THE EFFECT OF TWO CALCIUM SALTS ON THE BIOAVAILABILITY OF CALCIUM, MAGNESIUM AND IRON FROM BREAD

by

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INTRODUCTION

Interest in enrichment of bread and cereal products as a means of improving the nutritional health of a population dates back to the outbreak of World War II when armed service inductees were being turned down in great numbers for physical deficiencies, some of which were nutritionally-related (1). Recommendations of the American Medical Association's Council on Foods and Nutrition and the Food and Drug Administration prompted the National Research Council Committee on Foods and Nutrition at its first meeting in November 1940 to endorse a program favoring addition of thiamin, niacin, riboflavin and iron to flour (2).

A standard of identity for enriched flour became effective January 1, 1942 (3). This was amended in 1943 to provide currently effective standards requiring than thiamin, niacin, riboflavin and iron be added to flour with the option of including calcium and vitamin D (4). Levels established for calcium addition were: 500-625 mg calcium per pound flour and 300-800 mg calcium per pound bread. Enrichment of bread was inaugurated in May, 1941, although a standard of identity for enriched bread was not issued or made effective until 1952 (5). Today, 34 states have mandatory requirements for enrichment of flour and bread (2).

The Food and Nutrition Board of the National Academy of Sciences (NAS) reviewed the enrichment program in 1971 and proposed expanding the standards to include several additional nutrients, raise the amount of niacin, thiamin, riboflavin and iron to be added and to make calcium enrichment mandatory (2). In November, 1977, the Food and Drug Administration withdrew its 1971 recommendation to raise the iron level from 13-16.5 mg to 40 mg iron per pound flour (6).

Calcium was considered as one of the ten nutrients to be included in the proposed NAS fortification policy because of evidence of potential risk of deficiency of this mineral and also because calcium is among those nutrients for which recommended dietary allowances (RDA) have been established by the NAS Food and Nutrition Board (2). Several United States surveys have revealed inadequate intakes of calcium by selected groups of the population. The 1965-66 Household Food Consumption Survey (7) revealed daily calcium intake levels as low as 30% below the RDA for representative population groups, particularly for adolescent girls and women, elderly men, individuals residing in rural areas and the southern region of the United States and individuals from low-income households. Similarly, the Ten-State Nutrition Survey of 1968-70 indicated significant numbers of the population had intakes below the RDA for calcium (8).

Milk and dairy products are the major dietary sources of calcium, accounting for approximately 75% of the calcium consumed in the American diet (9). According to food consumption

profiles obtained during the first national Health and Nutrition Examination Survey (HANES), April 1971-June 1974 (10), 21% of the sample population (20,749 persons) seldom or never drank milk. Among the rest of the population representing United States civilians aged 1-74 years, 22% drank milk at least 1-6 times a week, 21% drank milk once daily and 36% drank milk 2 times or more a day. The selected findings showed a decline of milk consumption with age; one-third of persons aged 45 and over reported seldom or never consuming milk.

Cereal-based products comprise about 26% of daily caloric intake in the United States, with percentages varying according to geographic region and income (2). However, cereals are not generally considered good sources of calcium. Enrichment of bread and flour with calcium to the NAS proposed levels could make bread the second major contributor of this essential mineral in the American diet.

Of numerous food grade calcium salts available for calcium enrichment, calcium sulfate and calcium carbonate are the two sources which are the least expensive (11). Results of early studies (12-18) indicated little or no difference in availability of calcium from various sources, including calcium sulfate and carbonate, but none of these studies involved baked products such as bread.

Several studies concerning the relationship of dietary calcium with absorption of iron have been reported (19-23).

In some of these studies, an adverse effect of calcium carbonate on iron utilization was found (19,20,21,23).

This study was undertaken to investigate the availability of calcium added as calcium carbonate or calcium sulfate to bread. In addition, the effects of calcium fortification on iron and magnesium utilization were examined.

REVIEW OF LITERATURE

Contribution of Bread Ingredients

Usefulness of the present enrichment program was demonstrated by Jeans et al (25) in a study of dietary habits of 400 pregnant women in Iowa. Bread and potatoes contributed a large proportion of the calories consumed by these women; consumption of dairy products was low. The fact that the bread was enriched protected a majority of the subjects from deficiencies in iron, thiamin and riboflavin. They concluded that the addition of nonfat dry milk solids (NFDM) to the maximum amount not affecting loaf quality would enhance calcium intake, as well as protein and riboflavin intake of these women.

In addition to nutritional benefits, NFDM contributes flavor, crust color and desirable crumb characteristics when added at a level of 6% of the flour in bread (26). Until 5-10 years ago, most bread was made by the conventional dough method using 4-6% NFDM. Now, however, breads made by the continuous-mix method are made with lower levels (1-2%) of NFDM (27). In addition, the cost of NFDM has more than tripled the last few years, discouraging its use by bakers in bread formulations (26).

Substitutes for NFDM are available to bakers and include soy flour, whey protein and cheese whey either added separately or in blends. While replacements may have similar baking performance to that of NFDM, not all NFDM substitutes are nutritionally equivalent to NFDM (26).

If calcium is added to a replacement blend, then it may contribute as much as 80 mg calcium per pound of bread when the blend is added at a level of 2% of the flour. Addition of NFDM at the same level contributes 72 mg calcium per pound of flour. Field tests in commerical bakeries have resulted in bread made with replacement blend comparable in quality to bread made with NFDM solids.

In addition to NFDM and replacement blends, other ingredients contribute to the calcium content of bread. Assuming a 160 pound yield of bread per 100 pounds flour, white flour contributes approximately 60 mg calcium per pound of bread (28). The hardness of water used in baking determines how much calcium it contributes, with the average water supply contributing about 9 mg calcium per pound of bread. Yeast food contains calcium sulfate or calcium phosphate. Calcium propionate is used as a mold inhibitor (29). Calcium stearoyl-2-lactylate, used for its dough conditioning and emulsifying properties, is a source of calcium contributing 60 mg calcium per pound of bread (28). However, because of the calcium salt's limited emulsifying ability in water-oil systems, sodium stearoyl-2-lactylate is perferred by many bakers, especially in high fat, yeast-leavened breads (30).

The total amount of calcium contributed by dough conditioners, mold inhibitors, NFDM (2% level) and other bread ingredients amounts to approximately 350 mg calcium per pound

of bread (11), which is considerably reduced if calcium stearoyl-2-lactylate is replaced by the sodium salt in the formulation. Consequently, to bring the calcium level to that proposed by the NAS--900 mg per pound flour or about 565 mg per pound of bread--more calcium must be added in the form of a salt.

Both calcium sulfate and calcium carbonate meet the Food Chemicals Codex standards with high purities (31) and are listed as GRAS by the Food and Drug Administration (28). Calcium carbonate neutralizes acids produced by fermentation and therefore increases fermentation time, while calcium sulfate can be added at high levels without affecting the pH (28).

Calcium Availability from Calcium Salts

In a rat study by Steenbock and coworkers (12), calcium in the form of various salts was added at 0.3% and 0.47% of the basal diet rations. No differences were found in availability of calcium lactate, phosphate, silicate, carbonate or sulfate for rat growth.

Bethke et al (13) found no difference in availability of calcium in carbonate, sulfate and other calcium salts for bone formation in growing chicks. Calcium salts were administered so that each animal received the same amount of calcium but on a predetermined minimum requirement basis (2% calcium carbonate and 3.2% calcium sulfate).

Stearns and Jeans (14) reported good retention of calcium

in children 4-12 years old from calcium carbonate given as a supplement. However, much individual variation was observed in the quality of calcium retained.

Schroeder et al (15) reported efficient utilization of calcium sulfate in human subjects receiving from 319 to 795 mg calcium daily in supplemental form. Utilization of calcium from various sources was: evaporated milk, 29.1%; calcium sulfate, 23.7%; soybean milk (used in infant formulas), 22.6%; and whole cooked soybeans, 10.4%.

An interesting note concerning the study by Schroeder and coworkers (15) is that it was performed during World War II. Several events at this time prompted a request from the Civilian Food Requirements Board of the Food Distribution Administration for information concerning calcium availability from calcium sulfate. A shortage of dried milk products in the United States restricted the quantity of dried milk available to bakers. In addition, the armed forces and the allied countries' civilian populations were in need of milk. Calcium sulfate was the only calcium salt available in sufficient quantities to millers and bakers. However, an attempt to require that flour and bread be enriched with this calcium source was defeated (29).

In another study done during World War II, McCance and Widdowson (32) performed a series of balance experiments over a nine-month period on 5 female and 5 male adults. These experiments included a study of the absorption and excretion of calcium

when 40-50% of calories consumed by these subjects was provided by flours of 69% extraction fortified with monohydrogen calcium phosphate and calcium carbonate and of 92% extraction fortified with the same salts. The calcium salts were added to the flour so that 100 g of bread contained 0.1g added calcium, which is equivalent to the level proposed by NAS (2). Calcium absorption and utilization were found to be equally good from both salts, but only a small proportion of added calcium was absorbed which suggested that a large amount of this mineral must be added to increase calcium absorbance. Of the two salts, calcium carbonate was preferred for its abundance and its action in baking and cooking processes. Calcium carbonate appeared to improve taste and did not have a harmful effect on the appearance of products made from white flour fortified with this salt.

In a study with nine college women, Patton and Sutton (16) reported no significant difference in utilization of calcium from four calcium salts (gluconate, lactate, carbonate and sulfate). Calcium salts were given in supplement form supplying 400 mg calcium in addition to 347 mg supplied daily by the basal diet. Average percentage calcium utilized, as determined from calcium balances, was 18 from all salts.

A second study was conducted by Patton (17) to examine calcium balances of nine college women in relation to period variation, calcium source and effect of basal metabolic rate on

utilization. All sources of calcium, including calcium carbonate and sulfate, were found to be equally well utilized.

Effect of Calcium on Iron Utilization

Several studies have been reported concerning the relationship of dietary calcium with iron absorption. According to Chapman and Campbell (21), this relationship is dependent upon the ratio of calcium to iron in the diet. They added calcium carbonate and other salts to bread-based diets with calcium:iron ratios ranging from 167:1 to 343:1 and reported that calcium carbonate added at calcium:iron ratios of 182:1 and 226:1 interfered with iron utilization. Kletzien (19) reported that the addition of 1 and 3% calcium carbonate to a basal diet containing 42.5% ground corn and 42.5% wheat flour and to another basal diet containing 90% ground whole wheat resulted in lower tissue (liver, blood and carcass) iron values in rats, as compared to unsupplemented diets.

Hematological findings of Greig (20) showed that addition of 2% calcium carbonate to diets of breeding mice induced iron-deficiency anemia in both the dams and their litters. The anemia was less marked in second litters than in first litters and was prevented by adding 10 ppm iron as ferric citrate to the diet.

Amine and Hegstead (23) examined the effects of calcium carbonate on iron absorption in iron-depleted female albino rats. Retained iron was determined as the difference between

total body count two hours and nine days following ingestion of radioiron. When the salt mixture was added at 4% of the diet, removing either calcium (as calcium carbonate) or phosphorus from the salt mix resulted in greater inhibition of iron than when the balance salt mixture was used. Addition of 0.5 g calcium carbonate to 1.6 g labeled raw corn supplying 40 ug iron resulted in considerable reduction of the quantity of iron retained. Absorption of hemoglobin iron was greatly decreased by addition of 0.5 g calcium carbonate to 0.1646 g liver or 0.8088 g meat, either of which supplied 20 up iron.

Monsen and Cook (24) evaluated the influence of calcium and phosphorus salts on nonheme iron absorbance from a semi-synthetic meal. They performed a total of 116 absorbance tests on 34 male and female adult volunteers, each receiving 2-4 test meals. With addition of both calcium and phosphorus ($CaCl_2$, $CaHPO_4$ and K_2HPO_4), absorbance of nonheme iron was reduced 27-47% of that observed without adding these salts. Addition of the calcium or the phosphorus salts did not lower iron absorbed from the level observed when no salts were added.

Fourteen human metabolic studies were carried out by Apte and Venkatachalamps (22) to examine dietary calcium-iron interactions. The subjects consumed a cereal-rich diet. Results of these studies indicated a beneficial effect of calcium on iron absorption when the diet provided 1500 mg calcium and 15.5 mg iron (96:1 calcium-iron ratio). At these mineral levels, all

four subjects absorbed from 7 to 27% dietary iron. On the other hand, at 1000 mg calcium and 16.6 mg iron (60:1 ratio) intake per day, iron absorption was about 4% of intake. These subjects were in delicate iron balance. A daily intake of 16.6 mg dietary iron was found insufficient to meet daily iron requirements in individuals receiving 400 mg calcium (24:1 ratio) daily (22).

Effect of Calcium on Magnesium Utilization

The amount of magnesium absorbed in the small intestine decreases as dietary intake of calcium increases (33). Lee et al (34) reported reduction in apparent digestibility and urinary excretion of magnesium when dietary calcium was increased from a level of 0.4% to 0.8% in weanling rats. Hegstead et al (35) reported adverse effects of high dietary calcium intake on magnesium metabolism in rats but this effect was marked only at very low levels of magnesium intake. In their study, magnesium was added to diets as magnesium oxide at levels of 3-100 mg per 100 g diet and calcium as calcium carbonate at 200-1800 mg per 100 g diet.

To study the effect of dietary ratios on calcium, magnesium and phosphorus utilization, Forbes (36) performed growth and mineral balance studies on weanling rats. Two dietary levels of each mineral were investigated: calcium, 0.4 and 0.8%; magnesium, 142 and 420 ppm; and phosphorus, 0.19 and 0.50%. In

animals fed the low calcium diet, increased magnesium intake caused increases in percentage magnesium absorbed and excreted in the urine. Negative magnesium balance was observed only in rats fed high calcium, low magnesium, low phosphorus diets. Poor magnesium storage was observed in animals fed diets high in calcium and low in magnesium.

MATERIALS AND METHODS

Bread Preparation

Unenriched white flour[1] was made into bread (one-pound loaves) in the Baking Laboratory, Grain Science and Industry Department at Kansas State University. Table I shows the ingredients used for each batch of bread. White breads were baked using the standard sponge dough procedure (Appendix p 62). The yeast food (containing calcium sulfate), calcium propionate and NFDM were not omitted from the formula and were added at average levels in order to simulate a standard product. All minerals were added to the sponge portion of the flour. After baking, the bread was sliced, wrapped in polyethylene bags and kept in frozen storage.

Five types of bread were made and identified as follows:

Bread A. Ferrous sulfate from the Mallinckrodt Company was added according to current enrichment standards (4) to give 13-16.5 mg iron per pound flour.

Bread B. Ferrous sulfate was added as in bread A with calcium carbonate (ground limestone) from J.P. Bailey, Inc., added to meet proposed fortification level (2) of 900 mg calcium per pound flour.

Bread C. Ferrous sulfate was added as in bread A with calcium sulfate from U.S. Gypsum Company added at the proposed level (2) of 900 mg calcium per pound flour.

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[1]Flour milled at the ADM Milling Company, Kansas City, Mo.

TABLE I

Standard Sponge Dough Formula For White Breads

Ingredients	Total (g)	Sponge Ingredients (g)	Dough Ingredients (g)
Flour	9080	6356	2724
Water	5629	3768	1861
Yeast	227	227	
Salt, NaCl	182		182
Sugar	636		636
Shortening	272		272
Nonfat dry milk	182		182
Malted barley flour	7.3	7.3	
Arkady yeast food[1]	45	45	
Calcium propionate	18	18	
Sodium stearoyl-2-lactylate	27	27	
Ferrous sulfate	0.8752	0.8752	
Calcium carbonate[2]	44	44	
Calcium sulfate[3]	60	60	
Magnesium oxide[4]	3.6	3.6	
Zinc oxide[4]	0.180	0.180	

[1]8.8% calcium
[2]Added to breads B and D
[3]Added to breads C and E
[4]Added to breads D and E

Bread D. Ferrous sulfate and calcium carbonate were added as in bread B with magnesium oxide from the Mallinckrodt Company and zinc oxide from the New Jersey Zinc Company added according to proposed levels for fortification (2) of 200 mg magnesium and 10 mg zinc per pound flour.

Bread E. Ferrous sulfate, magnesium oxide and zinc oxide were added as in bread D with calcium sulfate used as the calcium source to supply 900 mg calcium per pound flour.

Diet Preparation

The sliced bread for rat diets was thawed and dried at room temperature for 24 hours on counter tops covered with plastic film. Each slice was turned over after 12 hours of drying. Dried bread was ground in the Experimental Milling Laboratory, Grain Science and Industry Department, Kansas State University. Bread slices were run through the Ross Experimental Roller Mill and then sifted through a 20w sieve.

Five diets were formulated from the five types of bread according to Chapman and Campbell (21). The diet composition is shown in Table II. Each diet was composed of 79.3% dried bread and 20.7% basal ration. In order to keep calcium and iron content at a minimum, iron and calcium salts were omitted from the salt mixture (Appendix p 63).

Ingredients for each diet were mixed in a Hobart Automatic Mixer for one hour. Sides and bottom of bowl were scraped frequently during mixing. Mixed diets were stored in poly-

TABLE II

Diet Composition

Constituents	%
Bread, dried	79.3
Basal ration	20.7
Casein	12.0
Corn oil	3.0
Alphacel	2.0
Salt mix[1]	1.5
Vitamin mix[2]	2.2

[1]U.S.P. XIV Salt Mixture, modified with the following salts omitted: ferric ammonium citrate, calcium carbonate, calcium citrate, and calcium diphosphate.

[2]Vitamin fortification mixture (#904654) supplied by Nutritional Biochemicals Division, ICN Pharmaceuticals, Inc., Cleveland, Ohio.

ethylene bags in a refrigerator. Quart-size, acid-rinsed plastic containers were used to store diets for individual animals.

Animals

Forty Sprague-Dawley male weanling rats weighing 40-50 g were obtained for the six-week study. Eight rats were assigned to each of five different diets in such a way that the mean weights of the five groups were equal. Animals were housed individually in randomly-numbered stainless steel metabolic cages. Diets and demineralized distilled water were available to the rats ad libitum. Feed intake and weight gain were recorded weekly for individual animals. Daily feed waste was collected and accounted for in feed intake calculations.

Balance Study

Two 96-hour collections of urine and feces were obtained from each rat during the third week (Days 18-22) and final week (Days 38-42) of the study. Feed intake was recorded throughout each period for each animal. Urine samples were collected every 24 hours in 120 ml acid-rinsed glass bottles using 0.2 ml of 10% (w/v) solution of thymol in proponal as a preservative (37). Every 24 hours, clean acid-rinsed collection bottles were provided and urinary-fecal separators were rinsed with distilled water following removal of samples. To remove feed particles from urine samples, the urine in each

collection bottle was filtered daily through ashless filter paper[1] into acid-rinsed polyethylene storage bottles, covered and refrigerated until the 96-hour period was completed. At the end of each period the pooled 24-hour samples were stored at 0°C for later analysis.

Fecal samples were collected every 24 hours during each collection period and stored at room temperature in 120 ml acid-rinsed glass bottles covered with cheesecloth. At the end of each period, pooled fecal samples were dried at room temperature for 96 hours and then dried under vacuum at 105°C for 24 hours. Dried samples were weighed, transferred to acid-rinsed plastic scintillation vials and stored at 0°C until analyzed.

Blood and Femur Samples

At the end of the six-week study, blood samples were collected from the tail of each animal for hemoglobin and hematocrit analysis, and the rats were sacrificed using chloroform. Right femurs were removed from each animal, stripped of all soft tissue, wrapped in heavy aluminum foil and stored at 0°C until analyzed.

Analytical Methods

Hemoglobin. The method of Hycel (38) was used for hemoglobin determination. Prior to obtaining blood samples, a

[1]Whatman No. 41, 0.000038 g maximum ash/7 cm circle.

standard curve was prepared using Hycel Cyanmethemoglobin Reagent to dilute Cyanmethemoglobin Standard and measuring absorbance of each solution at 540 nm on the Bausch and Lomb Spectrophotometer. Using a disposable micropipette, 0.2 ml whole blood was transferred to a colorimeter tube containing 6 ml Cyanmethemoglobin Reagent, mixed and allowed to rest 10 minutes before reading absorbance. The instrument was standardized with a blank tube containing 6 ml reagnet before each reading. Corresponding hemoglobin concentrations (g/100 ml) were determined from the standard curve. Hemoglobin measurements were performed in duplicate and values averaged for each animal.

Hematocrit. Heparinized capillary tubes were filled to approximately two-thirds volume with tail blood samples and one end sealed with plastic clay. Duplicate samples were prepared. After centrifuging the tubes 3 minutes in the Adams Autocrit Centrifuge (CT-2905), percentage packed cell volume was read from the reading chart on the instrument.

Calcium and Magnesium Determination. Calcium and magnesium were determined by atomic absorption spectrophotometry in the Soil Testing Laboratory, Agronomy Department, Kansas State University. A Perkin-Elmer Model 460 Atomic Absorption Spectrophotometer was used to measure the calcium and magnesium concentration in urine, fecal, femur, and diet samples. To prevent phosphorus interference, 0.5 ml of each digested sample

was diluted (1:20) with 0.5% lanthanum oxide solution prior to aspiring the sample into the flame and then directly reading the concentration (39, 40).

Urine Calcium and Magnesium. Frozen urine samples were defrosted at room temperature and total volume was determined using acid-rinsed graduated cylinders. A 5 ml aliquot of each sample was wet ashed in a nitric acid and perchloric acid mixture (1:1). The ash solution was diluted with demineralized distilled water to 25 ml volume.

Fecal Calcium and Magnesium. Fecal samples were thawed at room temperature, transferred to 200 ml acid-rinsed beakers and wet ashed in nitric acid and perchloric acid mixture (1:1). The ash solution was transferred to 50 ml acid-rinsed volumetric digestion tubes and diluted to volume with demineralized distilled water.

Femur Calcium and Magnesium. Femurs were thawed at room temperature, dried under vacuum in aluminum boats at 105°C for 14 hours and weighed prior to wet ashing. The ash solution was diluted with demineralized distilled water to 50 ml volume.

Diet Calcium and Magnesium. A representative 0.25 g sample of each diet was wet ashed in nitric acid and perchloric acid mixture (1:1). The ash solution was diluted to 25 ml volume with demineralized distilled water.

Diet Phosphorus. Digested diet samples were analyzed for phosphorus content in the Analytical Services Laboratory,

Animal Science and Industry Department, Kansas State University. The Fiske and SubbaRow method (41) and a Gilford model 240 spectrophotometer were used to measure phosphorus concentration in each sample.

Calcium and Magnesium Balance. Calcium intake was calculated from feed intake (g) recorded during each 96-hour collection period and calcium concentration (mg/g) in the diet as determined by atomic absorption spectrophotometry. The following formula was used to determine individual calcium balance during each collection period:

Ca Balance = Ca Intake - (Fecal Ca + Urinary Ca).

This method also was used to calculate magnesium intake and balance.

Statistical Analysis

Data from all measurements were subjected to analysis of variance, and the means were separated by Fischer's LSD with P=0.10, 0.05 and 0.01 as appropriate when the F-test rejected the hypothesis of equal means. Analyses of covariance were performed, followed by t-tests on adjusted means.

RESULTS AND DISCUSSION

Feed Intake and Weight Gain

Analysis of variance (Table III) revealed diet effects on both unadjusted feed intake and unadjusted weight gain ($P \leq 0.01$). Rats fed diet A (no calcium fortification) had significantly lower feed intakes ($P \leq 0.10$) and gained significantly less weight ($P \leq 0.10$) than rats fed the other diets to which had been fortified with calcium (Table IV). No significant differences were found for feed intake and weight gain among rats fed the other diets fortified with calcium salts. Chapman and Campbell (21) had reported that addition of calcium carbonate to bread-based diets resulted in a significant decrease in the amount of feed consumed by rats.

When weight gain was adjusted linearly for feed intake (Table IV) in covariant analysis (Table V), the significant difference due to diets disappeared. This indicated that the lower weight gain of the rats fed the bread without added calcium was related to the lower ad libitum feed intake of these animals.

TABLE III

Analysis of Variance for Feed Intake and Weight Gain of Rats[1]

Source of Variation	DF	Mean Squares and Significance	
		Feed Intake	Weight Gain
Diets	4	5967.5**	925.15*
Rats:Diets	35	761.12	250.50

[1] *$P \leq 0.05$ but > 0.01 **$P \leq 0.01$

TABLE IV

Mean Weight Gain (g) and Feed Intake (g) of Rats[1]
(n=8)

Diet	Mineral Salts Added to Bread	Unadjusted		Adjusted[2]
		Feed Intake	Weight Gain	Weight Gain
A	$FeSO_4$	497.12[a]	205.25[a]	218.14[a]
B	$FeSO_4$, $CaCO_3$	565.00[b]	232.00[b]	226.36[a]
C	$FeSO_4$, $CaSO_4$	548.75[b]	227.50[b]	226.30[a]
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	549.88[b]	219.00[b]	217.49[a]
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	561.00[b]	228.62[b]	224.08[a]

[1]Values in a column sharing a common letter are not significantly different ($P \leq 0.10$).

[2]Adjusted by linear regression for feed intake.

TABLE V

Analysis of Covariance Between Diets and Weight Gain (g)

Source of Variation	DF	Adjusted Mean Squares and Significance[1]
Diets	4	123.19[ns]
Rats:Diet	34	199.50

[1]ns Not significant.

Hemoglobin and Hematocrit

Both analysis of variance (Table VI) and covariance (Table VII) showed a significant effect ($P \leq 0.05$) effect of diet on hemoglobin but no significant effect of diet on hematocrit values. Mean hemoglobin and hematocrit values, unadjusted and adjusted for total feed intake, are given in Tables VIII and IX, respectively. Hemoglobin mean values ranged from 15.3 to 16.2 g/100 ml and hematocrit values ranged from 46.5 to 50.5%. All values for the various diets were within the normal ranges of 12.0 to 17.5 g/100 ml for hemoglobin and 39 to 53% for hematocrit for rats (44).

Unadjusted hemoglobin values were higher ($P \leq 0.10$) for rats fed diet E and lower ($P \leq 0.10$) for rats fed diet B than for diets A, C and D. When hemoglobin values were adjusted for total feed intake, hemoglobin of rats fed diet A was no longer signficantly different than those for rats fed diets B and E.

The calcium levels of breads making up diets B-E were approximately the same (Table X). However, higher hemoglobin values were observed for rats fed bread fortified with calcium sulfate than for those fed the bread fortified with calcium carbonate. Addition of magnesium and zinc to the breads appeared to be beneficial to hemoglobin formation.

Chapman and Campbell (21) found that addition of calcium carbonate to diets at an approximate level of 0.79% retarded

TABLE VI

Analysis of Variance for Hemoglobin and Hematocrit of Rats

Source of Variation	DF	Mean Squares and Significance[1]	
		Hemoglobin	Hematocrit
Diets	4	0.8119*	3.000[ns]
Rats:Diet	35	0.2415	3.871

[1] *$P \leq 0.05$ but > 0.01 [ns]Not significant

TABLE VII

Analysis of Covariance for Hemoglobin and Hematocrit Using Feed Intake as Covariate

Source of Variation	DF	Adjusted Mean Squares and Significance[1]	
		Hemoglobin	Hematocrit
Diets	4	0.8129*	3.867[ns]
Rats:Diet	34	0.2485	3.881

[1] *$P \leq 0.05$ but > 0.01 [ns]Not significant

TABLE VIII

Mean Hemoglobin Values (g/100 ml) of Rats[1] Fed Diets with Different Calcium Sources (n=8)

Diet	Mineral Salts Added to Bread	Mean Hemoglobin Values	
		Unadjusted	Adjusted[2]
A	$FeSO_4$	15.75^b	15.77^{abc}
B	$FeSO_4$, $CaCO_3$	15.31^a	15.30^a
C	$FeSO_4$, $CaSO_4$	15.75^b	15.75^b
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	15.72^b	15.72^{ab}
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	16.21^c	16.20^c

[1] Values followed by a common letter in a column are not significantly different ($P \leq 0.10$).

[2] Values adjusted for feed intake.

TABLE IX

Mean Hematocrit Values (%) of Rats Fed Diets with Different Calcium Sources[1] (n=8)

Diet	Mineral Salts Added to Bread	Mean Hematocrit Values Unadjusted	Adjusted[2]
A	$FeSO_4$	50.50^a	51.04^a
B	$FeSO_4$, $CaCO_3$	49.50^a	49.26^a
C	$FeSO_4$, $CaSO_4$	51.00^a	50.95^a
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	50.50^a	50.44^a
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	49.75^a	49.56^a

[1]Values followed by a common letter in a column are not significantly different ($P \leq 0.10$).

[2]Values adjusted linearly for feed intake.

TABLE X

Calcium and Magnesium Content of Breads[1]

Bread	Calcium Concentration (%)	Magnesium Concentration (ppm)
A	0.116	263
B	0.251	276
C	0.246	241
D	0.232	412
E	0.241	412

[1]Mineral content determined by atomic absorption spectrophotometry.

hemoglobin regeneration in anemic rats. Other researchers (19,20,23) have reported adverse effects of calcium carbonate on iron availability in rats. Generally, large calcium-iron ratios had a negative effect on iron utilization and relatively small ratios had no effect on, or actually promoted iron absorption (23). When calcium was added according to the proposed NAS level (2), as in diets B-E in the present study, the calcium-iron ratio in the flour was approximately 70:1. This ratio is considerably lower than the ratios used by Chapman and Campbell (23), who added calcium carbonate and other salts to bread-based diets with Ca:Fe ratios ranging from 167:1 to 343:1.

Several researchers have postulated that calcium interferes with iron utilization. These postulations refer to dietary calcium levels of 0.79% and above--levels considerably higher than those used in the present study (0.10 to 0.21%).

Chapman and Campbell (21) suggested that the mucosal cells become saturated with calcium, thereby blocking further iron absorption. Davis (42) attributed the calcium-iron interaction to the formation of insoluble compounds when calcium is present in the diet in excess, or above 1%. Apte and Venkatachalamps (22) suggested that excess calcium possibly interferes with iron absorption by competing directly with iron in common pathways of absorption. In vitro absorption of iron in rats was studied by Manis and Schachter (43), who attributed adverse

effects of calcium on iron absorption to competition for a similar process in an active phase of transport. Both iron and calcium cations use the same two-step transport mechanism: (1) mucosal uptake and (2) transport from tissue to bloodstream.

Femur Calcium Concentration (%)

Both analysis of variance ($P \leq 0.01$) and covariance ($P \leq 0.10$) showed an effect of diet on calcium concentration in the femur (Tables XI and XII, respectively). Unadjusted mean femur calcium concentrations ranged from 9.5% to 15.2% (Table XIII). Lee et al (34) reported higher concentrations of 20.3 to 21.4% calcium in femurs of weanling rats at the end of four weeks on diets with two higher levels (0.4 and 0.8%) of dietary calcium.

The calcium content of diet A was only about one-half that of the other diets (Table XIV). The combination of low dietary calcium concentration and the lower feed intake of rats fed diet A resulted in a much lower calcium intake (Table XV) for these rats. Consequently, unadjusted femur calcium concentrations (%) were significantly lower ($P \leq 0.01$) for rats fed diet A than for rats fed the other diets. When femur calcium concentration (%) was adjusted for calcium intake (Table XIII), a similar pattern was observed except that femur calcium concentrations (%) for diets A and C were no longer significantly different. Since there were no significant differences between

femur calcium concentrations (unadjusted or adjusted) among rats fed diets B-E with similar calcium levels, femur calcium concentration apparently was not affected by calcium source.

TABLE XI

Analysis of Variance for Femur Calcium Concentration (%)

Source of Variation	DF	Mean Squares and Significance[1] Femur Calcium
Diets	4	49.70**
Rats:Diet	35	2.367

[1] *P≤ 0.01

TABLE XII

Analysis of Covariance Between Diets and Femur Calcium Concentration (%)

Source of Variation	DF	Adjusted Mean Squares and Significance[1]
Diets	4	5.788+
Rats:Diet	34	2.433

[1] +P≤ 0.10 but> 0.05

TABLE XIII

Mean Femur Calcium Concentrations (%)[1]

Diet	Mineral Salts Added to Bread	Mean Femur Calcium (%) Unadjusted	Adjusted[1]
A	$FeSO_4$	9.496[a]	8.949[a]
B	$FeSO_4$, $CaCO_3$	15.16 [b]	15.32 [b]
C	$FeSO_4$, $CaSO_4$	14.44 [b]	14.64[ab]
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	15.23 [b]	15.33 [b]
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	15.24 [b]	15.33 [b]

[1]Values followed by a common letter in a column are not significantly different ($P \leq 0.10$).

[2]Values adjusted linearly for total calcium intake.

TABLE XIV

Dietary Calcium, Magnesium and Phosphorus Content and Calcium-Phosphorus Ratios

Diet	Mineral Salts Added to Bread	Mineral Content Ca (%)	Mg (ppm)	P (%)	Ca:P Ratio
A	$FeSO_4$	0.100	704	0.196	1.0:2.0
B	$FeSO_4$, $CaCO_3$	0.200	724	0.225	1.0:1.3
C	$FeSO_4$, $CaSO_4$	0.213	730	0.161	1.3:1.0
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	0.201	816	0.172	1.2:1.0
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	0.191	797	0.153	1.2:1.0

TABLE XV

Mean Calcium and Magnesium Intakes of Rats During Six-Week Period (n=8)

Diet	Mineral Salts Added to Bread	Calcium Intake (mg)	Magnesium Intake (mg)
A	$FeSO_4$	508.60	358.78
B	$FeSO_4$, $CaCO_3$	1131.1	409.06
C	$FeSO_4$, $CaSO_4$	1168.1	400.59
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	1080.1	438.50
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	1071.5	447.12

Femur Magnesium Concentration (%)

Femur magnesium concentrations (Tables XVI and XVII) were significantly lower ($P \leqslant 0.01$) in rats fed diet A than in rats fed other diets and significantly higher in rats fed diet D than in rats fed diet B ($P \leqslant 0.05$) and diets C and E ($P \leqslant 0.10$). There were no significant differences in femur magnesium concentrations among rats fed diets B, C and E. Unadjusted mean femur magnesium concentrations ranged from 0.26 to 0.34%, closely corresponding to values of 0.3 and 0.4% magnesium found by Lee et al (34) who attributed significant differences in femur magnesium concentration to protein source rather than to calcium level.

Breads making up diets D and E were fortified with magnesium oxide, thereby accounting for the higher magnesium levels

of these diets (Table XIV). Mean magnesium intakes of the rats throughout the six-week period are given in Table XV. When femur magnesium concentrations (%) were adjusted for magnesium intake (Tables XVII and XVIII), differences among diets were similar to those found in the unadjusted values. These results indicate that femur magnesium concentration (%) was increased with increased magnesium in the diet when calcium carbonate but not when calcium sulfate was added to the bread.

TABLE XVI

Analysis of Variance for Femur Magnesium Concentration (%)

Source of Variation	DF	Mean Squares and Significance[1]
Diets	4	0.0096**
Rats:Diet	35	0.0002

[1]**$P \leq 0.01$

TABLE XVII

Mean Femur Magnesium Concentration (%)[1] (n=8)

Diet	Mineral Salts Added to Bread	Mean Femur Magnesium (%) Unadjusted	Adjusted[2]
A	$FeSO_4$	0.2562[a]	0.2504[a]
B	$FeSO_4$, $CaCO_3$	0.3275[b]	0.3273[b]
C	$FeSO_4$, $CaSO_4$	0.3300[b]	0.3288[b]
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	0.3425[c]	0.3456[c]
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	0.3300[b]	0.3341[b]

[1]Values followed by a common letter in a column are not significantly different ($P \leq 0.10$).

[2]Means adjusted for total magnesium intake.

TABLE XVIII

Analysis of Covariance Between Diets and Femur Magnesium Concentrations (%)

Source of Variation	DF	Adjusted Mean Squares and Significance[1]
Diets	4	0.0051**
Rats:Diet	34	0.00016

[1] **$P \leq 0.01$

Femur Calcium (mg)

Analysis of variance (Table XIX) showed that mean femur calcium (mg) was significantly lower ($P \leq 0.01$) for rats fed diet A than for rats fed the other diets which were not significantly different from each other. Unadjusted mean femur calcium ranged from 54.58 to 58.06 mg in rats fed diets B-E (Table XX), which had similar calcium levels but different calcium sources. Lee et al (34) reported similar values of approximately 42 to 63 mg calcium in femurs of weanling rats consuming diets of 0.4% calcium for four weeks. Ranhotra et al (45) reported femur calcium values ranging from 43.8 to 75.0 mg in weanling rats at the end of four weeks on diets containing 500 mg calcium per 100 g diet.

In the present study, bread used in diet A was not fortified with calcium carbonate or calcium sulfate which explains the relatively low femur calcium (mg) and femur calcium concentration (%) of rats fed this diet in comparison to rats fed

the other diets. Diets B-E contained similar levels of calcium (Table XIV). No significant difference ($P \leq 0.10$) was found in either femur calcium (mg) or femur calcium concentration (%) between rats fed the diet containing calcium carbonate fortified bread (diet B) and those fed the calcium sulfate fortified bread (diet C). When adjusted linearly for calcium intake, femur calcium (mg) was significantly higher ($P \leq 0.01$) for rats fed diet E than for rats fed diet C (Tables XX and XXI). Therefore, increasing the magnesium and zinc content of the diet resulted in higher femur calcium (mg) but not higher femur calcium concentration (%) when breads were fortified with calcium sulfate.

TABLE XIX

Analysis of Variance for Femur Calcium (mg)

Source of Variation	DF	Mean Squares and Significance[1]
Diet	4	1538**
Rats:Diet	35	28.24

[1] $^{**}P \leq 0.01$

TABLE XX

Mean Femur Calcium (mg)[1]
(n=8)

Diet	Mineral Salts Added to Bread	Mean Femur Calcium (mg) Unadjusted	Mean Femur Calcium (mg) Adjusted[2]
A	$FeSO_4$	25.80^a	39.88^{ab}
B	$FeSO_4$, $CaCO_3$	57.43^b	53.38^{ab}
C	$FeSO_4$, $CaSO_4$	54.58^b	49.42^a
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	56.59^b	54.02^{ab}
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	58.06^b	55.75^b

[1]Values followed by a common letter in a column are not significantly different ($P \leq 0.10$).

[2]Adjusted linearly for calcium intake.

TABLE XXI

Analysis of Covariance Between Diets and Femur Calcium (mg)

Source of Variation	DF	Adjusted Mean Squares and Significance[1]
Diets	4	86.70*
Rats:Diet	34	26.69

[1]*$P \leq 0.05$ but > 0.01

Femur Magnesium (mg)

Analysis of variance (Table XXII and Table XXIII) showed that unadjusted mean femur magnesium (mg) was significantly lower ($P \leq 0.01$) for rats fed diet A than for those fed any of the other diets, which were not significantly different.

Ranhotra et al (45) added magnesium from various sources to rat diets at a level of 19 mg per 100 g diet, but femur magnesium (mg) was found to be higher when the dietary source of magnesium was wheat flour, magnesium oxide or magnesium chloride than when it was magnesium sulfate, magnesium phosphate, magnesium lactate, magnesium citrate or magnesium acetate. In the present study, magnesium was added to the rat diets as magnesium carbonate and magnesium sulfate in the salt mixture, as naturally-occurring magnesium in flour in the bread (Appendix p64), and also as magnesium oxide added to the bread in diets D and E. Dietary magnesium levels (Table XIV) were approximately 0.07% in diets A-C and 0.08% in diets D and E. Mean femur magnesium values ranged from 0.69 mg for rats fed diet A to 1.23-1.27mg for rats fed diets B-E; these values were similar to those observed by Lee et al (34) and Ranhotra et al (45).

When adjusted for magnesium intake, femur magnesium (mg) remained significantly lower ($P \leq 0.10$) in rats fed diet A than in rats fed other diets, but femur magnesium (mg) became significantly greater ($P \leq 0.10$) for rats fed diet C than those fed diets D and E (Tables XXIII and XXIV). Since diets A-C

contained similar levels of magnesium, the low femur magnesium (mg) and femur magnesium concentration (%) of animals fed diet A was affected by the low calcium intake of rats fed this diet. There was no significant difference in femur magnesium (mg) or femur magnesium concentration (%) due to type of calcium salt added to the bread. Fortification of the bread with magnesium oxide and zinc oxide resulted in significantly lower ($P \leq 0.10$) femur magnesium (mg) in rats fed calcium sulfate fortified bread but was not different in rats fed calcium carbonate fortified bread.

TABLE XXII

Analysis of Variance for Femur Magnesium (mg)

Source of Variation	DF	Mean Squares and Significance[1]
Diets	4	0.503**
Rats:Diet	35	0.00265

[1] **$P \leq 0.01$

TABLE XXIII

Mean Femur Magnesium (mg)[1]
(n=8)

Diet	Mineral Salts Added to Bread	Mean Femur Magnesium (mg) Unadjusted	Adjusted[2]
A	$FeSO_4$	0.6925^a	0.7795^a
B	$FeSO_4$, $CaCO_3$	1.232^b	1.235^{bc}
C	$FeSO_4$, $CaSO_4$	1.245^b	1.262^c
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	1.270^b	1.224^b
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	1.261^b	1.200^b

[1]Values followed by a common letter in a column are not significantly different ($P \leq 0.10$).

[2]Adjusted linearly for magnesium intake.

TABLE XXIV

Analysis of Covariance Between Diets and Femur Magnesium (mg)

Source of Variation	DF	Adjusted Mean Squares and Significance[1]
Diets	4	0.327**
Rats:Diet	34	0.00262

[1]**$P \leq 0.01$

Calcium Balance

The first 96-hr collection of urine and feces was taken at the end of three weeks of feeding the rats the experimental diets, and the second collection was taken at the end of the six-week study. Because of the obvious differences in age and weights of the growing animals at the time of each collection period, these periods were treated separately.

Analyses of variance (Table XXV) showed a significant effect ($P \leq 0.01$) of diet on calcium intake, urinary calcium, fecal calcium and calcium balance during both periods. Unadjusted mean urinary calcium values (Table XXVI) during period 1 ranged from 1.13-1.74 mg in rats fed diets B and D ($CaCO_3$-fortified bread) to 2.12-2.50 mg in rats fed diets C and E ($CaSO_4$-fortified bread), and, during period 2, ranged from 1.92-2.01 to 2.44-2.66 mg in rats fed diets B and D and diets C and E, respectively. Mean fecal calcium values during both periods ranged from 0.2 to 2.9 mg for rats fed diets B and D ($CaCO_3$-fortified bread) and from 0.3 to 4.3 mg for those fed diets C and E ($CaSO_4$-fortified bread). In rats fed diets with the calcium level at 0.4%, Lee et al (34) reported considerably higher fecal calcium levels, 50 to 75 mg, but calcium balance values in the present study were similar to the highest values reported by Lee and coworkers.

Although calcium was added at similar levels to diets B-E, rats fed diet C had significantly higher ($P \leq 0.10$) calcium intakes during the first period but not the second period

TABLE XXV

Analysis of Variance for Calcium Intake, Urinary Calcium, Fecal Calcium and Calcium Balance

Source of Variation	DF	Mean Squares and Significance[1]							
		Period 1				Period 2			
		Ca Intake	Urinary Ca	Fecal Ca	Ca Bal	Ca Intake	Urinary Ca	Fecal Ca	Ca Bal
Diets	4	577.2**	5.339**	11.51**	45.95**	8080**	4.410**	21.42**	4105**
Rats:Diet	35	92.38	0.725	0.2606[2]	59.70[2]	101.0	0.8095	1.852[3]	109.0[3]

[1] **P ≤ 0.01

[2] DF=32

[3] DF=29

TABLE XXVI

Unadjusted Mean Calcium Intake, Urinary Calcium, Fecal Calcium and Calcium Balance[1,2]

Diet	Mineral Salts Added to Bread	Collection Period 1			
		Ca Intake (mg)	Urinary Ca (mg)	Fecal Ca (mg)	Ca Bal (mg)
A	$FeSO_4$	48.15^{a}	0.43^{a}	0.24^{a}(7)	47.54^{a}(7)
B	$FeSO_4$, $CaCO_3$	105.60^{b}	1.13^{ab}	2.70^{b}(7)	102.85^{b}(7)
C	$FeSO_4$, $CaSO_4$	114.22^{c}	2.49^{c}	0.75^{c}	110.99^{c}
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	104.52^{b}	1.74^{bd}	2.89^{b}	99.89^{b}
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	106.00^{b}	2.12^{cd}	0.67^{ac}(7)	99.22^{b}(7)

Diet	Mineral Salts Added to Bread	Collection Period 2			
		Ca Intake (mg)	Urinary Ca (mg)	Fecal Ca (mg)	Ca Bal (mg)
A	$FeSO_4$	58.51^{a}	0.74^{a}	0.31^{a}(4)	57.08^{a}(4)
B	$FeSO_4$, $CaCO_3$	130.13^{b}	2.01^{b}	4.37^{b}(7)	125.09^{b}(7)
C	$FeSO_4$, $CaSO_4$	132.62^{b}	2.43^{b}	1.72^{a}	128.97^{b}
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	129.40^{b}	1.92^{b}	4.28^{b}	123.19^{b}
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	125.10^{b}	2.66^{b}	1.18^{a}(7)	121.60^{b}(7)

[1]Values followed by a common letter in a column are not significantly different ($P \leqslant 0.10$).

[2]Number of animals in parentheses when not equal to 8.

than rats fed other diets except for diet A (Table XXVI). In period 2, urinary calcium and calcium balance appeared to depend upon calcium intake since no significant differences were found among effects of diets B-E on these values. Unadjusted values showed that fecal excretion of calcium was significantly less ($P \leqslant 0.01$) in rats fed diets A (bread not fortified with either calcium salt), C and E ($CaSO_4$-fortified bread) than in rats fed diets B and D ($CaCO_3$-fortified bread), indicating slightly better apparent absorption of calcium when the bread was fortified with calcium sulfate than when fortified with calcium carbonate. All animals were in strong positive calcium balance throughout both collection periods.

Analyses of covariance (Table XXVII) showed significant effects ($P \leqslant 0.01$) of diet on fecal calcium and calcium balance adjusted for calcium intake during both collection periods. Although analysis of covariance showed a significant effect of diet on urinary calcium during period 1 ($P \leqslant 0.05$), no effects of diet on urinary calcium were found during period 2. Calcium salt used in fortification affected calcium metabolism during both periods, since similarities were observed between effects of diets B and D, both containing breads fortified with calcium carbonate, and between diets C and E, both containing breads fortified with calcium sulfate. In period 1, adjusted urinary calcium was significantly lower and fecal calcium significantly higher ($P \leqslant 0.05$) in rats fed bread fortified with calcium carbonate than in rats fed bread fortified with

TABLE XXVII

Analysis of Covariance Between Diets and
Urinary Calcium, Fecal Calcium and Calcium Balance

Source of Variation	DF	Adjusted Mean Squares and Significance[1]					
		Period 1			Period 2		
		Urinary Calcium	Fecal Calcium	Calcium Balance	Urinary Calcium	Fecal Calcium	Calcium Balance
Diets	4	1.765*	8.866**	5.388**	0.8754[ns]	15.07**	12.24**
Rats:Diet	34	0.5640	0.2243[2]	1.177[2]	0.7921	1.860[3]	3.120[3]

[1] [ns]Not significant; *P≤ 0.05 but> 0.01; **P≤ 0.01
[2] DF=31
[3] DF=28

calcium sulfate (Table XXVIII). No differences in fecal calcium and calcium balance were found between rats fed bread fortified with calcium carbonate and between rats fed bread fortified with calcium sulfate during either period.

Magnesium Balance

Analyses of variance (Table XXIX) showed a significant effect of diet on magnesium intake during both periods ($P \leqslant 0.01$). Since breads making up diets D and E were fortified with magnesium oxide, these diets had higher levels of dietary magnesium than diets A-C (Table XIV). The low level of magnesium intake for rats fed diet A during both periods can be attributed to significantly lower feed intakes in these animals (Table XXX).

Analyses of variance and covariance (Tables XXIX and XXXI) showed significant diet effects during both periods on urinary magnesium, fecal magnesium and magnesium balance. Unadjusted values and values adjusted for magnesium intake followed similar trends. When adjusted linearly for magnesium intake (XXXII), urinary magnesium levels during both periods were higher in rats fed bread fortified with calcium sulfate than in rats fed bread fortified with calcium carbonate. Addition of magnesium oxide and zinc oxide to bread resulted in higher levels of magnesium being excreted in the urine of rats when the bread was fortified with calcium sulfate than when it was fortified with calcium carbonate.

TABLE XXVIII

Effect of Diet on Calcium Metabolism during Two 96-hr Collection Periods[1,2]

Diet	Mineral Salts Added to Bread	Adjusted Means - Period 1			Adjusted Means - Period 2		
		Urinary Ca (mg)	Fecal Ca (mg)	Ca Balance[3] (mg)	Urinary Ca (mg)	Fecal Ca (mg)	Ca Balance[3] (mg)
A	$FeSO_4$	2.51^{b}	1.46^{ab} (7)	92.31^{ab} (7)	1.87^{a}	1.71^{ab} (4)	117.94^{ab} (4)
B	$FeSO_4$, $CaCO_3$	0.70^{a}	2.42^{bc} (7)	92.47^{a} (7)	1.71^{a}	4.14^{b} (7)	115.33^{a} (7)
C	$FeSO_4$, $CaSO_4$	1.68^{b}	0.28^{a}	93.80^{b}	2.09^{a}	1.47^{a}	118.19^{b}
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	1.35^{b}	2.67^{c}	91.81^{a}	1.64^{a}	4.10^{b}	115.52^{a}
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	1.67^{b}	0.52^{a} (7)	93.72^{b} (7)	2.46^{a}	1.09^{a} (7)	117.66^{b} (7)

[1]Values followed by a common letter in a column are not significantly different ($P \leq 0.10$).

[2]Number of animals in parentheses when not equal to 8.

[3]Means adjusted linearly for calcium intake during each collection period.

TABLE XXIX

Analysis of Variance for Magnesium Intake, Urinary Magnesium, Fecal Magnesium and Magnesium Balance

Source of Variation	DF	Mean Squares and Significance[1]							
		Period 1				Period 2			
		Mg Intake	Urinary Mg	Fecal Mg	Mg Bal	Mg Intake	Urinary Mg	Fecal Mg	Mg Bal
Diets	4	126.8**	110.4**	11.71**	93.84**	179.4**	114.1**	39.38**	67.56*
Rats:Diet	35	16.33	24.04	1.481[2]	21.03[3]	16.46	21.78	5.733[4]	10.77[4]

[1] *P≤ 0.05 but >0.01; **P≤ 0.01
[2] DF=32
[3] DF=31
[4] DF=30

TABLE XXX

Unadjusted Mean Magnesium Intake, Urinary Magnesium, Fecal Magnesium and Magnesium Balance[1,2]

Diet	Mineral Salts Added to Bread	Period 1			
		Mg Intake (mg)	Urinary Mg (mg)	Fecal Mg (mg)	Mg Bal (mg)
A	$FeSO_4$	33.97^{a}	13.12^{a}	5.87^{a} (7)	15.56^{a} (7)
B	$FeSO_4$, $CaCO_3$	38.19^{b}	11.79^{a}	6.51^{ab}(7)	20.44^{bc}(7)
C	$FeSO_4$, $CaSO_4$	39.15^{bd}	19.52^{b}	6.00^{a}	15.74^{a} (7)
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	43.43^{cd}	11.76^{a}	7.14^{b}	23.53^{b}
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	44.23^{c}	18.24^{b}	9.01^{c} (7)	16.35^{ac}(7)

Diet	Mineral Salts Added to Bread	Period 2			
		Mg Intake (mg)	Urinary Mg (mg)	Fecal Mg (mg)	Mg Bal (mg)
A	$FeSO_4$	41.27^{a}	19.97^{ab}	6.46^{a}(4)	15.95^{ab}(4)
B	$FeSO_4$, $CaCO_3$	47.06^{b}	18.63^{a}	7.96^{ab}(7)	20.30^{b}(7)
C	$FeSO_4$, $CaSO_4$	45.62^{b}	22.26^{ab}	5.43^{a}	17.93^{b}
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	52.53^{c}	23.04^{b}	10.00^{bc}	19.49^{b}
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	52.20^{c}	28.44^{c}	10.91^{c}	12.85^{a}

[1]Values followed by a common letter in a column are not significantly different ($P \leqslant 0.10$).

[2]Number of animals in parentheses when not equal to 8.

TABLE XXXI

Analysis of Covariance Between Diets and Urinary Magnesium, Fecal Magnesium and Magnesium Balance

Source of Variation	DF	Adjusted Mean Squares and Significance[1]					
		Period 1			Period 2		
		Urinary Mg	Fecal Mg	Mg Bal	Urinary Mg	Fecal Mg	Mg Bal
Diets	4	99.22**	5.999**	72.25**	61.46**	12.36+	73.65*
Rats:Diet	34	22.72	1.239[2]	18.83[3]	16.75	4.808[4]	20.50[4]

[1] +P≤0.10 but>0.05; *P≤0.05 but>0.01; **P≤0.01
[2] DF = 31
[3] DF = 30
[4] DF = 29

TABLE XXXII

Effect of Diet on Magnesium Metabolism during Two 96-hr Collection Periods[1,2]

Diet	Mineral Salts Added to Bread	Adjusted Means - Period 1			Adjusted Means - Period 2		
		Urinary Mg (mg)	Fecal Mg (mg)	Mg Bal (mg)	Urinary Mg (mg)	Fecal Mg (mg)	Mg Bal[3] (mg)
A	$FeSO_4$	15.07^{ab}	6.70^{a}(7)	18.24^{ab}(7)	23.71^{bc}	8.36^{ab}(4)	17.73^{ab}(4)
B	$FeSO_4$, $CaCO_3$	12.28^{ab}	6.63^{a}(7)	20.85^{b}(7)	19.02^{a}	8.21^{b}(7)	20.54^{b}(7)
C	$FeSO_4$, $CaSO_4$	19.67^{c}	6.03^{a}	15.69^{a} (7)	23.48^{bc}	6.17^{a}	18.62^{b}
D	$FeSO_4$, $CaCO_3$ MgO, ZnO	10.78^{a}	6.68^{a}	22.12^{b}	20.26^{b}	9.01^{b}	18.56^{b}
E	$FeSO_4$, $CaSO_4$ MgO, ZnO	16.63^{bc}	8.55^{b}(7)	14.92^{a}(7)	25.86^{c}	10.00^{b}	12.00^{a}

[1]Values followed by a common letter in a column are not significantly different ($P \leq 0.10$).

[2]Number of animals in parentheses when not equal to 8.

[3]Means adjusted for magnesium intake during each period.

During both periods, increasing the dietary level of magnesium resulted in higher ($P \leqslant 0.10$) fecal magnesium, unadjusted and adjusted, when the bread was fortified with calcium sulfate but not when it was fortified with calcium carbonate. During period 1, adjusted fecal magnesium was greater ($P \leqslant 0.05$) for rats fed bread fortified with calcium sulfate, magnesium oxide and zinc oxide than for rats fed any of the other breads. In period 2, it was not significantly different from those of rats fed any of the breads except the calcium sulfate fortified bread.

Analyses of both unadjusted and adjusted values indicated that rats fed breads fortified with calcium carbonate were in more positive magnesium balance ($P \leqslant 0.10$) than were rats fed breads fortified with calcium sulfate. During period 2, rats fed bread fortified with calcium sulfate, magnesium oxide and zinc oxide had a less positive magnesium balance ($P \leqslant 0.05$) than rats fed other fortified breads.

Differences among diet effects were not consistent from period 1 to period 2. However, results obtained during period 1 suggest an effect of fortification with either calcium salt on magnesium metabolism, since rats fed bread fortified with calcium carbonate were in less positive calcium balance but in more positive magnesium balance than rats fed bread fortified with calcium sulfate.

The effects of varying dietary ratios of calcium and magnesium on magnesium metabolism has been reported (33-36, 45),

but the effect of calcium source on magnesium utilization has not been reported. The balance results of the present study did not agree with those obtained by Forbes (36) who found that increasing dietary magnesium level from 142 ppm to 420 ppm resulted in increased percentage absorbed and excreted in the urine of weanling rats fed a low calcium diet (0.4% calcium). In the present study, calcium was in the diets at considerably lower levels of 0.1-0.2% and magnesium at higher levels of 704-816 ppm (Table XIV).

According to O'Dell (46), the antagonistic relationship between calcium and magnesium is dependent upon dietary intake of phosphorus. When dietary intake of phosphorus is low, excess magnesium causes loss of calcium from the body. Phosphorus cuases decreased magnesium absorption and counteracts the harmful effects of excess magnesium. Harmful effects were described by Duckworth (47) who attributed bone calcium depletion to high magnesium intake, thus high levels of magnesium in the diet may have a direct action on calcification and also may accelerate phosphatase activity.

Dietary calcium-phosphorus ratios used in the present study ranged from 1.0:2.0 to 1.3:1.0 (Table XIV). A dietary ratio of 1 part calcium to 1 part phosphorus promotes the highest level of absorption, and ratios below 1:2 and above 2:1 are not recommended (9). The ratios in the diets used were within the recommended range, thereby possibly counteracting any deleterious effects of high magnesium:calcium ratios, or excess magnesium, on calcium absorption.

CONCLUSIONS

Based on the results obtained in this study, both calcium carbonate and calcium sulfate are suitable for calcium fortification of bread. Both salts were well utilized by rats when added to white bread at the NAS proposed level. Hemoglobin generation in rats was greater when calcium sulfate was added than when calcium carbonate was added to the breads at the proposed level, but it was within the normal range for all rats. Balance studies and femur analysis indicated better utilization of calcium and less efficient utilization of magnesium in rats fed calcium sulfate fortified bread than in rats fed calcium carbonate fortified bread, but all animals were in positive calcium and magnesium balance.

SUMMARY

The effects of two calcium salts (calcium carbonate and calcium sulfate) added to white bread at NAS proposed levels on the availability of calcium, iron and magnesium were investigated. Five different diets each containing approximately 80% dried bread were fed to Sprague-Dawley weanling rats for a period of six weeks. Criteria of utilization were: weight gain, feed intake, levels of calcium and magnesium excreted in the urine and feces, calcium and magnesium balance, femur calcium and magnesium content and blood hemoglobin and hematocrit levels.

Rats fed bread with no calcium fortification had significantly lower feed intake and weight gain than rats fed other breads. However, when weight gain was adjusted for feed intake, there were no significant differences among diets. Diet had no significant effect on hematocrit values, but calcium carbonate had a slightly adverse effect on hemoglobin generation and fortification with magnesium and zinc had a beneficial effect on hemoglobin generation. However, all hemoglobin and hematocrit values were within the normal range for rats. Femur calcium concentration (%) and femur calcium (mg) were not affected by fortification with either calcium salt. Increasing the magnesium and zinc content of the diet resulted in higher femur calcium (mg) but lower femur magnesium concentration (%) and femur magnesium (mg) when breads were fortified

with calcium sulfate than when fortified with calcium carbonate.

All animals were in positive calcium balance during both balance periods, but significant differences were observed among diets. Unadjusted values showed greater fecal excretion of calcium in rats fed bread fortified with calcium carbonate than rats fed other breads. Urinary calcium adjusted for calcium intake during period 1 was significantly lower and fecal calcium adjusted for calcium intake during both periods was significantly higher in rats fed diets with calcium carbonate than in rats fed calcium sulfate fortified breads. Better absorption of calcium was attained when the bread was fortified with calcium sulfate than when it was fortified with calcium carbonate.

All animals were in positive magnesium balance during both balance periods but significant differences were observed among diets. Urinary magnesium was greater for rats fed the calcium sulfate fortified breads during both periods. During period 1, but not during period 2, fortification of bread with calcium sulfate resulted in a less positive magnesium balance as compared to fortification of bread with calcium carbonate.

Both calcium carbonate and calcium sulfate were found to be suitable for calcium fortification of bread.

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APPENDIX

STANDARD SPONGE DOUGH PROCEDURE FOR WHITE BREADS

1. Ingredients are weighed separately for the sponge and dough and, except for water, yeast and shortening, emptied into separate containers.
2. Sponge ingredients are placed in the mixing bowl.
3. The sponge is mixed for 3 min at low speed and then for 1 min at Speed 2. The sponge temperature is at $76 \pm 1^{\circ}F$.
4. The mixed sponge is placed in a lightly greased fermentation jar and allowed to ferment for 4 hrs.
5. The dough ingredients and the fermented sponge are then placed into a mixer bowl and mixed for $1\frac{1}{4}$ min at Speed 1, and then at Speed 2 until the dough has reached optimum development. The temperature at this stage is $82^{\circ}F$.
6. A resting period of 30 min is allowed. Each piece of dough is then scaled to 539 g.
7. Each dough is hand punched, sheeted and rolled triple-fold; relaxed for 10 min, moulded, panned and proofed to height (1.5 cm above pan) at $105^{\circ}F$ and relative humidity of 92%.
8. Breads are baked at $425^{\circ}F$ for 20 min, cooled for 1 hr and sacked in plastic bags.

TABLE XXXIII

Composition of Salt Mixture[1]

Ingredient	Weight (g)
Cupric sulfate	0.0776
Manganese sulfate	0.2009
Ammonium alum	0.0923
Potassium iodide	0.0405
Sodium fluoride	0.5071
Alphacel	4.9816
Magnesium carbonate	35.2
Magnesium sulfate	38.3
Potassium chloride	124.7
Potassium phosphate dibasic	218.8
Sodium chloride	77.1

[1]U.S.P. XIV, modified

TABLE XXXIV

Proximate Analysis of Flour and Diets[1]

Constituent	Flour (%)	Diet A (%)	B (%)	C (%)	D (%)	E (%)
Moisture	11.6	5.5	6.2	6.2	5.4	5.6
Ash	0.4	3.4	3.8	3.8	3.9	3.8
Protein	12.4	19.1	18.6	18.2	18.9	20.8
Fat	0.9	4.5	4.8	4.3	4.6	4.7
Calcium	0.01	0.10	0.20	0.21	0.20	0.19
Magnesium	0.024	0.070	0.072	0.073	0.082	0.080

[1]Calcium and magnesium analyses by atomic absorption spectrophotometry in Dept. of Agronomy, Kansas State University. Moisture, ash, protein and fat analyses made by Dr. David Wetzel, Dept. of Grain Science and Industry, Kansas State University.

TABLE XXXV

Total Weight Gain, Feed Intake, Calcium and Magnesium Intake, Hemoglobin, Hematocrit, Femur Calcium and Femur Magnesium of Individual Animals After 42 Days on Experimental Diets[1]

Rat No.	Weight Gain (g)	Feed Intake (g)	Ca Intake (mg)	Mg Intake (mg)	Hb (g/100ml)	Hct (%)	Femur Calcium (%)	Femur Calcium (mg)	Femur Magnesium (%)	Femur Magnesium (mg)
					Diet A					
1	199	510	508.98	359.04	16.2	51	10.08	27.35	0.25	0.67
2	194	486	485.03	342.14	15.5	47	10.85	26.85	0.24	0.60
3	194	508	506.98	357.63	14.2	48	10.86	29.40	0.27	0.72
4	212	509	507.98	358.34	16.1	51	11.51	30.30	0.26	0.68
5	220	548	546.90	385.79	16.1	52	9.29	26.20	0.26	0.74
6	202	505	503.99	355.52	16.0	50	5.18	13.55	0.28	0.74
7	236	549	547.90	386.50	15.7	54	9.76	29.80	0.25	0.76
8	185	462	461.08	325.25	16.2	51	8.44	22.95	0.24	0.63
					Diet B					
1	222	527	1055.05	381.55	15.9	52	16.16	57.40	0.33	1.12
2	220	553	1107.11	400.37	15.0	47	17.45	63.55	0.34	1.22
3	216	567	1135.13	410.51	15.0	51	16.73	63.65	0.33	1.22
4	219	526	1053.05	380.82	15.5	50	13.77	49.65	0.32	1.16
5	270	604	1209.21	437.30	15.7	50	13.78	58.70	0.30	1.29
6	233	560	1121.12	405.44	14.8	48	13.96	52.50	0.33	1.23
7	251	581	1163.16	420.64	15.3	51	13.59	52.90	0.33	1.30
8	225	602	1205.20	435.85	15.3	47	15.83	61.10	0.34	1.32
					Diet C					
1	220	527	1122.51	384.71	15.3	51	14.25	54.25	0.32	1.24
2	241	550	1171.50	401.50	15.5	52	14.75	59.80	0.32	1.28
3	229	562	1197.06	410.26	16.2	53	13.93	52.35	0.33	1.24
4	216	542	1154.46	395.66	15.7	52	12.50	45.90	0.34	1.24
5	223	535	1139.55	390.55	15.7	50	14.13	52.55	0.33	1.23
6	229	534	1137.42	389.82	15.9	50	14.00	52.35	0.33	1.19
7	219	562	1197.06	410.26	15.3	48	15.21	56.35	0.34	1.28
8	243	578	1231.14	421.94	16.4	52	16.79	63.05	0.33	1.26

TABLE XXXV

(continued)

Rat No.	Weight Gain (g)	Feed Intake (g)	Ca Intake (mg)	Mg Intake (mg)	Hb (g/100ml)	Hct (%)	Femur Calcium (%)	Femur Calcium (mg)	Femur Magnesium (%)	Femur Magnesium (mg)
					Diet D					
1	236	584	1173.84	476.54	15.5	52	14.65	56.00	0.34	1.30
2	204	511	1027.11	416.98	16.2	52	13.00	45.20	0.38	1.31
3	228	554	1113.54	452.06	16.2	52	15.66	58.60	0.33	1.23
4	236	541	1087.41	441.46	15.2	50	14.80	57.10	0.33	1.29
5	204	518	1041.18	422.69	15.3	48	15.88	56.60	0.34	1.22
6	241	578	1161.78	471.65	15.5	50	15.61	61.90	0.33	1.32
7	214	533	1071.33	434.93	16.5	52	15.67	59.05	0.34	1.27
8	189	480	964.80	391.68	15.4	48	16.59	58.25	0.35	1.22
					Diet E					
1	224	575	1098.25	458.28	16.0	52	14.90	56.55	0.33	1.25
2	219	571	1090.61	455.09	16.0	52	12.26	48.15	0.32	1.28
3	230	544	1039.04	433.57	16.0	47	17.00	63.65	0.33	1.23
4	227	520	993.20	414.44	16.1	49	16.35	60.15	0.32	1.18
5	235	566	1081.06	451.10	16.1	48	16.35	62.35	0.33	1.27
6	208	530	1012.30	422.41	15.7	48	15.36	55.45	0.35	1.25
7	250	576	1100.16	459.07	16.7	49	13.39	53.90	0.34	1.36
8	236	606	1157.46	482.98	17.1	53	16.32	64.30	0.32	1.27

[1]Diet A - Bread + $FeSO_4$ (current enrichment level)
Diet B - Bread + $FeSO_4$ (current level) + $CaCO_3$ (proposed level)
Diet C - Bread + $FeSO_4$ (current level) + $CaSO_4$ (proposed level)
Diet D - Bread + $FeSO_4$ (current level) + $CaCO_3$, MgO and ZnO (proposed levels)
Diet E - Bread + $FeSO_4$ (current level) + $CaSO_4$, MgO and ZnO (proposed levels).

TABLE XXXVI

Calcium Intake, Fecal Calcium, Urinary Calcium and Calcium Balance In Individual Animals at End of Two 96-hr Collection Periods[1]

Rat No.	Collection Period 1				Collection Period 2			
	Ca Intake (mg)	Fecal Ca (mg)	Urinary Ca (mg)	Ca Balance (mg)	Ca Intake (mg)	Fecal Ca (mg)	Urinary Ca (mg)	Ca Balance (mg)
				Diet A				
1	51.90	0.37	0.30	51.23	55.89	---	0.38	---
2	50.90	0.40	0.39	50.11	54.89	0.25	0.57	54.07
3	47.90	---	0.54	---	56.89	0.43	1.39	55.07
4	48.90	0.14	0.21	48.55	59.88	0.28	0.51	59.09
5	51.90	0.17	0.53	51.20	60.88	0.29	0.52	60.07
6	34.93	0.13	0.56	34.32	62.87	---	1.01	---
7	50.90	0.23	0.48	50.19	63.87	---	0.98	---
8	47.90	0.28	0.42	47.20	52.89	---	0.57	---
				Diet B				
1	96.10	2.52	0.73	92.85	132.13	2.09	1.91	128.19
2	102.10	2.96	0.33	96.81	132.13	6.25	1.08	124.80
3	96.10	---	1.22	---	116.12	3.23	1.36	111.53
4	98.10	1.90	0.89	95.31	120.12	---	1.29	---
5	122.12	3.05	1.54	117.53	144.14	2.23	1.18	140.73
6	104.10	1.71	0.55	101.84	116.12	6.80	4.42	104.90
7	108.11	2.48	1.26	104.37	134.13	4.57	1.41	128.15
8	118.12	4.31	2.55	111.23	146.15	5.40	3.42	137.33
				Diet C				
1	106.50	0.53	2.43	103.54	132.32	5.31	1.71	129.30
2	119.28	0.74	1.99	116.55	136.32	0.74	2.40	133.18
3	123.54	0.80	2.54	120.20	125.67	1.98	1.89	121.80
4	110.76	0.51	3.11	107.14	127.80	1.31	3.12	123.37
5	119.28	1.12	3.79	114.37	125.67	1.76	2.05	121.86
6	106.50	0.58	1.00	104.92	127.80	1.15	2.33	124.32
7	104.37	0.64	1.64	102.09	138.45	0.05	2.03	136.37
8	123.54	1.06	3.40	119.08	146.97	1.45	3.98	141.54

TABLE XXXVI

(continued)

Rat No.	Collection Period 1					Collection Period 2			
	Ca Intake (mg)	Fecal Ca (mg)	Urinary Ca (mg)	Ca Balance (mg)		Ca Intake (mg)	Fecal Ca (mg)	Urinary Ca (mg)	Ca Balance (mg)
					Diet D				
1	116.58	2.57	1.48	112.53		126.63	3.93	1.01	121.69
2	100.50	3.02	3.99	93.49		120.60	2.75	2.43	115.42
3	112.56	4.12	2.18	106.26		122.61	4.42	1.80	116.39
4	100.50	2.13	0.28	98.09		140.70	3.72	1.64	135.34
5	112.56	2.76	1.64	108.16		122.61	3.27	1.75	117.59
6	108.54	2.28	1.51	104.75		142.71	6.25	1.68	134.78
7	100.50	3.69	1.82	94.99		148.74	6.55	3.73	138.46
8	84.42	2.57	0.99	80.86		110.55	3.36	1.32	105.87
					Diet E				
1	106.96	0.76	2.68	103.52		137.52	1.51	3.76	132.25
2	108.87	0.69	1.57	106.61		122.24	---	4.24	---
3	103.15	0.57	3.42	99.15		120.33	0.71	2.11	115.38
4	99.32	0.67	1.47	97.18		106.96	0.66	1.23	105.07
5	99.32	0.50	1.67	97.15		131.79	1.86	2.61	127.32
6	97.41	0.79	1.43	95.19		114.60	1.17	3.50	109.93
7	97.41	0.69	0.94	95.78		137.52	1.03	1.80	134.69
8	135.61	---	3.82	---		129.88	1.34	2.01	126.53

[1]Period 1 - at end of 3 weeks on experimental diets; Period 2 - at end of 6 weeks on experimental diets.

TABLE XXXVII

Magnesium Intake, Fecal Magnesium, Urinary Magnesium and Magnesium Balance In Individual Animals At End of Two 96-hr Collection Periods[1]

Rat No.	Collection Period 1					Collection Period 2			
	Mg Intake (mg)	Fecal Mg (mg)	Urinary Mg (mg)	Mg Balance (mg)		Mg Intake (mg)	Fecal Mg (mg)	Urinary Mg (mg)	Mg Balance (mg)
1	36.61	7.30	20.60	8.71	Diet A	39.42	---	18.18	---
2	35.90	6.30	5.81	23.79		38.72	5.45	9.18	24.09
3	33.79	---	16.98	---		40.13	7.55	28.41	4.17
4	34.50	4.90	12.10	17.50		42.24	7.75	17.64	16.85
5	36.61	5.75	18.90	11.96		42.94	5.10	19.14	18.70
6	24.64	4.70	11.33	8.61		44.34	---	25.70	---
7	35.90	6.05	10.64	19.21		45.06	---	24.01	---
8	33.79	6.10	8.58	19.11		37.31	---	17.50	---
1	34.75	5.55	9.96	19.24	Diet B	47.78	7.10	17.64	23.04
2	36.92	7.35	7.16	22.41		47.78	7.90	14.49	25.39
3	34.75	---	12.21	---		41.99	7.05	19.27	15.67
4	35.48	5.75	8.60	21.13		43.44	---	13.86	---
5	44.16	8.05	18.65	17.46		52.13	7.35	23.62	21.16
6	37.65	5.60	7.06	24.99		41.99	10.30	12.29	19.40
7	39.10	5.10	15.69	18.31		48.51	7.90	23.34	17.27
8	42.74	8.20	14.98	19.54		52.85	8.10	24.55	20.20
1	36.50	6.15	17.78	12.57	Diet C	46.72	6.65	19.06	21.01
2	40.88	5.40	17.25	18.23		46.72	6.80	23.83	16.09
3	42.34	6.00	22.59	13.75		43.07	5.85	22.70	14.52
4	37.96	5.60	20.15	12.21		43.80	7.65	22.21	13.94
5	40.88	6.65	17.58	16.65		43.07	4.40	22.70	15.97
6	36.50	5.60	12.12	18.78		43.80	4.55	21.19	18.06
7	35.77	4.80	32.00	---		47.45	0.70	23.69	23.06
8	42.34	7.70	16.66	17.98		50.37	6.85	22.70	20.82

TABLE XXXVII

(continued)

Rat No.	Collection Period 1					Collection Period 2			
	Mg Intake (mg)	Fecal Mg (mg)	Urinary Mg (mg)	Mg Balance (mg)		Mg Intake (mg)	Fecal Mg (mg)	Urinary Mg (mg)	Mg Balance (mg)
					Diet D				
1	47.33	7.80	10.33	29.20		51.41	10.15	18.41	22.85
2	40.80	7.25	10.96	22.59		48.96	7.60	32.42	8.94
3	45.70	6.35	11.86	27.49		49.78	8.55	24.97	16.26
4	40.80	7.10	6.23	27.47		57.12	8.40	23.41	25.31
5	45.70	7.30	12.20	26.20		49.79	6.15	16.03	27.61
6	44.06	6.15	16.08	21.83		57.94	14.05	27.12	16.77
7	40.80	10.00	16.38	14.42		60.38	17.20	24.67	18.51
8	34.27	5.15	10.06	19.06		44.88	7.90	17.29	19.69
					Diet E				
1	44.63	9.50	20.47	14.66		57.38	13.65	27.90	15.83
2	45.43	9.25	7.85	28.33		51.01	12.65	24.94	13.42
3	43.04	8.85	21.29	12.90		50.21	8.50	27.97	13.74
4	41.44	10.80	14.91	15.73		44.63	9.25	25.59	9.79
5	41.44	6.00	19.70	15.74		54.99	13.05	30.10	11.84
6	40.65	9.45	15.94	15.26		47.82	10.35	23.26	14.21
7	40.65	9.25	19.58	11.82		57.38	9.60	35.52	12.26
8	56.59	---	26.20	---		54.20	10.20	32.28	11.72

[1]Period 1 - at end of 3 weeks on experimental diets; Period 2 - at end of 6 weeks on experimental diets.

THE EFFECT OF TWO CALCIUM SALTS ON THE BIOAVAILABILITY OF CALCIUM, MAGNESIUM AND IRON FROM BREAD

by

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The effects of two calcium salts (calcium carbonate and calcium sulfate) added to white bread at NAS proposed levels on the availability of calcium, iron and magnesium were investigated. Five different diets each containing approximately 80% dried bread were fed to Sprague-Dawley weanling rats for a period of six weeks. Criteria of utilization were: weight gain, feed intake, levels of calcium and magnesium excreted in the urine and feces, calcium and magnesium balance, femur calcium and magnesium content and blood hemoglobin and hematocrit levels.

Rats fed bread with no calcium fortification had significantly lower feed intake and weight gain than rats fed other breads. However, when weight gain was adjusted for feed intake, there were no significant differences among diets. Diet had no significant effect on hematocrit values, but calcium carbonate had a slightly adverse effect on hemoglobin generation and fortification with magnesium and zinc had a beneficial effect on hemoglobin generation. However, all hemoglobin and hematocrit values were within the normal range for rats. Femur calcium concentration (%) and femur calcium (mg) were not affected by fortification with either calcium salt. Increasing the magnesium and zinc content of the diet resulted in higher femur calcium (mg) but lower femur magnesium concentration (%) and femur magnesium (mg) when breads were fortified

with calcium sulfate than when fortified with calcium carbonate.

All animals were in positive calcium balance during both balance periods, but significant differences were observed among diets. Unadjusted values showed greater fecal excretion of calcium in rats fed bread fortified with calcium carbonate than rats fed other breads. Urinary calcium adjusted for calcium intake during period 1 was significantly lower and fecal calcium adjusted for calcium intake during both periods was significantly higher in rats fed diets with calcium carbonate than in rats fed calcium sulfate fortified breads. Better absorption of calcium was attained when the bread was fortified with calcium sulfate than when it was fortified with calcium carbonate.

All animals were in positive magnesium balance during both balance periods but significant differences were observed among diets. Urinary magnesium was greater for rats fed the calcium sulfate fortified breads during both periods. During period 1, but not during period 2, fortification of bread with calcium sulfate resulted in a less positive magnesium balance as compared to fortification of bread with calcium carbonate.

Both calcium carbonate and calcium sulfate were found to be suitable for calcium fortification of bread.