CALCIUM ABSORPTION FROM MECHANICALLY-DEBONED MEAT VERSUS MILK BY MALE RATS

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Bachelor of Science

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Stillwater, Oklahoma

1974

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1979

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PREFACE

This study is concerned with testing the calcium availability of mechanically-deboned meat against milk, a well known source of calcium. Male rats are the experimental animals used to decide the extent of calcium absorption from the two sources. The outcome of the study will determine if mechanically-deboned meat can supply dietary calcium equal to milk.

The author wishes to express her gratitude to her major adviser, Dr. Esther Winterfeldt, for her support and assistance during this study. Appreciation is also expressed to Dr. Fred Owens for his guidance throughout the project and to Dr. Bernice Kopel for her assistance in completing the manuscript.

A special thanks goes to Dr. Lester Reed and the staff at the Oklahoma State University Soils Laboratory for the use of their facilities. Appreciation is also expressed to Dr. Larry Claypool of the Statistics Department. Special appreciation is extended to Mrs. Joyce Gazaway for typing the final manuscript and additional services.

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CHAPTER I

PROBLEM DEFINITION

Introduction

Mechanically-deboned red meat is a relatively new concept in the meat industry. Although deboning has been used by the poultry industry for approximately 10 years, the equipment allowing deboning of red meat has not been perfected until recently (1).

One advantage of mechanically-deboned meat (MDM) is that it recovers meat that is otherwise lost by conventional hand boning. An additional one billion more pounds of meat could be saved per year by the MDM process (2). The MDM process utilizes the bones and scraps of meat remaining on bones from hand boning. The bones are chopped, shredded and forced under pressure through holes approximately 0.5 mm in diameter. The meat remaining on the bone passes through the holes along with bone marrow and any bone particles smaller than 0.5 mm. One potential benefit of the bone particles in MDM is its higher calcium content. While regular meat contains 0.01 percent calcium, MDM could contain as much as 1.75 percent calcium (3). This increased calcium could possibly benefit those suffering from osteoporosis or lactase deficiency.

Milk is widely known as a source of available calcium; however, many people are unable to consume milk. Such people need to maintain a positive calcium balance, a difficult task without consuming milk. If the

calcium in MDM is as well absorbed as the calcium in milk, MDM could be a practical source of calcium for those who have difficulty consuming milk.

Purpose of the Study

At the present time, little research has been reported concerning MDM and its possible nutritional benefits to the consumer. Therefore, it will be the purpose of this experiment to determine if the calcium available in MDM is as well absorbed as the calcium in milk.

Objectives of the Study

The purpose of the study will be implemented through specific objectives. Designing appropriate feed treatments will be the initial objective. The feed treatments must be consistent in ingredients, differing only in their source of calcium. Procedures to measure calcium consumption and excretion are other objectives which will aid in the determination of calcium absorption. Finally, an appropriate statistical design which will test the null hypothesis must be chosen.

Hypotheses

Null Hypothesis (H_o): The average amount of calcium absorption will not differ from treatment to treatment. Alternative Hypothesis (H_A): The average amount of calcium absorption will differ from treatment to treatment.

Definition of Terms

Lactase deficiency: a decrease in the activity of the lactase

enzyme which hydrolyzes lactose (milk sugar) into its component monosaccharides, glucose and galactose.

Mechanically-deboned meat: meat which is produced by a mechanical deboning machine. The bones and meat scraps are shredded and forced under pressure through holes 0.5 mm in diameter. Large bone fragments and white connective tissue do not pass through the holes. The texture of MDM is finer and more uniform than that of ground beef.

Osteoporosis: a disease characterized by a reduction in the amount of bone, caused by calcium being withdrawn from the bone.

CHAPTER II

REVIEW OF LITERATURE

Introduction

In this section, literature is reviewed pertaining to calcium availability from bone. Literature is also reviewed concerning osteoporosis and lactose intolerance. Those who suffer from these two conditions may benefit from any alternative dietary calcium sources. Current literature summarizing the advantages and disadvantages of mechanically-deboned meat is also included.

Calcium Availability from Bone

A review of literature comparing calcium availability from bone with availability from milk uncovered only one such experiment. The experiment was conducted with humans. One group received calcium from bone meal and the other group from milk. The calcium from bone was absorbed equally as well as milk (4). In the same experiment, a parallel study was conducted using rats. They found calcium retention from bone to be 83.3 percent compared with 93 percent from dried whole milk.

The remaining literature revealed bone and bone products to be an adequate source of calcium. Fifteen patients with osteoporosis tested whole-bone extract as a carrier of radioactive calcium. The bone extract significantly increased absorption of the calcium tracer (5).

In another work, calcium and phosphorus balance studies were conducted on three children (6). Experimental periods of five days were used to compare raw whole bone, incinerated bone and boiled whole bone. Only raw whole bone beneficially influenced calcium and phosphorus balances.

Six patients given a calcium deficient diet supplemented with bone meal found the bone meal to be tasteless and acceptable (7). The patients, ranging in age from 18 to 40 years, were placed on balance experiments for four three-day periods. In the first two periods the diets were deficient in calcium and in the last two periods bone meal supplements were added. Negative or slightly positive balances of calcium and phosphorus changed to strongly positive balances after the addition of bone meal.

Comparison of bone or bone products with calcium carbonate supplements in various animal studies revealed little difference between the two. Five different sources of calcium were tested in 200 day-old male chicks (8). The study was conducted over a four-week period, at which time the chicks were slaughtered and analyzed for total body ash and calcium content of ash. Statistical analysis of the results indicated that sterilized bone flour may be a better source of calcium than calcium carbonate for growing chicks. Fraser (9) tested limestone, calcium sulphate derived from bone and bone meal as supplements to calcium-deficient rations in growing swine. A total of seven groups with five pigs each were fed basal rations with calcium supplements. All calcium supplements appeared to promote growth. Ramsbottom (10) tested pig rations supplemented with limestone, bone meal and "dicapha". Calcium and phosphorus retention appeared positive for all three rations. Udall

and McCay (11) supplemented the diets of beagle pups with fresh ground bone. Two litters of beagles were used to assess the calcium availability of bone. They found the calcium requirement satisfied by fresh bone meal at a level of 0.4 percent of the dry weight of the diet. All four animal studies indicated bone or bone products to be available sources of calcium.

McQuarrie, Ziegler and Moore (12) compared the nutritional value of calcium enriched ground beef with milk in two hospital patients. The calcium supplemented meat was found equal to milk as a source of calcium, phosphorus, and protein. The findings were of value in recommending milk substitutes in the diet of infants and growing children.

The literature indicates bone to be an available source of calcium. Ingestion of bone might improve calcium levels in lactose deficient individuals or those suffering from osteoporosis.

Lactose Intolerance

Lactose, the disaccharide of milk, is hydrolyzed in the gastrointestinal tract by the enzyme lactase to form glucose and galactose. If low levels of lactase activity exist, lactose remains undigested in the intestinal lumen where it exerts osomotic action (13). This action causes accumulation of fluids in the stomach and small intestine resulting in abdominal distension, cramps and increased gastrointestinal motility (14).

The frequency of lactose intolerance increases with age and is higher among certain races and nationalities. Numerous studies have been conducted in an attempt to explain the cause and occurrence of lactose intolerance.

It is estimated that 80 percent of the non-Caucasian adult population of the world is lactose intolerant (15). In other words, more people throughout the world are lactose intolerant than tolerant.

The occurrence of lactose intolerance seems to be racially linked. Bayless, Rothfeld, Messa, Wise, Page and Bedine (16) tested 166 hospitalized male patients for lactose intolerance. Eighty-one percent of the 98 blacks tested exhibited abnormal lactose tolerance tests while only 12 percent of the 59 whites were intolerant. Another test by Bayless and Rosnesweig (17), using 20 blacks and 20 whites, substantiated these results. Nineteen of the blacks were lactose intolerant and only two of the whites exhibited intolerance.

Gilat, Ruhn, Gelman and Mizrahy (18) found lactose intolerance to be prevalent in Jewish communities. Of the biopsied subjects studied, 60 percent were found to have lactase deficiency. The authors felt their subjects ethnic origin was the main determinant of lactase deficiency, not the geographic location or milk drinking habits. Due to the high incidence of lactose intolerance, it was suggested that approximately 66 percent of the Jewish population in Israel is lactase deficient.

Kanaghinis and Hatzioannou (19) found lactose intolerance common among Greek adults. Subjects tested from continental Greece exhibited a 44.7 percent tolerance compard with 56 percent in Cretans and 66 percent in Greek Cypriots. The prevalence of lactose intolerance explained the low levels of milk consumed in Greece.

Mexican-Americans and Orientals are other national groups found to have a high incidence of lactose intolerance. Sowers and Winterfeldt (20) tested 33 Mexican-Americans for lactose intolerance. They found

47 percent of the nonrelated and 50 percent of the related Mexican-American subjects were lactose intolerant. Twenty healthy Oriental adults residing in the United States were tested for symptoms of lactose intolerance: abdominal cramps and diarrhea (21). After ingestion of lactose equivalent to that found in one quart of milk, 19 of the 20 subjects reported symptoms. Consumption of one or two glasses of milk caused 14 to report symptoms.

Lactose deficiency has been proposed to be genetically predetermined. This theory could account for the racial difference in lactase deficiency found by Bayless and Rosensweiger (17). Nineteen of their 20 Negro subjects compared with two of their 20 Caucasian subjects gave a history of milk intolerance. The findings of Sowers and Winterfeldt (20) also supported the theory. Two successive generations were tested for lactose intolerance in four family groups. In Family I, neither parents nor children were intolerant. In Family II, both parents and three of their five children were intolerant. In Family III, one parent was intolerant and three of the five children were intolerant. In Family IV, one parent was intolerant and two of the four children were intolerant.

Another popular explanation for lactase deficiency is lack of milk consumption after weaning. The conditions in Nigeria are not favorable for dairy cattle and as a result milk consumption after weaning does not occur. A study conducted in Nigeria found lactase deficiency widespread (22). Lack of milk intake could have caused decreased lactase production in the intestine throughout adult life. Jones and Latham (25) speculated that lactose deficiency would be delayed in children who continue to drink milk.

Calcium and phosphorus metabolism was tested in four lactose tolerant subjects and one lactose intolerant subject (24). When lactose was ingested by the lactose tolerant subjects, faecal and urinary calcium and phosphorus fell with an improvement in calcium and phosphorus balance. When lactose was ingested by the lactose intolerant subject, faecal calcium rose and calcium balance became more negative. When lactose ingestion stopped, faecal calcium fell and calcium balance became less negative. This suggests that ingestion of milk by intolerant individuals would result in a negative calcium balance great enough to eventually produce osteoporosis.

The literature indicates it would be beneficial for lactose intolerant individuals, a majority of the world's population, to have another source of calcium other than milk or milk products. Adequate calcium intake may help prevent osteoporosis develpoment in later life.

Osteoporosis

Osteoporosis is characterized by a decrease in bone forming activity with a reduction in the amount of bone (25). The groups most commonly affected are women after menopause and men over 50 years (26).

The only source of calcium available to the body is through the diet. Any excess calcium ingested is stored in bone, which acts as a reservoir for 99 percent of the total body calcium (27). Calcium must be constantly supplied to fulfill its metabolic roles and to account for that which is lost daily. A daily loss of 100 to 200 mg in the urine, 125 to 180 mg in the feces, and 20 mg in the sweat occurs whether or not calcium is being ingested (27). Failure to supply calcium through the diet results in calcium being withdrawn from the skeleton (27).

Continual resorption of calcium from the skeleton over a prolonged period of time is thought to lead to osteoporosis (28). Some estimate a daily loss of 50 mg of calcium per day for 20 years will result in osteoporosis (29).

Studies conducted on rats found a low-calcium diet decreased both bone ash content and concentration of serum calcium (30). Patton (31) experimented with low intakes of calcium in nine college women and found all subjects lost calcium. These women could well benefit from additional calcium sources.

Johnson, Alcantara and Linkswiler (32) found an increase in protein caused a two-fold increase in urinary calcium when both calcium and phosophorus were held constant. These findings could have serious implications for persons with osteoporosis, if calcium intake were low and protein intake were high. Another study using three levels of calcium substantiated these findings (33).

Studies conducted on lactase deficient individuals suffering from osteoporosis found them to have a low milk diet (34). The literature suggests diets too low in calcium cause a negative balance resulting in calcium being withdrawn from the bone. Since milk is the best known source of calcium available to man, ideally one could obtain all needed calcium from milk. If this is not possible, another source of calcium should be available to safeguard against loss of needed bone calcium and development of osteoporosis in later life.

Mechanically-Deboned Meat

Mechanically-deboned meat would be beneficial to the world food supply by recovering meat that is otherwise discarded by hand boning.

The hand boning process loses meat remaining on neck bones, ribs and backbones. Mechanical deboning could recover 13 to 16 pounds of meat from each beef carcass (2).

Specific differences exist between MDM and hand-boned meat. These differences are a direct result of the deboning process. One major difference is the calcium content of MDM. Hand-bonded meat is approximately 0.01 percent calcium (3). Bones from cattle analyzed on a dry fat-free basis are 18 to 24 percent calcium (3). The United States Department of Agriculture (USDA) would allow 0.75 percent calcium content for MDM (3). The percent of calcium is dependent upon the amount of meat attached to the bone, the equipment used, and the extent to which the bones are broken prior to deboning.

Hand-boned meat contains minimal amounts of ascorbic acid. Bone marrow is a good source of ascorbic acid. Therefore, the bone marrow incorporated in MDM increases the ascorbic acid content. Fresh MDM contains two to three milligrams ascorbic acid per 100 grams of meat (3).

Meat is considered a good source of iron. The iron content of hand-boned beef is 2.6 to 3.1 gm per 100 grams of beef. MDM contains twice as much iron at 4.3 to 6.3 gm per 100 grams of MDM (3).

Flouride intake could be increased through MDM consumption. The fixed level of calcium in MDM would prevent any danger of fluoride toxicity. Many areas of the country without flouridated water could benefit from the fluoride incorporated in MDM (35).

Problems arise from the processing of MDM. MDM is subject to oxidation due to the high level of unsaturated fatty acids contained in bone marrow lipids (3). Microbiological quality of MDM is another

problem caused by improper refrigeration of bones and carcasses. It is important the bones are kept cold and are deboned soon after removal of the carcass.

The use of mechanically-deboned red meat has been under attack from consumer groups since its first production in April, 1976 (36). The opposition objected to the bone content and questioned the digestibility and mouthfeel of MDM. When tested, the standard particle size (ranging from 0.001 to 0.018 inches) was not detected by mouthfeel (2).

The USDA has proposed not over 20 percent MDM can be added to certain processed meats. The USDA has proposed the addition of MDM to beef patties, canned corned beef, sausage products, bologna, braunschweiger, spaghetti and meatballs, chow mein, potted meat and deveiled meet. Hamburger, ground beef and fabricated steaks may not contain MDM (3).

Summary

In summary, a review of literature indicated that calcium availability from bone and bone products is adequate. With a majority of the world's population being more lactose intolerant than tolerant, an alternative calcium source to milk could be beneficial. The literature revealed osteoporosis, a reduction in the amount of bone, may be aggravated by inadequate calcium intake. The addition of MDM to the diet could increase calcium blance and decrease the possibility of osteoporosis.

CHAPTER III

METHOD AND PROCEDURE

Introduction

The purpose of the study is to determine if the calcium available in MDM is as well absorbed as the calcium in milk. Milk, a well known source of calcium, cannot be consumed by certain individuals. MDM could be a beneficial calcium source for anyone omitting milk from their diet. This chapter will discuss in detail the three major objectives of the study: to design appropriate feed treatments, to measure calcium consumption and excretion, and to analyze the data statistically.

Composition of Feed Treatments

The two major calcium sources used in the experiment were nonfat dry milk (NFDM) and mechanically-deboned meat (MDM). Four feed treatments, containing three different levels of calcium, were used to test calcium availability. Composition and calcium percentages of each treatment can be found in Tables I and II respectively. Treatments B and C contained the same calcium level set at 0.4 percent. The optimum calcium level for growing rats is 0.56 percent (37). A level of 0.4 percent, below the requirement, was chosen to insure maximum calcium absorption. Treatments B and C differed in their calcium sources: MDM supplied the calcium in Treatment B, while NFDM supplied the calcium in Treatment C.

TABLE I

COMPOSITION OF FEED TREATMENTS

Ingredient	A	B	C	D
MDM				
Percent of Diet	10.7	10.7	0.0	0.0
Gram Weight	642.0	642.0	0.0	0.0
Ground Chuck				
Percent of Diet	0.0	0.0	10.7	10.7
Gram Weight	0.0	0.0	642.0	642.0
NFDM				
Percent of Diet	18.6	0.0	18.6	0.0
Gram Weight	1116.0	0.0	1116.0	0.0
Limestone				
Percent of Diet	0.424	0.424	0.424	0.424
Gram Weight	25.4	25.4	25.4	25.4
Solka Floc				
Percent of Diet	10.0	10.0	10.0	10.0
Gram Weight	600.0	600.0	600.0	600.0
Salt				
Percent of Diet	0.5	0.5	0.5	0.5
Gram Weight	30.0	30.0	30.0	30.0
Vitamin D				
Percent of Diet	0.0083	0.0083	0.0083	0.0083
Gram Weight	0.498	0.498	0.498	0.498
Corn Meal				
Percent of Diet	59.77	78.36	59.76	78.36
Gram Weight	3586.0	4702.0	3586.0	4702.0
Total				
Percent of Diet	100.0	100.0	100.0	100.0
Gram Weight	6000.0	6000.0	6000.0	6000.0

To eliminate any error in food consistency, ground chuck was added to Treatment C in the same amount as MDM in Treatment B. Treatment A represented a high calcium diet of 0.64 percent. It contained the same amount of MDM as in Treatment B and the same amount of NFDM as in Treatment C. Treatment D represented the low level of calcium at 0.16 percent. Ground chuck was the only source of calcium.

TABLE II

CALCIUM PERCENTAGE OF FEED TREATMENTS

Treatment	Calcium Source		Percent Calcium
A	642.00 g MDM	(2.43% Ca) =	15.60
	1116.00 g NFDM	(1.30% Ca) =	14.50
	25.44 g Limestone	(33.00% Ca)	8.40
			38.50 g Ca / 6000.00 g feed = .64%
B	642.00 g MDM	(2.43% Ca) =	15.60
	25.44 g Limestone	(33.00% Ca) =	8.40
			24.00 g Ca / 6000.00 g feed = .4%
C	642.00 g Ground Chuck	(0.17% Ca) =	1.09
	1116.00 g NFDM	(1.30% Ca) =	14.51
	25.44 g Limestone	(33.00% Ca) =	8.38
			23.98 g Ca / 6000.00 g feed = .4%
D	642.00 g Ground Chuck	(0.17% Ca) =	1.09
	25.44 g Limestone	(33.00% Ca) =	8.40
			9.49 g Ca / 6000.00 g feed = .16%

Treatment calculations began by setting the level of NFDM at approximately 20 percent for Treatment C, low enough to prevent diarrhea in rats (38). This level plus the calcium contained in ground chuck failed to supply the needed 0.4 percent calcium; therefore, an additional 0.424 percent limestone supplemented the NFDM. All treatments contained the same percentage of limestone, to eliminate limestone as a variable.

Both MDM and ground chuck were analyzed for calcium by the Oklahoma State University Soils Laboratory. They contained 2.43 percent and 0.17 percent calcium respectively. The level of MDM in Treatment B was formulated to supply the same amount of calcium as NFDM and ground chuck in Treatment C.

Solka floc was added as 10 percent of each treatment as a source of fiber in the diet. The addition of 0.5 percent salt to each treatment assured adequate sodium intake. Varying percentages of corn meal were added to obtain a total value of 6000 grams of feed per treatment. Assuming that growing rats consume 20 grams of feed per day, 10 rats per treatment would consume 6000 grams over a 30-day period (37). Corn meal was used because it is virtually free of calcium and supplies approximately four calories per gram (39).

Preparation of Feed Treatments

Drying and grinding of the MDM and ground chuck occurred before these ingredients could be added to the feed mixture. The meats were spread on large sheet pans, dried at approximately 93° C, and frozen. The high fat content made grinding the meat in a Wiley Mill impossible. The addition of dry ice lowered the temperature, allowing both fat and meat to be ground to a fine powder.

Each ingredient was weighed separately. The gram weight of each ingredient contained in the four treatments is shown in Table I. Mixing was done in a Hobart Mixer beginning with the lowest calcium content, Treatment D, and proceeding to the highest calcium content, Treatment A. This method assured the higher calcium feeds would not contaminate the lower calcium feeds.

Feeds were transferred to plastic bags and marked with their corresponding Treatment letter. Feeds remained in storage at -18° C throughout the entire experimental period.

Experimental Animals

Forty male Sprague-Daley white rats, approximately 28 days old, were used in the experiment. Male rats were chosen to eliminate any difference in eating habits, activity, and calcium absorption due to sex.

Ten rats were randomly assigned to each treatment. Upon arrival, the rats were weighed and placed in individual cages. All cages were contained in a battery of 48 cages, 24 cages per side. Forty slips of paper bearing a Treatment letter and rat number were placed in a jar and one slip of paper drawn at a time. Each cage was assigned a Treatment letter and number according to the chosen slip of paper. Twenty rats were housed on each side of the battery. Figure 1 diagrams the exact arrangement of rats by Treatment letter and rat number.

Of the original 40 rats ordered, seven were dead upon arrival. The experiment began without the seven rats; however, replacements arrived within three days. The experiment was extended three days beyond completion for the seven rats only.

B6	A8	B8	C9	D6	A7
A10	B4	D3	B3	C4	D8
C5	D2	A1	C10	A4	D5
A3	C2				

A2	C7	D10	B2	D1	B7
C1	D7	B5	A5	C6	A9
B1	D4	C3	B10	A6	C8
D9	B9				

Figure 1. Random Arrangement of Rats by Treatment Letter and Rat Number

Care and Feeding

As explained previously, the rats were housed in a battery of 48 individual cages, 20 rats per side. Cages were suspended over stainless steel liners. The wire floor of the cages permitted waste products to drop onto the liners for collection. A constant temperature of approximately 25° C was maintained in the room at all times. Artificial lighting was operated for approximately 12 hours per day.

Each cage was equipped with a water bottle for ad libitum water consumption. When the water level reached half full, the bottles were refilled using cool tap water.

Glass feeding jars containing three days' feed supply were placed in each cage, allowing ad libitum feeding. Before placing the feeding jars in the cages, the total weight of the jar plus feed was recorded.

Following the three-day feeding period, the jars with remaining feed were weighed and recorded. The loss in weight during the three-day feeding period represented the feed consumed. The total experiment consisted of nine three-day feeding periods.

Collection and Analysis for Calcium

Collection of feces occurred at the end of each three-day feeding period. The samples were labeled with their corresponding Treatment letter, rat number, and collection date, and stored in plastic containers until analysis. Cleaning of the stainless steel liners occurred twice weekly. A total of nine collections corresponded with the nine feeding periods. Only the feces were analyzed for calcium. Calcium excreted in the feces represents that not absorbed from the diet plus some metabolic fecal calcium, while calcium excreted in the urine represents metabolized calcium from endogenous calcium sources (13).

After collection, feces were placed in a drying oven at approximately 90° C until completely dry. A Mettler balance was used to obtain the total dry weight of each sample. Next, one gram of each sample was weighed into a 200-milliliter beaker. Digestion of each sample began by heating in 25 milliliters in a 67 percent nitric acid 33 percent perchloric acid mixture. Samples were digested to almost dry, then diluted to a volume of 100 milliliters with deionized water. Before reading by atomic absorption, one milliliter of lanthanum chloride was added to four milliliters of the digested and diluted sample. The lanthanum chloride allowed a more accurate reading by forming a calcium chloride complex. Calcium chloride is easily detected by the flame of the atomic absorption spectrophotometer (40).

The Perkin-Elmer 403 atomic absorption spectrophotometer was used to detect the sample calcium levels. The reading from the atomic absorption machine was in micrograms per milliliter.

Each feed treatment was analyzed for calcium to determine the percentage of calcium in each treatment. The feed was analyzed using the above mentioned method. Table III contains the percentage calcium per feed treatment. These percentages were used for all calculations.

TABLE III

PERCENTAGE OF CALCIUM AFTER ANALYSIS OF FEED TREATMENTS

Treatment	Percentage Calcium
A	.60
B	.30
C	.34
D	.14

Calcium Consumption Calculations

The method for determining the amount of feed consumed was explained previously in Care and Feeding. Calcium consumption was determined by multiplying the amount of feed consumed by the percentage of calcium contained in each treatment. Table IV represents a sample three-day feed and calcium consumption for each treatment.

TABLE IV

FEED AND CALCIUM CONSUMPTION

Treatment	Feeding Grams	-	Weighing Grams	=	Consumed Grams	x	Percent Ca	=	Ca Consumed Grams
A	195.39	-	148.62	=	46.77	x	.5697	=	.2664
B	182.73	-	149.48	=	33.25	x	.2996	=	.0998
C	188.50	-	141.61	=	46.89	x	.3396	=	.1594
D	196.07	-	148.98	=	47.09	x	.1398	=	.0659

Calcium Excretion Calculations

The reading from the atomic absorption spectrophotometer was in micrograms per milliliter. Approximately one gram of the sample was digested and analyzed; therefore, the reading could also be in micrograms per gram of sample. Table V provides sample calculations beginning with the atomic absorption reading and concluding with the gram weight of calcium excreted.

Analysis of Data

The data was analyzed statistically by the Oklahoma State University Computer Center. The statistical test of analysis of variance (ANOV) was used to test the null hypothesis (H_o): The average amount of calcium absorbed will not differ from treatment to treatment. ANOV was chosen because all data was measured quantitatively in gram weight: gram weight of calcium consumed minus gram weight of calcium excreted equals gram weight of calcium absorbed (41).

TABLE V

CALCIUM EXCRETION

	A	B	C	D
Atomic Absorption Reading	18700/g .0187 g/g	12200/g .0122 g/g	5800/g .0058 g/g	6200/g .0062 g/g
Sample Weight	$\frac{.0187\ g}{1.01121\ g}$	$\frac{.0122\ g}{1.01799\ g}$	$\frac{.0058\ g}{1.03015\ g}$	$\frac{.0062\ g}{1.01374\ g}$
Percent Calcium Excreted	1.8492	1.1984	0.5630	0.6110
Total Weight of Sample	13.41420 g	4.39274 g	14.41905 g	12.14850 g
Total Calcium Excreted	0.2481 g	0.0526 g	0.0812 g	0.0742 g

The ANOV confirmed or denied there was a difference in calcium absorption from treatment to treatment. It did not specify which treatments had different absorption levels. Therefore, the least significant difference (lsd) was used. The lsd compared the mean calcium absorption of the four treatments and determined which treatments, if any, had similar absorption levels. Analysis of variance and least significant difference were also run on the mean amount of feed consumed by each treatment group.

The four treatment groups were also analyzed for mean initial weight, mean final weight, mean daily weight, mean daily weight gain, mean daily feed intake, mean daily calcium intake, mean daily calcium excretion, mean daily calcium retention, calcium retention as a

percentage of calcium intake, and calcium retention per gram of daily weight gain. A discussion of the findings are presented in Chapter IV.

CHAPTER IV

RESULTS AND DISCUSSION

The results of the analysis of variance (ANOV) and least significant difference (lsd) for calcium absorption and feed consumption are discussed in detail. Mean analysis for each treatment group is also discussed in this chapter.

Calcium Absorption

The ANOV was used to test the mean calcium absorption for each three-day feeding period. Table VI contains a sample of ANOV for one three-day feeding period. A total of nine feeding periods were tested using the ANOV.

TABLE VI

SAMPLE ANALYSIS OF VARIANCE FOR CALCIUM ABSORPTION

Source	df	SS	MS	F Value	Prob F
Treatment	3	.07069	.02356	22.64752	.0001
Error	29	.03017	.00104		
Corrected Total	32	.10087			

As shown in Table VI, the probability of a larger F value was .0001. The number is smaller than .05, this implies rejecting the H_o and concluding the H_A: The average amount of calcium absorption will differ from treatment to treatment. For all nine feeding periods, the probability of a larger F was found to be .0001. Therefore, through the entire experiment there was a difference in calcium absorption from treatment to treatment.

The ANOV did not specify which treatments differed in calcium absorption; therefore, the least significant difference (lsd) was used to detect specific treatment differences. Table VII gives the results of the lsd for Treatments B and C throughout the entire experiment. Treatments B and C will receive the majority of attention, because they contained the same percentage of calcium. Their only variation was the source of calcium. As shown in Table VII, there was a significant difference in calcium absorption from Treatment B (MDM) and Treatment C (NFDM) through the entire experimental period.

Feed Consumption

Analysis of variance and lsd were also tested for feed consumption during each three-day feeding period. The ANOV for all nine feeding periods was found to be significantly different at the .05 level. Table VIII indicates the lsd findings for each feeding period.

The feed consumption for Treatments B and C differed throughout the entire period, as shown by Table VIII. The rats in Treatment C (NFDM) consumed significantly more feed than those in Treatment B (MDM).

Table IX contains mean values for each treatment over the entire experimental period. As seen in Table IX, the initial weight of the

TABLE VII

LEAST SIGNIFICANT DIFFERENCE FOR TREATMENTS B AND C

Date	Difference in Absorption	lsd .05	Results
8/31	.077	.033	Significant difference at 5% level
9/4	.088	.042	Significant difference at 5% level
9/7	.124	.031	Significant difference at 5% level
9/10	.119	.021	Significant difference at 5% level
9/15	.175	.033	Significant difference at 5% level
9/18	.133	.027	Significant difference at 5% level
9/21	.188	.021	Significant difference at 5% level
9/24	.113	.027	Significant difference at 5% level
9/27	.122	.035	Significant difference at 5% level

TABLE VIII

LEAST SIGNIFICANT DIFFERENCE FOR FEED CONSUMPTION

Date	Significant Difference	Not Significant
8/31	A&C, B&C	A&B, A&D, B&D, C&D
9/4	A&D, B&C, B&D	A&B, A&C, C&D
9/7	A&B, B&C, B&D	A&C, A&D, C&D
9/10	A&B, B&C, B&D	A&C, A&D, C&D
9/15	A&B, B&C, B&D	A&C, A&D, C&D
9/18	A&B, B&C, B&D	A&C, A&D, C&D
9/21	A&B, B&C, B&D	A&C, A&D, C&D
9/24	A&B, B&C, B&D	A&C, A&D, C&D
9/27	A&B, B&C, B&D	A&C, A&D, C&D

TABLE IX

MEAN CALCULATIONS BY TREATMENT

Mean Calculation	A	B	C	D
Initial Weight, g	69.35	69.45	73.72	69.78
Final Weight, g	223.38	101.38	265.49	203.27
Daily Weight Gain, g	4.5	0.99	5.7	3.9
Daily Feed Intake, g	18.21	11.15	19.26	18.35
Daily Ca Intake, g	0.037	0.0334	0.0654	0.0257
Daily Ca Excretion, g	0.0524	0.0269	0.0123	0.0058
Daily Ca Retention, g	0.0513	0.0065	0.0531	0.0199
Ca Retention as Percent Ca Intake	49.47	19.46	81.19	77.43
Ca Retention/g Daily Weight Gain	0.010	0.007	0.009	0.005

rats was approximately the same for all four treatments. However, the mean final weight was significantly less for Treatment B. The mean daily weight gain for Treatment B was again significantly less than the other treatments: 0.99 grams for Treatment B (MDM) opposed to 5.7 grams for Treatment C (NFDM). The difference in weight gain can be explained by the fact that the daily feed intake for Treatment B was also significantly less than the other treatments. Treatment B consumed 11.15 grams per day as opposed to 19.26 grams per day for Treatment C. Because the feed intake for Treatment B was less than Treatment C, the calcium intake was also less: 0.0334 grams and 0.0654 grams respectively. While the calcium intake for Treatment B was half the intake of Treatment C, the calcium excretion was twice that of Treatment C. Treatment B excreted 0.0269 grams and Treatment C excreted only 0.0123 grams. As a result, the calcium retention was significantly less for Treatment B than for Treatment C. This is in agreement with the ANOV and lsd statistical analysis. Expressing calcium retention as a percent of calcium intake, again Treatment B was less than Treatment C: 19.46 percent and 81.19 percent respectively. When the mean daily calcium retention per gram of daily weight gain was calculated, the range between Treatments B and C became smaller: 0.007 grams and 0.009 grams respectively.

From the results of the statistical analysis and the daily mean values one must conclude the calcium availability of mechanically-deboned meat to be less than that of nonfat dry milk.

CHAPTER V

SUMMARY AND CONCLUSIONS

The technique used in the mechanical deboning process incorporates minute bone fragments into the ground meat. A review of literature indicated bone meal to be an adequate source of calcium. Research has not been conducted regarding the calcium availability of MDM. If the calcium contained in the bone particles can be absorbed, MDM would be an additional calcium source.

The objective of the project was to test the calcium availability of MDM against that of NFDM, a well known source of calcium. The project design consisted of four feed treatments with three levels of calcium: high, moderate, and low. The moderate calcium level was represented by two of the feed treatments; however, their calcium sources varied. The calcium source in Treatment B was MDM and in Treatment C was NFDM. Ten rats were randomly assigned to each feed treatment. The calcium absorption was determined by the difference in the calcium consumed and the calcium excreted.

Statistical analysis of the mean calcium absorption was conducted through analysis of variance and least significant difference. The statistical tests revealed a significant difference in calcium absorption at the 0.5 level between Treatments B (MDM) and C (NFDM). Therefore, it would appear the calcium in MDM is not as well absorbed as the calcium in NFDM.

Although the calcium availability of mechanically-deboned meat was found to be less than that of nonfat dry milk, a certain percentage of calcium from MDM was absorbed. Any additional calcium source would benefit persons consuming calcium deficient diets.

Problems and Suggestions

A major problem that occurred during the initial mixing of the feed was because dried meat still contains a certain amount of fat. The high fat content made grinding the meat impossible without the addition of dry ice to keep the fat in solid form.

After the feed was mixed, the ground meat particles were larger than the other feed ingredients. This presented a problem throughout the entire experiment. The rats objected to the MDM and ground chuck flavors. They tended to eat more of the other ingredients and leave the MDM and ground chuck particles. Freeze-drying the meats would have been a more satisfactory method. This would have allowed the meat particles to be ground more finely and the feed mixture would have been more uniform.

Because of the significant difference in feed intake and the growth rate between treatments, pair feeding of rats would have been beneficial. If the feed intakes of the other treatments had been the same as Treatment B, growth rates and calcium intake between treatments would not have been as drastic.

Within each treatment group all 10 rats consumed similar quantities of feed and exhibited similar growth rates. Therefore, any future studies of this type can be conducted using fewer than 10 rats per treatment.

Calcium to phosphorus ratios in the feed rations were not determined. The ratio of these two minerals could be important in future studies using MDM.

The presence of lactose has beneficial effects on calcium absorption. Consideration should be given to this fact when milk is used as a calcium source.

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