significance. Relative to the treatment differences, however, the interaction was not large. The analyses of variance of wet weight and dry weight of the femurs are contained in Appendix B. This experiment provided no real differences among the treatment means for organic weight of the femurs.

Breaking Load of the Femurs

Femoral breaking load means are presented in Table 5 on the following page. These means are indicative that the femurs of the calcium deprived rats were more fragile and did break under less weight than did the femurs of the control animals. The increment in breaking loads of the femurs was attributed to the presence of calcium in the diet.

A response surface for breaking load was plotted in Figure 6 from mean values for the femoral breaking loads. The effects of calcium deprivation in the diet and cage type differences were qualitatively revealed. Variation of each factor, especially dietary treatment, produced a realistic effect on breaking load.

Statistical evidence demonstrated that the breaking loads of femurs for rats receiving the dietary treatments behaved differently at the 0.5 per cent level as shown in

TABLE 5   Mean breaking load of femurs for each rat

RATS	BREAKING LOAD
	gm.
Cage type I, Treatment I	
2	12,261
4	11,884
5	11,951
11	12,655
16	11,801
17 [1]	12,249
26	11,302
Mean	12,008
Cage type I, Treatment II	
3	996
12	1,103
14	1,151
18	949
20	1,139
24 [1]	1,048
28	969
Mean	1,050
Cage type II, Treatment I	
1	11,482
7	11,560
8	12,491
13	11,797
19	11,345
23	11,152
25	11,282
Mean	11,587
Cage type II, Treatment II	
6	878
9	1,432
10	694
15	1,589
21	1,088
22	1,313
27 [1]	842
Mean	1,119

[1]
  One femur missing.
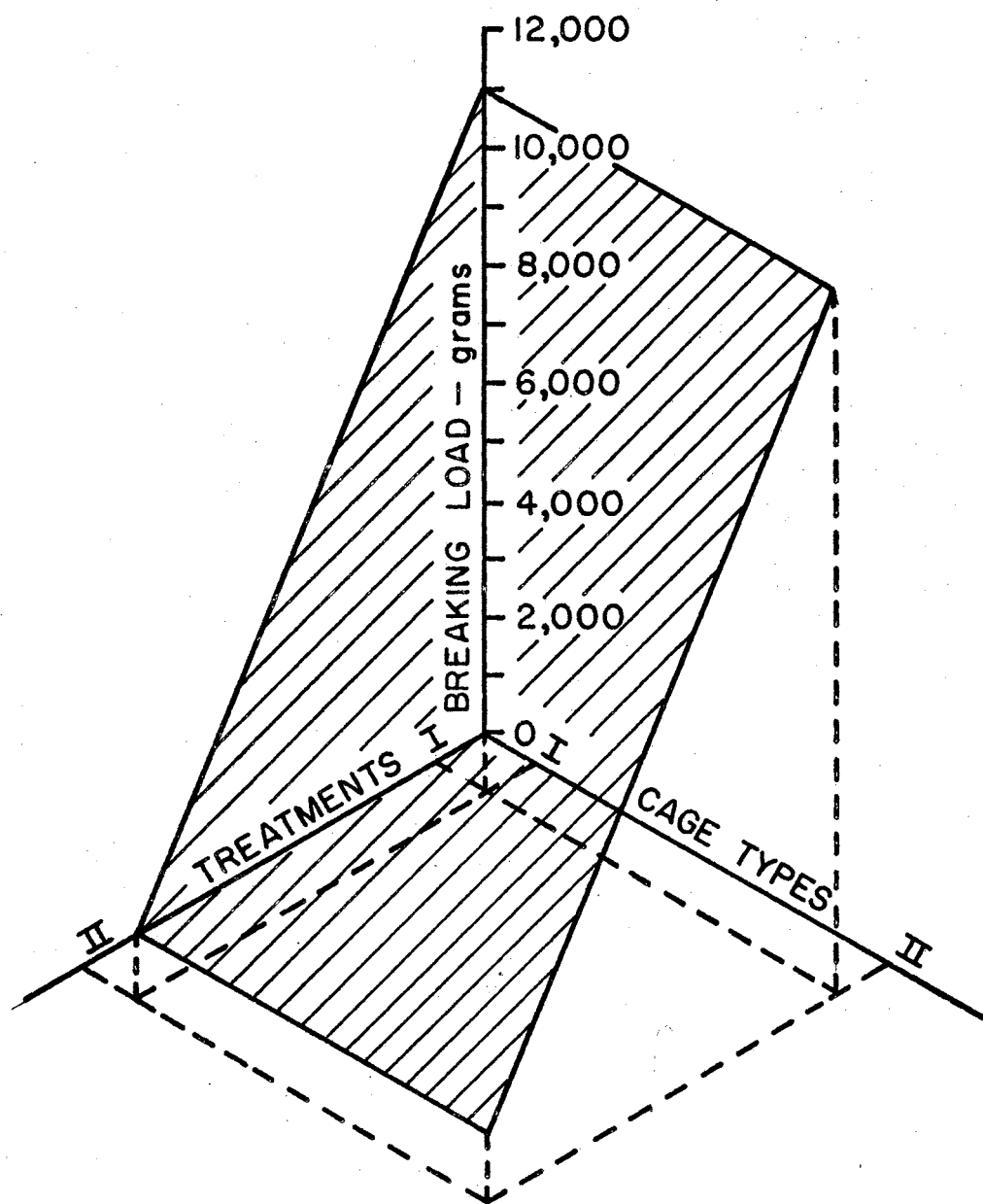
Fig. 6. Response surface depicting the effect of calcium deprivation in the diet and cage type differences on femoral breaking load.

Table 6. Although there was not enough evidence to detect the effect of the rectangular and round cage types on femoral breaking load, there was some interaction between treatments and cage types at the 10 per cent level.

Total Ash Content of the Femur

Dietary rations with inadequate calcium content allow growth of the soft tissues to proceed while the skeletal structure is inadequately supplied with calcium. The result is a decrease in bony tissue per unit volume or a dimunition in total bone mass (osteoporosis). Total ash content determinations of the femurs were the criteria used for indicating the bone density effects produced by the different dietary treatments.

Individual mean values of ash content of the femurs are given in Table 7. Ash content was recorded in grams weight and in percentages of the total dry weight of the bone. As shown in Table 8, the total ash content of the bones of the calcium deprived animals was decreased in contrast to that of the controls. The statistical evidence revealed a highly significant difference, exceeding the 0.5 per cent level, in the dietary treatment effects. The calculated F for cage type effects was less than the tabulated $F_{(1,18)}$ which demonstrates that no difference exists in the ash content attributed to cage type. In addition, there was no evidence to indicate that treatments mean square and the cage types mean square are different. Hence, no evidence is available

TABLE 6   Analysis of variance of femoral breaking load

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F
Total	55			
Blocks	6	1,194,091	199,015	
Cages	1	436,485	436,485	1.6281
Treatments	1	1,606,714,314	1,606,714,314	5,993.3474**
Treatments x Cages	1	842,802	842,802	3.1437 x
Error A (Main Plot)	18	4,825,673	268,093	
Femur	1	1,134	1,134	.0219
Femur x Treatments	1	11,832	11,832	.2285
Femur x Cages	1	10,478	10,478	.2024
Femur x Treatments x Cages	1	77,555	77,555	1.4980
Error B (Sub Plot)	24	1,242,547	51,773	

** Significant at the 0.5% level
x Significant at the 10% level

TABLE 7   Mean ash content of femurs for each rat

RATS	ASH CONTENT	
	gm.	%
Cage type I, Treatment I		
2	.1351	40.28
4	.1443	55.23
5	.1639	54.16
11	.2105	76.38
16	.1560	61.60
17[1]	.1345	58.15
26	.1429	79.77
Mean	.1553	60.80
Cage type I, Treatment II		
3	.0522	47.85
12	.0479	29.66
14	.0317	28.59
18	.0486	34.06
20	.0458	30.45
24	.0603	31.74
28	.0563	38.57
Mean	.0490	34.42
Cage type II, Treatment I		
1	.1649	53.71
7	.1709	55.03
8	.1691	80.90
13	.1658	54.59
19	.1515	54.31
23	.1431	58.36
25	.1296	53.56
Mean	.1565	58.64
Cage type II, Treatment II		
6	.0553	34.20
9	.0538	34.73
10	.0514	34.29
15	.0561	32.28
21	.0536	31.45
22	.0574	32.64
27	.0468	32.09
Mean	.0535	33.10

[1]
One femur missing

TABLE 8   Analysis of variance of femoral ash content

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F
Total	55			
Blocks	6	0.00067	0.00011	
Cages	1	0.00002	0.00002	0.0343
Treatments	1	0.15852	0.15852	331.5736 **
Treatments x Cages	1	0.00016	0.00016	.3406
Error A (Main Plot)	18	0.00861	0.00048	
Femur	1	0.00022	0.00022	1.3381
Femur x Treatments	1	0.00029	0.00029	1.7104
Femur x Cages	1	0.00034	0.00034	2.0204
Femur x Treatments x Cages	1	0.00017	0.00017	1.0452
Error B (Sub Plot)	24	0.00401	0.00017	

** Significant at the 0.5% level

to conclude that a treatment by cage interaction exists.

In Figure 7, the effects of the two factors, dietary treatments and cage types, on mean ash content (in grams) were plotted in a response surface. As shown by this response surface, dietary treatments had the greatest bearing on the total ash content of the bone. No interaction between the two factors can be detected.
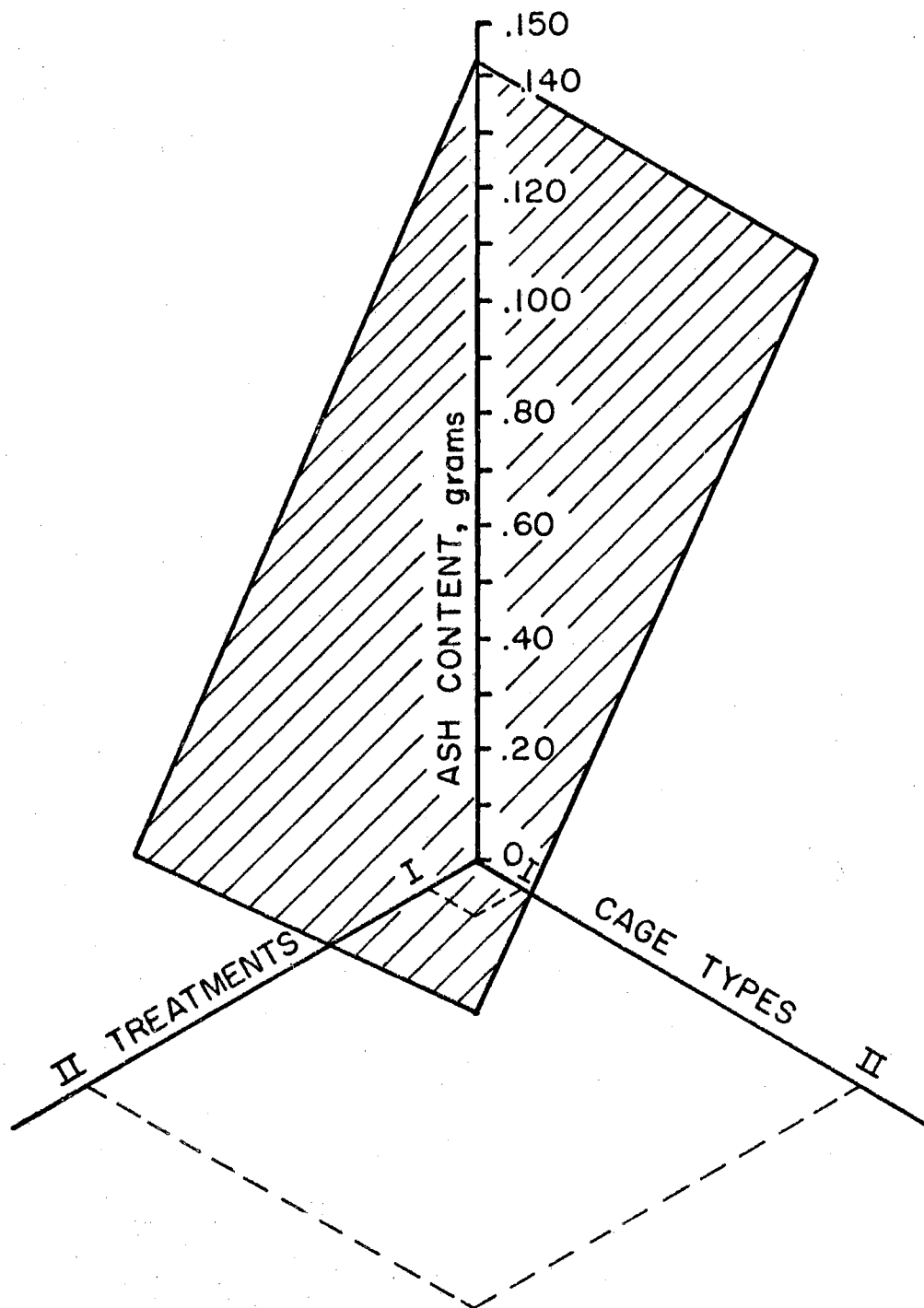
Fig. 7. Response surface depicting the effects of calcium deprivation in the diet and cage type differences on femoral ash content.

CHAPTER V

SUMMARY AND CONCLUSIONS

Calcium deprivation in the diet in relation to bone
density changes in the weanling albino rat was investigated.
The experimental units were the 28 albino rats, 14 of which
served as the control group and 14 as the experimental
group. The animals were arranged in a randomized block
design having a 2^2 factorial arrangement of treatments.
The combination of the two dietary treatments and the two
cage types gave the factorial arrangement of treatments
for each rat in the seven replicates.

After approximately one month of feeding, the animals
in each replicate were sacrificed and were evaluated accord-
ing to final body weight, animal length, physical character-
istics, and femoral characteristics. Statistical analysis
revealed a significant difference between the dietary treat-
ments on femoral breaking load. No difference between cage
types on breaking load was evident. There was a significant
difference in the ash content of the control and experi-
mental animals which revealed a decrease in the total bone
mass in the calcium deprived animals.

The results of this research support the theory that
calcium in the diet throughout the lifetime is indeed

important in relation to the prevention of the demineralization process. Because calcium deficiency produces osteoporotic bone loss in animals, it seems reasonable that this bone loss would also occur in humans. However, there is a dire need for more epidemiological studies to be conducted so that the etiology of this complex skeletal disorder can be evaluated more thoroughly.

Some suggestions for future research are the following:

1. To study the importance of varying levels of fluorine in the diet in relation to bone density changes in the white rat.

2. To study the relationship between calcium and fluorine in the diet and the interplay between the minerals in the body.

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APPENDIX A

DAILY RECORDS OF ANIMAL FEEDING

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 1

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			58.0	No Marking
2/22-23	30.0	8.0	7.0		Healthy
2/24	15.0	6.0	22.0		
2/25	15.0	3.0	12.0		Active
2/26	20.0	4.0	11.0		
2/27	20.0	6.0	14.0	89.0	Alert
2/28	20.0	13.0	7.0		
3/1-2	40.0	6.0	14.0		Playful
3/3	20.0	10.0	30.0		
3/4	20.0	8.0	12.0		
3/5	20.0	9.0	11.0		Coat glossy
3/6	24.0	4.0	16.0	136.0	
3/7	20.0	9.0	15.0		Playful
3/8-9	40.0	5.0	15.0		Active
3/10	20.0	13.0	27.0		
3/11	20.0	9.0	11.0		Alert
3/12	20.0	6.0	14.0		Curious
3/13	20.0	7.0	13.0	158.0	
3/14	20.0	6.0	14.0		Healthy
3/15-16	40.0	1.0	19.0		
3/17	20.0	8.0	32.0		Alert
3/18	20.0	6.0	14.0		Fur shiny
3/19	40.0	4.0	16.0		Healthy
3/20		12.0	28.0	192.0	Sacrificed

TABLE A   Daily Record of Animal Feeding – Treatment I

Rat No. 2

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			60.0	Notch one – right ear
2/22-23	30.0	8.0	7.0		Healthy
2/24	15.0	14.0	16.0		
2/25	15.0	10.0	5.0		Calm
2/26	20.0	2.0	13.0		
2/27	20.0	8.0	12.0	92.0	Alert
2/28	20.0	6.0	14.0		
3/1-2	40.0	8.0	12.0		Healthy
3/3	20.0	9.0	31.0		
3/4	20.0	10.0	10.0		Alert
3/5	20.0	6.0	14.0		Healthy
3/6	20.0	4.0	16.0	146.0	
3/7	20.0	4.0	16.0		
3-8/9	40.0	4.0	16.0		Alert
3/10	20.0	7.0	33.0		Healthy
3/11	20.0	4.0	16.0		
3/12	20.0	5.0	15.0		Shiny coat
3/13	20.0	4.0	16.0	183.0	
3/14	20.0	3.0	17.0		Active
3/15-16	40.0	1.0	19.0		
3/17	20.0	4.0	36.0		Healthy
3/18	20.0	3.0	17.0		
3/19	40.0	2.0	18.0		Alert
3/20		24.0	16.0	228.0	Sacrificed

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 4

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			54.0	Notch one - both ears
2/22-23	30.0	8.0	7.0		Healthy
2/24	15.0	14.0	16.0		
2/25	15.0	13.0	2.0		Inactive
2/26	20.0	8.0	7.0		
2/27	20.0	13.0	7.0	65.0	
2/28	20.0	18.0	2.0		Quiet
3/1-2	40.0	12.0	8.0		More alert
3/3	20.0	20.0	20.0		
3/4	20.0	13.0	7.0		
3/5	20.0	7.0	13.0		Better appetite
3/6	20.0	7.0	13.0	113.0	More active
3/7	20.0	8.0	12.0		
3/8-9	40.0	9.0	11.0		Healthy
3/10	20.0	16.0	24.0		
3/11	20.0	6.0	14.0		Active
3/12	20.0	7.0	13.0		Curious
3/13	20.0	5.0	15.0	142.0	
3/14	20.0	8.0	12.0		Fur healthy
3/15-16	40.0	2.0	18.0		
3/17	20.0	10.0	30.0		Alert
3/18	20.0	6.0	14.0		Healthy
3/19	40.0	5.0	15.0		Curious
3/20		22.0	18.0	192.0	Sacrificed

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 5

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			58.0	Notch two - right ear
2/22-23	30.0	6.0	9.0		Healthy
2/24	15.0	8.0	22.0		
2/25	15.0	7.0	8.0		Active
2/26	20.0	4.0	11.0		
2/27	20.0	8.0	12.0	88.0	Alert
2/28	20.0	12.0	8.0		
3/1-2	40.0	10.0	10.0		Healthy
3/3	20.0	14.0	26.0		
3/4	20.0	10.0	10.0		Alert
3/5	20.0	6.0	14.0		Coat shiny
3/6	20.0	7.0	13.0	118.0	
3/7	20.0	7.0	13.0		
3/8-9	40.0	7.0	13.0		Healthy
3/10	20.0	16.0	24.0		
3/11	20.0	4.0	16.0		Curious
3/12	20.0	5.0	15.0		Alert
3/13	20.0	3.0	17.0	160.0	
3/14	20.0	6.0	14.0		Healthy
3/15-16	40.0	0.0	20.0		
3/17	20.0	2.0	38.0		Fur shiny
3/18	26.0	2.0	18.0		Alert
3/19	40.0	6.0	20.0		Healthy
3/20		10.0	30.0	200.0	Sacrificed

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 7

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			56.0	Notch two - right ear notch one - left ear
2/22-23	30.0	9.0	6.0		
2/24	15.0	8.0	22.0		Healthy
2/25	15.0	5.0	10.0		Active
2/26	20.0	4.0	11.0		
2/27	20.0	8.0	12.0	92.0	
2/28	20.0	10.0	10.0		Alert
3/1-2	40.0	8.0	12.0		
3/3	20.0	16.0	24.0		
3/4	20.0	8.0	12.0		Healthy
3/5	20.0	4.0	16.0		Shiny coat
3/6	26.0	5.0	15.0	138.0	Healthy
3/7	20.0	15.0	11.0		
3/8-9	40.0	5.0	15.0		Cage turned over
3/10	26.0	1.0	39.0		
3/11	26.0	10.0	16.0		Active
3/12	26.0	10.0	16.0		
3/13	26.0	8.0	18.0	162.0	
3/14	26.0	15.0	11.0		Food jar overturned
3/15-16	40.0	8.0	18.0		
3/17	26.0	14.0	26.0		Alert
3/18	26.0	8.0	18.0		Healthy
3/19	40.0	9.0	17.0		Knocked over water
3/20		16.0	24.0	188.0	Sacrificed

TABLE A   Daily Record of Animal Feeding – Treatment I

Rat No. 8

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			52.0	Notch one – right ear notch two – left ear
2/22-23	30.0	8.0	7.0		
2/24	15.0	12.0	18.0		Healthy
2/25	15.0	7.0	8.0		Active
2/26	20.0	4.0	11.0		
2/27	20.0	6.0	14.0	84.0	Alert
2/28	20.0	14.0	6.0		
3/1-2	40.0	8.0	12.0		Healthy
3/3	20.0	16.0	24.0		Active
3/4	20.0	10.0	10.0		
3/5	20.0	3.0	17.0		Coat shiny
3/6	20.0	6.0	14.0	136.0	
3/7	20.0	7.0	13.0		
3/8-9	40.0	5.0	15.0		Curious
3/10	20.0	8.0	32.0		
3/11	20.0	4.0	16.0		Active
3/12	26.0	2.0	18.0		
3/13	26.0	6.0	20.0	172.0	Healthy coat
3/14	26.0	10.0	16.0		Active
3/15-16	40.0	4.0	22.0		
3/17	26.0	8.0	32.0		Alert
3/18	20.0	11.0	15.0		Curious
3/19	40.0	5.0	15.0		
3/20		16.0	34.0	230.0	Sacrificed

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 11

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			56.0	Red color - head, notch one - right ear
2/22-23	30.0	5.0	10.0		Healthy
2/24	15.0	10.0	20.0		
2/25	15.0	7.0	8.0		Active
2/26	20.0	4.0	11.0		
2/27	20.0	8.0	12.0	84.0	Alert
2/28	20.0	8.0	12.0		
3/1-2	40.0	6.0	14.0		Healthy
3/3	20.0	18.0	22.0		
3/4	20.0	10.0	10.0		
3/5	20.0	4.0	16.0		Shiny coat
3/6	26.0	2.0	18.0	138.0	
3/7	26.0	7.0	19.0		
3/8-9	40.0	10.0	16.0		Healthy
3/10	20.0	8.0	32.0		
3/11	26.0	1.0	19.0		Active
3/12	26.0	6.0	20.0		Curious
3/13	26.0	8.0	18.0	190.0	
3/14	26.0	8.0	18.0		Contented
3/15-16	40.0	4.0	22.0		
3/17	26.0	2.0	38.0		Shiny fur
3/18	26.0	4.0	22.0		Healthy
3/19	40.0	9.0	17.0		Content
3/20		20.0	20.0	234.0	Sacrificed

TABLE A   Daily Record of Animal Feeding – Treatment I

Rat No. 13

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			58.0	Red color - head, notch one - both ears
2/22-23	30.0	10.0	5.0		Healthy
2/24	15.0	8.0	22.0		
2/25	15.0	4.0	11.0		Active
2/26	15.0	2.0	13.0		
2/27	20.0	5.0	10.0	96.0	Active
2/28	20.0	10.0	10.0		Alert
3/1-2	40.0	8.0	12.0		
3/3	20.0	10.0	30.0		
3/4	20.0	9.0	11.0		Healthy
3/5	20.0	5.0	15.0		
3/6	24.0	4.0	16.0	136.0	Active
3/7	20.0	10.0	14.0		
3/8-9	40.0	6.0	14.0		Alert
3/10	20.0	20.0	20.0		
3/11	20.0	6.0	14.0		Curious
3/12	20.0	5.0	15.0		Shiny fur
3/13	20.0	5.0	15.0	154.0	
3/14	20.0	8.0	12.0		Active
3/15-16	40.0	2.0	18.0		
3/17	20.0	10.0	3.0		Healthy
3/18	20.0	6.0	14.0		
3/19	40.0	5.0	15.0		Alert
3/20		18.0	22.0	180.0	Sacrificed

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 16

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			58.0	Red color - head, notch two - both ears
2/22-23	30.0	8.0	7.0		
2/24	15.0	10.0	20.0		Healthy
2/25	15.0	6.0	9.0		Alert
2/26	20.0	3.0	12.0		
2/27	20.0	6.0	14.0	90.0	
2/28	20.0	10.0	10.0		
3/1-2	40.0	8.0	12.0		
3/3	20.0	12.0	28.0		Noisy
3/4	20.0	11.0	9.0		
3/5	20.0	13.0	7.0		Alert
3/6	20.0	9.0	11.0	127.0	
3/7	20.0	7.0	13.0		Healthy
3/8-9	40.0	6.0	14.0		Alert
3/10	20.0	12.0	28.0		
3/11	20.0	4.0	16.0		Turned over food
3/12	20.0	7.0	13.0		Shiny fur
3/13	20.0	5.0	15.0	150.0	
3/14	20.0	10.0	10.0		Curious
3/15-16	40.0	1.0	19.0		
3/17	20.0	6.0	32.0		Healthy
3/18	20.0	2.0	18.0		
3/19	40.0	6.0	14.0		Alert
3/20		20.0	20.0	180.0	Sacrificed

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 17

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			52.0	Blue color - head
2/22-23	30.0	7.0	8.0		Healthy
2/24	15.0	14.0	16.0		
2/25	15.0	8.0	7.0		Alert
2/26	20.0	5.0	10.0		
2/27	20.0	6.0	14.0	82.0	Active
2/28	20.0	13.0	7.0		
3/1-2	40.0	10.0	10.0		Healthy
3/3	20.0	20.0	20.0		
3/4	20.0	13.0	7.0		Alert
3/5	20.0	9.0	11.0		Coat shiny
3/6	20.0	6.0	14.0	122.0	
3/7	20.0	9.0	11.0		Healthy
3/8-9	40.0	7.0	13.0		
3/10	20.0	18.0	22.0		
3/11	20.0	4.0	16.0		Curious
3/12	20.0	9.0	11.0		Active
3/13	20.0	6.0	14.0	154.0	
3/14	20.0	8.0	12.0		Very cautious
3/15-16	40.0	2.0	18.0		
3/17	20.0	8.0	32.0		Alert
3/18	20.0	6.0	14.0		Healthy
3/19	40.0	6.0	14.0		Active
3/20		26.0	14.0	190.0	Sacrificed

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 19

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			56.0	Blue color - head, notch one - left ear
2/22-23	30.0	6.0	9.0		Healthy
2/24	15.0	5.0	25.0		
2/25	15.0	7.0	8.0		Active
2/26	20.0	4.0	11.0		
2/27	20.0	6.0	14.0	85.0	Alert
2/28	20.0	9.0	11.0		
3/1-2	40.0	7.0	13.0		Healthy
3/3	20.0	12.0	28.0		
3/4	20.0	12.0	8.0		Coat glossy
3/5	20.0	8.0	12.0		Alert
3/6	20.0	7.0	13.0	118.0	
3/7	20.0	9.0	11.0		
3/8-9	40.0	9.0	11.0		Curious
3/10	20.0	10.0	30.0		
3/11	20.0	6.0	14.0		Alert
3/12	20.0	7.0	13.0		
3/13	20.0	6.0	14.0	144.0	
3/14	20.0	8.0	12.0		Healthy
3/15-16	40.0	3.0	17.0		
3/17	20.0	8.0	32.0		Fur Shiny
3/18	20.0	6.0	14.0		Alert
3/19	40.0	9.0	11.0		
3/20		9.0	31.0	164.0	Sacrificed

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 23

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			57.0	Blue color - head notch two - both ears
2/22-23	30.0	10.0	5.0		Healthy
2/24	15.0	15.0	15.0		
2/25	15.0	8.0	7.0		Active
2/26	20.0	4.0	11.0		
2/27	20.0	8.0	12.0	85.0	Alert
2/28	20.0	12.0	8.0		
3/1-2	40.0	9.0	11.0		
3/3	20.0	20.0	20.0		
3/4	20.0	12.0	8.0		
3/5	20.0	10.0	10.0		Shiny fur
3/6	20.0	8.0	12.0	113.0	Alert
3/7	20.0	8.0	12.0		
3/8-9	40.0	8.0	12.0		Curious
3/10	20.0	18.0	22.0		
3/11	20.0	14.0	6.0		Healthy
3/12	20.0	9.0	11.0		Active
3/13	20.0	10.0	10.0	135.0	Very active
3/14	20.0	10.0	10.0		
3/15-16	40.0	2.0	18.0		Alert
3/17	20.0	12.0	28.0		
3/18	20.0	6.0	14.0		Fur shiny
3/19	40.0	9.0	11.0		
3/20		16.0	24.0	152.0	Sacrificed

TABLE A   Daily Record of Animal Feeding - Treatment I

Rat No. 25

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			52.0	Green color - head notch one - right ear
2/22-23	30.0	10.0	5.0		
2/24	15.0	6.0	24.0		Healthy
2/25	15.0	3.0	12.0		Active
2/26	20.0	4.0	11.0		
2/27	20.0	6.0	14.0	76.0	Alert
2/28	20.0	12.0	8.0		
3/1-2	40.0	8.0	12.0		
3/3	20.0	11.0	29.0		Curious
3/4	20.0	8.0	12.0		
3/5	30.0	7.0	13.0		Coat healthy
3/6	20.0	10.0	10.0	113.0	
3/7	20.0	10.0	10.0		
3/8-9	40.0	7.0	13.0		Squeaking
3/10	20.0	18.0	22.0		
3/11	20.0	11.0	9.0		Active
3/12	20.0	7.0	13.0		Alert
3/13	20.0	6.0	14.0	133.0	
3/14	20.0	7.0	13.0		Healthy
3/15-16	40.0	2.0	18.0		
3/17	20.0	12.0	28.0		Active
3/18	20.0	7.0	13.0		
3/19	40.0	9.0	11.0		Coat healthy
3/20		20.0	20.0	163.0	Sacrificed

TABLE A   Daily Record of Animal Feeding – Treatment I

Rat No. 26

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			56.0	Green color – head notch one – left ear
2/22-23	30.0	8.0	7.0		Healthy
2/24	15.0	10.0	20.0		
2/25	15.0	8.0	7.0		Active
2/26	20.0	3.0	12.0		
2/27	20.0	9.0	11.0	82.0	
2/28	20.0	14.0	6.0		
3/1-2	40.0	10.0	10.0		Alert
3/3	20.0	20.0	20.0		
3/4	20.0	10.0	10.0		
3/5	20.0	12.0	8.0		Noisy
3/6	20.0	8.0	12.0	113.0	Healthy
3/7	20.0	12.0	8.0		
3/8-9	40.0	9.0	11.0		Active
3/10	20.0	20.0	20.0		
3/11	20.0	7.0	13.0		Coat healthy
3/12	20.0	10.0	10.0		Alert
3/13	20.0	4.0	16.0	138.0	
3/14	20.0	12.0	8.0		Curious
3/15-16	40.0	5.0	15.0		
3/17	20.0	12.0	28.0		Active
3/18	20.0	6.0	14.0		Healthy
3/19	40.0	5.0	15.0		
3/20		18.0	22.0	174.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 3

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			56.0	Notch one - left ear
2/22-23	30.0	10.0	5.0		Healthy
2/24	15.0	12.0	18.0		
2/25	15.0	8.0	7.0		Active
2/26	15.0	5.0	10.0		
2/27	15.0	7.0	8.0		Alert
2/28	15.0	8.0	7.0		
3/1-2	30.0	7.0	8.0		Healthy
3/3	15.0	18.0	12.0		
3/4	15.0	10.0	5.0		
3/5	15.0	8.0	7.0		Fur dull
3/6	20.0	3.0	12.0	100.0	
3/7	20.0	7.0	13.0		Inactive
3/8-9	40.0	9.0	11.0		
3/10	20.0	8.0	32.0		
3/11	20.0	6.0	14.0		Curious
3/12	20.0	6.0	14.0		Alert
3/13	20.0	7.0	13.0	138.0	
3/14	20.0	7.0	13.0		Fur Sparse
3/15-16	40.0	7.0	13.0		
3/17	20.0	14.0	26.0		Teeth stained
3/18	20.0	6.0	14.0		Nervous
3/19	30.0	6.0	14.0		Shedding fur
3/20		12.0	18.0	164.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 6

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			53.0	Notch two - left ear
2/22-23	30.0	4.0	11.0		
2/24	15.0	6.0	24.0		Healthy
2/25	15.0	6.0	9.0		Active
2/26	15.0	4.0	11.0		
2/27	15.0	4.0	11.0	88.0	Alert
2/28	15.0	6.0	9.0		
3/1-2	30.0	6.0	9.0		Healthy
3/3	15.0	10.0	20.0		
3/4	15.0	7.0	8.0		
3/5	15.0	4.0	11.0		Yellowish fur
3/6	20.0	2.0	13.0	124.0	Alert
3/7	20.0	10.0	10.0		
3/8-9	40.0	11.0	9.0		Nervous
3/10	20.0	24.0	16.0		
3/11	20.0	15.0	5.0		Active
3/12	20.0	10.0	10.0		Nervous
3/13	20.0	8.0	12.0	133.0	Nose crusty
3/14	20.0	11.0	9.0		Fur lost on abdomen
3/15-16	40.0	7.0	13.0		Fur yellowish
3/17	20.0	10.0	30.0		Teeth stained
3/18	20.0	5.0	15.0		Nervous
3/19	30.0	7.0	13.0		Blood on feed jar
3/20		7.0	23.0	163.0	Sacrificed

TABLE B   Daily Record of Animal Feeding – Treatment II

Rat No. 9

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			57.0	Notch two – both ears
2/22-23	30.0	6.0	9.0		
2/24	15.0	12.0	18.0		Healthy
2/25	15.0	8.0	7.0		
2/26	15.0	5.0	10.0		
2/27	15.0	4.0	11.0	85.0	Alert
2/28	15.0	6.0	9.0		
3/1-2	35.0	4.0	11.0		
3/3	15.0	10.0	25.0		
3/4	15.0	8.0	7.0		Active
3/5	15.0	4.0	11.0		Yellowish fur
3/6	20.0	2.0	13.0	117.0	
3/7	20.0	10.0	10.0		Active
3/8-9	40.0	13.0	7.0		
3/10	20.0	15.0	25.0		
3/11	20.0	7.0	13.0		Nose crusty
3/12	20.0	7.0	13.0		Scratching nose
3/13	20.0	9.0	11.0	132.0	
3/14	20.0	9.0	11.0		Fur dry and rough
3/15-16	40.0	4.0	16.0		Shedding fur
3/17	20.0	12.0	28.0		
3/18	20.0	4.0	16.0		Nervous
3/19	30.0	8.0	12.0		Teeth stained
3/20		10.0	20.0	166.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 10

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			56.0	Red color - head
2/22-23	30.0	6.0	9.0		
2/24	15.0	12.0	18.0		
2/25	15.0	7.0	8.0		Healthy
2/26	15.0	3.0	12.0		
2/27	15.0	4.0	11.0	87.0	Active
2/28	15.0	7.0	8.0		
3/1-2	30.0	5.0	10.0		
3/3	15.0	6.0	24.0		
3/4	15.0	5.0	10.0		
3/5	15.0	4.0	11.0		Fur dull
3/6	20.0	2.0	13.0	130.0	Active
3/7	20.0	4.0	16.0		Alert
3/8-9	40.0	9.0	11.0		Blood on cage
3/10	20.0	13.0	27.0		
3/11	20.0	7.0	13.0		Crusty nose
3/12	20.0	6.0	14.0		Scratching nose
3/13	20.0	6.0	14.0	140.0	
3/14	20.0	8.0	12.0		
3/15-16	40.0	3.0	17.0		Very nervous
3/17	20.0	10.0	30.0		Teeth stained
3/18	20.0	7.0	13.0		Crusty nose
3/19	30.0	10.0	10.0		
3/20		10.0	20.0	158.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 12

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight	Remarks
2/21	15.0			56.0	Red color - head, notch one - left ear
2/22-23	30.0	6.0	9.0		Healthy
2/24	15.0	9.0	21.0		
2/25	15.0	8.0	7.0		Active
2/26	15.0	4.0	11.0		
2/27	15.0	7.0	8.0	85.0	Alert
2/28	15.0	10.0	5.0		
3/1-2	30.0	11.0	4.0		Not very active
3/3	15.0	12.0	18.0		
3/4	15.0	11.0	4.0		Loss of appetite
3/5	15.0	11.0	4.0		
3/6	20.0	2.0	13.0	106.0	Yellowish fur
3/7	20.0	8.0	12.0		Nervous
3/8-9	40.0	13.0	7.0		Increased urination
3/10	20.0	20.0	20.0		
3/11	20.0	10.0	10.0		Drank all water
3/12	20.0	10.0	10.0		Increased urination
3/13	20.0	10.0	10.0	122.0	Fur rough
3/14	20.0	12.0	8.0		
3/15-16	40.0	7.0	13.0		Increased urination
3/17	20.0	20.0	20.0		
3/18	20.0	10.0	10.0		Crusty nose
3/19	30.0	11.0	9.0		Teeth stained
3/20		20.0	10.0	140.0	Sacrificed

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TABLE B   Daily Record of Animal Feeding – Treatment II

Rat No. 15

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			58.0	Red color – head notch two – left ear
2/22-23	30.0	8.0	7.0		Healthy
2/24	15.0	10.0	20.0		
2/25	15.0	8.0	7.0		Active
2/26	15.0	8.0	7.0		
2/27	15.0	4.0	11.0	83.0	Alert
2/28	15.0	5.0	10.0		
3/1-2	30.0	5.0	10.0		Active
3/3	15.0	8.0	22.0		
3/4	15.0	7.0	8.0		Alert
3/5	15.0	4.0	11.0		
3/6	15.0	4.0	11.0	108.0	Yellowish fur
3/7	15.0	9.0	6.0		
3/8-9	40.0	7.0	8.0		
3/10	20.0	18.0	22.0		
3/11	20.0	8.0	12.0		Active
3/12	20.0	8.0	12.0		Sneezing
3/13	20.0	8.0	12.0	130.0	
3/14	20.0	10.0	10.0		Nervous
3/15-16	40.0	5.0	15.0		Losing fur
3/17	20.0	12.0	28.0		
3/18	20.0	5.0	15.0		Crusty nose
3/19	30.0	3.0	17.0		Teeth stained
3/20		7.0	23.0	178.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 18

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			54.0	Blue color - head, notch one - right ear
2/22-23	30.0	8.0	7.0		Healthy
2/24	15.0	12.0	18.0		
2/25	15.0	9.0	6.0		Alert
2/26	15.0	4.0	11.0		
2/27	15.0	7.0	8.0	74.0	Active
2/28	15.0	7.0	8.0		
3/1-2	30.0	6.0	9.0		Healthy
3/3	15.0	8.0	22.0		
3/4	15.0	10.0	5.0		
3/5	15.0	7.0	8.0		Fur dull
3/6	15.0	3.0	12.0	102.0	
3/7	15.0	9.0	6.0		
3/8-9	40.0	8.0	7.0		Nervous
3/10	20.0	30.0	10.0		
3/11	20.0	14.0	16.0		Active
3/12	20.0	8.0	12.0		Crusty nose
3/13	20.0	14.0	6.0	104.0	Rough fur
3/14	20.0	19.0	1.0		Loss of appetite
3/15-16	40.0	13.0	7.0		
3/17	20.0	20.0	20.0		Losing fur
3/18	20.0	10.0	10.0		Nervous
3/19	30.0	9.0	11.0		Teeth stained
3/20		16.0	14.0	132.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 20

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			52.0	Blue color - head, notch one - both ears
2/22-23	30.0	8.0	7.0		Healthy
2/24	15.0	8.0	22.0		
2/25	15.0	7.0	8.0		Active
2/26	15.0	3.0	12.0		
2/27	15.0	5.0	10.0	84.0	Alert
2/28	15.0	6.0	9.0		
3/1-2	35.0	4.0	11.0		Healthy
3/3	15.0	10.0	25.0		Alert
3/4	15.0	10.0	5.0		
3/5	15.0	2.0	13.0		Fur dull
3/6	20.0	3.0	12.0	122.0	
3/7	20.0	7.0	13.0		Active
3/8-9	40.0	9.0	11.0		
3/10	20.0	15.0	25.0		
3/11	20.0	11.0	9.0		Nervous
3/12	20.0	10.0	10.0		Inactive
3/13	20.0	7.0	13.0	130.0	Fur shedding
3/14	20.0	10.0	10.0		
3/15-16	40.0	7.0	13.0		Yellowish fur
3/17	20.0	12.0	28.0		
3/18	20.0	7.0	13.0		Nervous
3/19	30.0	10.0	10.0		Teeth stained
3/20		26.0	4.0	146.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 21

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			59.0	Blue color - head notch two - right
2/22-23	30.0	8.0	7.0		
2/24	15.0	9.0	21.0		Healthy
2/25	15.0	7.0	8.0		
2/26	15.0	4.0	11.0		Active
2/27	15.0	3.0	12.0	93.0	Alert
2/28	15.0	4.0	11.0		
3/1-2	35.0	3.0	12.0		Healthy
3/3	20.0	6.0	29.0		
3/4	15.0	8.0	12.0		
3/5	15.0	4.0	11.0		Dull coat
3/6	20.0	2.0	13.0	133.0	
3/7	20.0	7.0	13.0		Very active
3/8-9	40.0	9.0	11.0		
3/10	20.0	6.0	32.0		
3/11	20.0	9.0	11.0		Losing fur
3/12	20.0	8.0	12.0		Nose itching
3/13	20.0	6.0	14.0	143.0	Much fur shed
3/14	20.0	7.0	13.0		Fur very yellow
3/15-16	40.0	6.0	14.0		
3/17	20.0	10.0	30.0		Nervous
3/18	20.0	8.0	22.0		
3/19	30.0	6.0	14.0		Teeth stained
3/20		12.0	18.0	160.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 22

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			51.0	Blue color - head, notch two - left ear
2/22-23	30.0	10.0	5.0		
2/24	15.0	14.0	16.0		Healthy
2/25	15.0	7.0	8.0		Active
2/26	15.0	4.0	11.0		
2/27	15.0	4.0	11.0	85.0	Active
2/28	15.0	5.0	10.0		
3/1-2	30.0	4.0	11.0		Very active
3/3	15.0	4.0	26.0		
3/4	15.0	3.0	12.0		
3/5	15.0	3.0	12.0		Fur dull
3/6	20.0	1.0	14.0	125.0	Blood on food jar
3/7	20.0	3.0	17.0		
3/8-9	40.0	3.0	17.0		
3/10	20.0	6.0	34.0		Blood on food jar
3/11	20.0	6.0	14.0		
3/12	20.0	4.0	16.0		Crusty nose
3/13	20.0	4.0	16.0	146.0	Blood on food jar
3/14	20.0	4.0	16.0		Blood on food jar
3/15-16	40.0	3.0	17.0		
3/17	20.0	4.0	36.0		Very nervous
3/18	20.0	17.0	3.0		Lost appetite
3/19	30.0	6.0	14.0		Teeth stained
3/20		14.0	16.0	170.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 24

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			60.0	Green color - head
2/22-23	30.0	8.0	7.0		Healthy
2/24	15.0	10.0	20.0		
2/25	15.0	12.0	3.0		Inactive
2/26	15.0	2.0	13.0		
2/27	15.0	7.0	8.0	87.0	Active
2/28	15.0	2.0	13.0		
3/1-2	30.0	4.0	11.0		Alert
3/3	15.0	10.0	20.0		
3/4	15.0	10.0	5.0		Fur dull
3/5	15.0	5.0	10.0		
3/6	20.0	4.0	11.0	114.0	
3/7	20.0	8.0	12.0		Blood spots on feed jar
3/8-9	40.0	14.0	6.0		
3/10	20.0	22.0	18.0		Crusty nose
3/11	20.0	12.0	8.0		Inactive
3/12	20.0	12.0	8.0		Sneezing
3/13	20.0	14.0	6.0	124.0	Yellowish fur
3/14	20.0	7.0	13.0		Blood on food jar
3/15-16	40.0	10.0	10.0		
3/17	20.0	20.0	20.0		Blood on food jar
3/18	20.0	10.0	10.0		Fur shedding
3/19	30.0	9.0	11.0		Teeth stained
3/20		18.0	12.0	144.0	Sacrificed

TABLE B   Daily Record of Animal Feeding – Treatment II

Rat No. 27

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			56.0	Green color – head, notch one – both ears
2/22-23	30.0	5.0	10.0		Right eye pinched
2/24	15.0	11.0	19.0		
2/25	15.0	7.0	8.0		Right eye normal
2/26	15.0	3.0	12.0		Healthy
2/27	15.0	3.0	12.0	81.0	Alert
2/28	15.0	4.0	11.0		
3/1-2	35.0	3.0	12.0		Active
3/3	20.0	4.0	31.0		
3/4	15.0	5.0	15.0		
3/5	15.0	6.0	9.0		Yellowish fur
3/6	20.0	2.0	13.0	110.0	
3/7	20.0	8.0	12.0		
3/8-9	40.0	8.0	12.0		Active
3/10	20.0	14.0	26.0		
3/11	20.0	7.0	13.0		Curious
3/12	20.0	7.0	13.0		Crusty nose
3/13	20.0	7.0	13.0	120.0	
3/14	20.0	6.0	14.0		Fur Rough
3/15-16	40.0	2.0	18.0		
3/17	20.0	8.0	32.0		Fur shedding
3/18	20.0	4.0	16.0		
3/19	30.0	4.0	16.0		Teeth stained
3/20		4.0	26.0	146.0	Sacrificed

TABLE B   Daily Record of Animal Feeding - Treatment II

Rat No. 28

Date	Food Given gm.	Food Left gm.	Food Eaten gm.	Weight gm.	Remarks
2/21	15.0			55.0	Green color - head, notch two - right ear
2/22-23	30.0	8.0	7.0		Healthy
2/24	15.0	7.0	23.0		
2/25	15.0	8.0	7.0		Alert
2/26	15.0	2.0	13.0		
2/27	15.0	3.0	12.0	87.0	Active
2/28	15.0	4.0	11.0		
3/1-2	40.0	2.0	13.0		
3/3	15.0	8.0	32.0		Alert
3/4	15.0	8.0	7.0		
3/5	15.0	8.0	7.0		Fur dull
3/6	20.0	2.0	13.0	128.0	
3/7	20.0	6.0	14.0		
3/8-9	40.0	12.0	8.0		Nervous
3/10	20.0	10.0	30.0		
3/11	20.0	11.0	9.0		Active
3/12	20.0	5.0	15.0		Nose crusty
3/13	20.0	13.0	7.0	148.0	Yellow fur
3/14	20.0	12.0	8.0		Losing fur
3/15-16	40.0	4.0	16.0		
3/17	20.0	15.0	25.0		Nervous
3/18	20.0	6.0	14.0		Fur shedding
3/19	30.0	7.0	13.0		Teeth stained
3/20		6.0	24.0	166.0	Sacrificed

APPENDIX B

ANALYSES OF VARIANCE

TABLE A   Analysis of variance of final body weight

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F
Total	27			
Blocks	6	1,234.93	205.82	
Cages	1	82.29	82.29	.1895
Treatments	1	7,755.57	7,755.57	17.8662 **
Treatments x Cages	1	1,575.00	1,575.00	3.6290 x
Error	18	7,813.64	434.09	

** Significant at the 0.5% level
 x Significant at the 10% level

TABLE B   Analysis of variance of weight gain

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F
Total	27			
Blocks	6	1,069.50	178.25	
Cages	1	63.00	63.00	.1403
Treatments	1	7,689.14	7,689.14	17.1276 **
Treatments x Cages	1	1,515.57	1,515.57	3.3759 x
Error	18	8,080.79	448.93	

** Significant at the 0.5% level
 x Significant at the 10% level

TABLE C   Analysis of variance of change in length

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F
Total	27			
Blocks	6	11.00	1.83	
Cages	1	2.89	2.89	1.0556
Treatments	1	22.32	22.32	8.1519*
Treatments x Cages	1	1.75	1.75	.6392
Error	18	49.29	2.74	

*Significant at the 2.5% level

TABLE D   Analysis of variance of wet weight of femur

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F
Total	55			
Blocks	6	0.095346	0.015891	
Cages	1	0.001026	0.001026	0.2348
Treatments	1	0.281685	0.281685	64.3704**
Treatments x Cages	1	0.017244	0.017244	3.9406 x
Error A	18	0.078772	0.004376	
Femur	1	0.000039	0.000039	0.0767
Femur x Treatments	1	0.000004	0.000004	0.0081
Femur x Cages	1	0.000138	0.000138	0.2722
Femur x Treatments x Cages	1	0.000143	0.000143	0.2820
Error B	24	0.012157	0.000507	

** Significant at 0.5% level
x Significant at 10% level

TABLE E   Analysis of variance of dry weight of femur

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F
Total	55			
Blocks	6	0.017917	0.002986	
Cages	1	0.001750	0.001750	0.9320
Treatments	1	0.185829	0.185829	9.8972 **
Treatments x Cages	1	0.000644	0.000644	0.3429
Error A	18	0.0038797	0.001878	
Femur	1	0.000022	0.000022	0.0003
Femur x Treatments	1	0.000156	0.000156	0.0268
Femur x Cages	1	0.000314	0.000314	0.0540
Femur x Treatments x Cages	1	0.000131	0.000131	0.2255
Error B	24	0.139451	0.005810	

** Significant at the 1% level

VITA

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Master of Science

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