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Effects of Acid Fermentation, Dry-Heat and Wet-Heat Processing on the Bioavailability of Calcium, Iron and Fluoride in Mechanically-Deboned Beef

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To my parents, Mr. and Mrs. C. F. Tso

EFFECTS OF ACID FERMENTATION, DRY-HEAT AND WET-HEAT PROCESSING ON THE BIOAVAILABILITY OF CALCIUM, IRON AND FLUORIDE IN MECHANICALLY-DEBONED BEEF

by

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Tzy-Bin Nancy Tso

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ABSTRACT

Effects of Acid Fermentation, Dry-Heat and Wet-Heat Processing on the Bioavailability of Calcium, Iron and Fluoride in Mechanically-Deboned Beef

by

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Mechanically-deboned beef shank (MDS) and hand-deboned beef shank (HDS) were prepared into thuringer (acid fermented), canned meat (retorted) and bologna (dry-heated). Raw meat and these products were then lyophilized and mixed into balanced diets and fed to weanling male rats for three weeks to evaluate the bioavailability of calcium, iron and fluoride. Bioavailability of calcium from MDS and its processed products was determined by feeding growing male rats with 5.01 to 5.53 g Ca/kg from MDS. HDS diets with 0.18 to 7.13 g Ca/kg added from calcium carbonate were also fed to rats for a relative comparison. The bioavailability of calcium in MDS appeared to be in the same range as that of calcium carbonate as evidenced by apparent absorption, relative biological values, bone ash and calcium content, and breaking

strength of femora. Processing by acid fermentation, dry-heat or wet-heat did not affect calcium bioavailability of MDS and HDS. Iron bioavailability from MDS and HDS was evaluated by apparent iron absorption value, terminal hemoglobin level, and liver iron storage. This study indicates that the iron from HDS was more efficiently utilized than the iron from MDS; however, MDS contains 30 percent more metabolizable iron than HDS. Fermentation processing improved, whereas retorting and high level of dietary calcium (7.13 g Ca/kg) depressed the absorption and utilization of iron from MDS and HDS. Apparent fluoride absorption, retention and bone (vertebra and femur) fluoride content were assayed to evaluate fluoride bioavailability. Processing by acid fermentation, dry-heat or wet-heat tended to decrease fluoride apparent absorption and retention. Bone fluoride contents were more related to dietary fluoride levels rather than to pocessing procedures. This study indicates that bone fluoride content is probably a more reliable measure to evaluate fluoride bioavailability than the percent absorption and retention values.

(119 pages)

INTRODUCTION

The mechanical deboner, used to separate edible meat from bone, is a relatively new machine used in meat processing. This equipment which started as a fish deboning machine was modified for use in the poultry and red meat industry (USDA Issue Guideline for MDM). Mechanically-deboned poultry meat products have been approved for human consumption for several years. In November, 1974, mechanically-deboned red meats (MDRM) were also approved for human consumption (Mulhern, 1976). However, a court injunction was passed to prohibit its further use in products for human consumption in September, 1976 (Bryant, 1976). A public hearing was held concerning the proposed standards and labeling requirements for tissue from ground bone in February, 1978. As a result of this hearing, a regulation was setforth (Federal Register, 1978).

The current regulation prescribes that at least 98 percent of the bone particles present shall have a maximum size no greater than 0.5 mm in their greatest dimension and there shall be no bone particles larger than 0.85 mm in their greatest dimension. The MDRM shall not have a calcium content exceeding 0.75 percent; shall have a minimum protein content of no less than 14.0 percent with a minimum protein efficiency ratio (PER) of 2.5 and fat content of not more than

30 percent. The name of the finished product containing MDM shall be further qualified by the phrase "With Mechanically Processed (Species) Product" and "Contains Up To __ percent Powered Bone." This product of MDRM may not be used in baby, toddler or junior foods.

Mechanical deboning has the potential of saving all the lean, red meat that is produced, while the method of hand deboning wastes some lean, red meat. This potentially important food source of mechanically-deboned meat (MDM) could amount to 2,090,757 metric tons per year (Field, 1976a). According to Noble (1974), it is estimated that current animal agriculture could yield 25-92 percent more meat products if meat and bone were mechanically separated. Therefore, mechanical deboning can save red meat and consequently reduce protein waste in the world facing a protein crisis. Since some connective tissue is removed and some bone particles and bone marrow are added to the meat by mechanical deboners, the net result is that the essential amino acid content of MDM is very similar to that of hand-deboned meat (HDM) (Essary and Ritchey, 1968; Field, 1976b). Lean hand-deboned beef has a protein efficiency ratio (PER) of 2.85 (Happich et al., 1975), while MDM from beef, pork and lamb neck bones has a PER value close to 2.8 if the calcium content is 1.0 percent or lower (Field, 1976b).

MDM differs from HDM in mineral content, particularly calcium, iron and fluoride because fine bone particles and bone marrow are incorporated into the product during the mechanical deboning process.

Field et al. (1974a) showed an increase in calcium in MDM compared with HDM of 500 percent when carcasses were deboned prior to rigor and 1000 percent when deboned post rigor. The amount of calcium in MDM has been reported from 2.17 to 17.2 g/kg (Hendricks et al., 1977b; Kruggel and Field, 1977; Field et al., 1976; Chat et al., 1977). Calcium level in MDM varied depending on the amount of meat attached to the bone at the time of deboning, the equipment used, the extent to which the bones are broken prior to being mechanically-deboned, the yield of meat and the type of bone used (Field et al., 1974b; Goldstrand, 1975). For regulatory purposes, the average concentration of calcium in fresh MDRM is considered to be about 0.5 percent by weight (Field et al., 1974b).

"Recommended Dietary Allowance" (Food and Nutrition Board, 1974) has suggested a minimum requirement for calcium at 800 mg/day for adults and 1200 mg/day for pregnant, lactating women and teenagers. Osteoporosis is a disease of bone, and particularly involves elderly people. It afflicts about 25 percent of Americans over 65 years old (Garn et al., 1967). It has been suggested that osteoporosis may be the result of trying to adapt to a low calcium intake (Albanese and Edelson, 1973). Presently Americans derive more than half of their dietary calcium from milk and dairy products (Comar and Bronner, 1964). However, in many other parts of the world, the supply of milk and milk products is not sufficient to meet the recommended dietary allowance for calcium. Many of the 138 articles reviewed by Walker

(1972) point out calcium deficiencies in human diets. Research conducted by Lutwak (1975) showed that a continuation of the present trend of low calcium in the American diet will lead to an increased incidence of peridontal disease in adults in their 30s and 40s, followed by increased osteoporosis in their 50s and 60s. Therefore, MDM which contains a higher amount of calcium than HDM can be nutritionally beneficial.

The amount of iron in MDM is higher than in HDM mainly due to the presence of bone marrow in MDM. Mechanically-deboned beef from commercial sources contains 4.3-6.3 mg of iron per 100 g of fresh meat (Field, 1976b), while hand-deboned beef with equivalent fat content contains 2.6-3.1 mg of iron per 100 g of fresh meat. Therefore, approximately twice as much iron is present in the commercial samples of MDM compared with HDM. Iron in mechanically-deboned turkey meat (18.8 ppm) and in hand-deboned turkey meat (10.8 ppm) was equally utilized when tested in hemoglobin regeneration of anemic rats (Allred et al., 1976). On the other hand, Anderson et al. (1977) indicated that iron from hand-deboned shank and hand-deboned plate was more efficiently utilized than the iron from mechanically-deboned shank and mechanically-deboned plate by anemic rats. However, because iron content is higher in MDM more metabolizable iron was available.

"Recommended Dietary Allowance" (Food and Nutrition Board, 1974) recommended a daily intake of 18 mg of iron for women and 10 mg for men. The frequency of iron deficiency anemia in many populations

emphasizes the importance of dietary iron. It has been estimated that more than 5 million adult women in this country have iron deficiency anemia and that at least twice this number do not have enough iron reserves to meet the physiologic needs of menstruation and pregnancy (Cook, 1977). In this case, higher iron content of MDM makes it a more significant source of iron than HDM.

Fluoride could be present in MDM because some microscopic bone particles are present. The fluoride content of animal bone depends on the age of slaughter (National Academy of Science, 1974) and on type of food consumed. Concentrations of fluoride in the skeleton varied almost linearly with the concentration of fluoride ingested (Shupe et al., 1963). The consumption of fluoride from MDM and other foods combined could be far below 20-80 mg (Kruggel and Field, 1977) that would be consumed daily to produce toxicity (Food and Nutrition Board, 1974). A frankfurter containing 10 percent MDM would contain about 1.7 ppm fluoride (Kruggel and Field, 1977). Since the daily fluoride intake in many areas of the United States is not sufficient to afford optimal protection against dental cavities (Food and Nutrition Board, 1974), products which contain MDM should be of value in furnishing needed fluoride.

MDM is similar to HDM in many respects, but bone particles and bone marrow give it some unique properties. In recent years, MDM products are becoming increasingly popular and only a limited

amount of research on this item is available; thus, the measurement of the bioavailability of the nutrients in MDM is very important.

The major objectives of the present study were (1) To determine the bioavailability of calcium, phosphorus, iron and flouride in mechanically-deboned beef shank, and (2) To evaluate the effects of food processing (acid fermentation, dry-heat and wet-heat) on the calcium, phosphorus, iron and fluoride bioavailability of mechanically-deboned beef shank.

REVIEW OF LITERATURE

Food Processing

Today, over 95 percent of the food consumed has been subjected to some form of processing (Harris and Karmas, 1975). Processed meats account for 35 percent of the meat shipped from federally inspected plants. They accounted for 8 percent of the total food dollar, with a value of $7 billion in 1969 (Kramlich et al., 1975). Processed pork products ranked second in grocery sales for 1973 with sales of 8 billion dollars. This sale increased by $1.6 million from 1972 to 1973 (Meat Board Reports, 1974). With this rapid increase in the use of processed meats it is necessary to know the nutritional consequences of the different methods of processing.

The processing methods employed in this study were acid fermentation, dry-heat cookery and wet-heat cookery.

Acid fermentation

Acid fermented meat products are usually made in the form of semidry sausage and require considerable knowledge of the art. They can be prepared using starter cultures comparable to those used in the cheese industry, or they can be held under specific conditions that preferentially promote the growth of organisms that impart flavor, texture and preservative qualities. The most common acid fermented

sausages are thuringer with a final pH of 4.8 to 5.0 and lebanon bologna with a final pH of 4.7 to 5.9 (Kramlich et al., 1975).

Selection of lean meat, as the basic ingredient for sausage formulation, is important in obtaining quality products. Cooked sausages such as frankfurters, bologna and similar comminuted sausage products are limited by government regulation to a maximum of 30 percent fat (Price and Schweigert, 1972). The other important ingredients in the fermented sausages are salts (1-5 percent), fermentable carbohydrates (sucrose and dextrose are used primarily), seasonings, extenders (binders, fillers and stabilizers), nitrate and nitrite. The formation of acid by the reaction between sugar and starter increases the acidity of the finished products.

Dry-heat cookery

During dry-heat cooking, the meat is surrounded by hot air, such as occurs in oven-roasting or broiling. Deep-fat frying and pan frying are also considered to be dry-heat methods of cookery (Kramlich et al., 1975). Dry air is a less efficient conductor of heat than water vapor. Since the atmosphere surrounding the meat is dry, the rate of which heat energy is supplied to the external surface is slower than that for wet-heat. With dry-heat, the external surface temperature may be considerable above 100°C. Dry-heat methods have been recommended for tender cuts of meat (Paul and Palmer, 1972).

Wet-heat cookery

Cooking by moist heat makes use of hot water or steam. The moisture is generally kept in contact with the meat by recirculating it to prevent its loss. In fresh meat, this is usually accomplished by covering the container with a lid, which causes most of the steam to condense and to be available again for generation to steam (Kramlich et al., 1975). Stewing, braising, pot-roasting, simmering and swissing are all commonly used moist-heat cooking procedures. In water vapor, the temperature to which the external surface of the meat is exposed will not exceed 100°C unless the system is under pressure. Large amounts of connective tissue in tough meat require long slow cooking in a moist atmosphere to tenderize the meat by degradation of collagenous tissue (Paul and Palmer, 1972).

Calcium

Bone particles in MDM

Mechanical deboners cause the inclusion of tiny bone particles in the final products. The bone particles from cattle, pigs and sheep are 18-24 percent calcium when expressed on a dry-fat-free basis (Field et al., 1974c). Young (1976) prepared a protein isolate from bone residue of mechanically-deboned chicken meat. The isolate contained in percent, 60-65 protein, 23-25 lipid, 5-10 ash and 4-6 moisture. Field et al. (1977) had characterized bone particles from five

lots of MDM from beef neck bones. The percentage of isolated bone particles ranged from 2.8 to 4.1 percent, and the calcium value ranged from 0.76 to 1.04 percent.

Basically, two approaches have been investigated in monitoring bone levels in MDM. The first one involves a physical separation of the tissue from bone particles and calculation of the percent bone residue present by weight (Kamm and Coffin, 1968; Hill and Hite, 1968). The second approach involves a chemical determination of calcium present from which a percent bone particle is calculated. This latter approach has been found to be more accurate than the physical method (Kamm and Coffin, 1968).

One of the major factors involved in product acceptance of MDM is the amount of microscopic bone particles remaining in the meat. The amount of bone present in MDM probably should be limited to 1.0 percent calcium to maintain a standard of identity for any meat item containing MDM or MDM for processing (Field, 1976b). More important, however, would be a limit on bone particle size. Field et al. (1977) observed that the bone particle diameters of MDM from beef neck bones ranged from 76.6 μ to 117.7 μ. Field (1976b) indicated that a maximum particle size of 0.5 mm would be desirable. Fried (1976) reported that particle sizes measured microscopically ranged from 0.025 to 0.457 mm were not detectable as gritty texture in the mouth.

Placing a limit on the amount of MDM incorporated into formulated products seemed to be desirable, and there is a suggestion of a limit of 20 percent on MDM and a limit of 15 percent on MDM for processing (Fried, 1976). Triangle tests indicated that no significant differences existed in bologna containing 10 percent MDM or 10 percent HDM (Field and Riley, 1972). A sensory panel was used to determine the quality and acceptability of ground turkey samples (Chongdarakul, 1974). It was recommended that the products should be ground through a 9.5 mm opening and contain levels of deboned meat not to exceed 10 percent.

Calcium bioavailability in bone meal

"Bone meal" refers to the fine dry powder obtained by boiling the bones to remove fat and a good deal of organic material, drying with hot air, and grinding (Drake et al., 1949). Edible bone meal has long been used as a source of calcium and phosphorus in baby foods, some enriched flours and livestock feedings as a dietary supplement (Blosser et al., 1954). Blosser et al. (1954) indicated that bone meal added at 1 percent to a grain ration completely devoid of calcium, would still supply 0.30 percent calcium in the grain ration (in excess of recommended amount suggested by Loosli et al. 1950). Sixteen samples of edible bone meal from three processors contained an average of 33.0 percent calcium, 15.4 percent phosphorus, 572 ppm

fluoride and 129 ppm zinc (Bartlet et al., 1952). Elliott (1962) reported that 600 mg calcium from bone meal (33 percent calcium) can be incorporated into food without appreciably affecting acceptability.

The use of bone as a source of calcium in man is not new. In fact, man in the past was dependent on animal and fish bone for a large portion of his calcium intake. Today many people in underdeveloped society still depend on bone as their chief source of calcium. The bioavailability of the calcium in bone meal appeared to be the same range as that of milk for the human (Drake et al., 1949). Mechanical deboning can provide an alternative natural source of calcium for any people who dislike or can't digest milk and milk products. Especially, 100 g of mechanically-deboned poultry meat contains about as much calcium as 160 g (2/3 cup) of milk (Hendricks et al., 1977b).

Forbes et al. (1921) and Mitchell et al. (1937) were some of the first to report that retention of calcium from bone sources was high. Udall and McCay (1953) studied the absorption efficiency of calcium from fresh beef bone. The bones were coarsely ground (particle size ranged from 0.318 to 0.635 cm) and were incorporated into diets to provide 2.96 to 4.94 g Ca/kg diet. Dogs at 80 and 110 days of age absorbed 56 and 58 percent of the dietary bone calcium. Drake et al. (1949) measured retentions of calcium by rats fed $CaCO_3$, whole milk or various bone sources. Relative to the 80.5 percent calcium retention from $CaCO_3$ and 94.2 percent from whole milk, calcium retention from bone sources were as follows: Straight run bones 92.6 percent,

soft pork and beef bones 80.2 percent, hard pork and beef bones 85.4 percent, cooked ground bone 73.3 percent. Thus, the retention of the calcium from bone is about 90 percent of the retention from whole milk for young rats.

Microscopic bone particles are easily broken down in the weak hydrochloric acid solution which is found in the stomach (Johnson et al., 1970). Posner (1969) observed that nonreversible hydrolysis of bone readily occurs in aqueous media at physiological pH value. The above studies make it clear that bone in the human diet can be beneficial.

Influence of acidity on calcium absorption

An important factor affecting utilization of calcium is pH. The solubility of the calcium source in the diet may be an important part of maintaining a positive calcium balance by affecting the calcium bioavailability (Mahoney et al., 1975). Bone salt belongs to a class of minerals called hydroxyapatite which has the composition $Ca_{10}(PO_4)_6(OH)_2$. Bone particles were stable in MDM but they were readily solubilized in 0.018 - 0.15 M HCl (Field et al., 1977). Bone is solubilized in HCl concentrations even lower than this range. Johnson et al. (1970) used a 0.007 M HCl solution to leach mineral from the surface of bone. Solubilization of bone by the 0.9 percent lactic acid in meat had also been reported (Lawire, 1974). Irving (1926) indicated that calcium absorption is influenced strongly by the solubility conditions prevalent in the intestine. He found that under

comparable conditions absorption of calcium salts proceeds in this order: acid acetate > neutral chloride > acid citrate > acid lactate. Increasing acidity by food processing (i.e., fermentation) may favor the solubilization of calcium from bone particles in MDM, reduce the bone particle size and thus increase calcium bioavailability.

Increased diet acidity may also favor calcium absorption. Verdaris and Evans (1975) designed an experiment of four diet groups combining either 0.2 or 2.1 percent calcium and pH of either 4.5 or 6.1 to study the effect of diet calcium and acidity on voluntary intake of dry matter and calcium metabolism. They concluded that within the same calcium treatment, the more acid diet tended to increase the absolute amount of calcium ingested, absorbed and retained. Therefore, the stimulating effect of lower pH on calcium absorption with low or high dietary calcium indicates a favorable effect of low pH on calcium metabolism. Ender and Dishington (1970) demonstrated a favorable effect of feeding acid and calcium-rich diets to dairy cows pre- and postpartum as a preventative measure against milk fever. It is also concluded that the increase in calcium absorption in the ileum with a low buffer capacity was due to the prevailing acidic pH (Ali and Evans, 1967). The increased bioavailability of calcium for absorption in the stomach and ileum with decreased pH is considered an indication of a relationship between increased solubility and availability of calcium for absorption in the gastrointestinal tract.

Iron

Iron bioavailability in meat

Meat is by far the best source of food iron utilized by man. Studies of food iron absorption in normal individuals fed a single food in which radioiron was incorporated biologically have shown that iron absorption from vegetables ranged from 3 to 8 percent and from animal products from 8 to 20 percent (Layrisse et al., 1968). Various types of meat have different patterns of iron absorption because of the various proportions of hemoglobin, myoglobin and ferritin content. Heinrich et al. (1969) found that iron absorption from rabbit muscle was 12 percent in normal and 23 percent in iron-deficient subjects. Moore (1964) found a mean of 10 percent absorption in normal subjects fed chicken muscle. The iron absorption from 2 to 4 mg veal muscle iron in 107 adults (both normal and iron deficiency) was 21.5 percent which was very close to that observed from iron ascorbate (23.8 percent) (Martinez-Torres and Layrisse, 1971).

Most of chemical iron in meat is found in heme compounds (myoglobin and hemoglobin). The use of radioactive method showed that approximately 60 to 65 percent of the total radioactivity (Fe^{59}) was found in the fractions corresponding to myoglobin, 33 to 37 percent to hemoglobin, and approximately 5 percent to ferritin (Layrisse and Martinez-Torres, 1972). In order to know the bioavailability of meat

iron, it is necessary to understand the mechanism of iron absorption from heme compounds.

Heme moiety from hemoglobin and myoglobin is directly absorbed by the mucosal cell instead of elemental iron. It was shown that ascorbic acid did not increase and nonabsorbable chelating agents (phytate, EDTA, deferioxamine) did not reduce hemoglobin absorption in both human and animal studies (Callender et al., 1957; Turnbull et al., 1962; Hallberg and Sölvell 1967). These indicate that little, if any, iron is split from hemoglobin in the intestinal lumen. Conrad et al. (1967) found that 16 percent of the hemoglobin iron is present as nonheme iron and 84 percent is present as heme iron in the lumen of small intestine during digestion. There was no significant difference in the rate of heme splitting from hemoglobin over a 3-hour period between iron-deficient rats (64 percent) and iron-loaded rats (59 percent). Splitting of heme from hemoglobin was greatest in duodenum and least in ileum (Conrad et al., 1966a).

When heme enters mucosa, iron is split from it by heme-splitting activity. Up to 1.5 times more heme enter iron deficient mucosa during 3-hour study and more iron is split from the heme entering iron-deficient mucosa. Total mucosal uptake of heme was greatest in duodenum and least in ileum in the ratio of 8:3:1, respectively (Wheby et al., 1970). The rate at which the iron split from heme is transported from mucosa to plasma determines the inhibitory feedback effect on heme-splitting substance and is the rate limiting step in the

absorption of heme iron (Manis and Schacter, 1964). In iron-deficient mucosa, this transport step is rapid so that little inhibition develops. In iron-loaded mucosa, transport of iron to plasma is very slow so that significant inhibition develops to heme-splitting activity. Thus, as less mucosal heme is split, this, in turn, inhibits further entry of heme into mucosa. This serosal transport ratio of duodenum to jejunum to ileum was 81:15:1, respectively (Wheby et al., 1970).

There was a greater absorption of iron from test doses of hemoglobin than from chemically purified heme by guinea pigs. On the other hand, if oral doses of heme dialysates containing globin degradation products were ingested, absorption was greater than for a test dose of hemoglobin (Conrad et al., 1966b). This indicated that the globin degration products enhanced the absorption of the heme.

Meat enhances the apparent absorption of iron from hemoglobin. The mean absorption of hemoglobin given alone (12 percent) was about half the absorption of the same food when given with veal (22 percent), whereas there was no difference between the iron absorption from meat given alone and when administered with hemoglobin (Martinez-Torres and Layrisse, 1971). This seems to indicate that the digestion of meat protein plays some role in meat iron absorption. It was suggested that the formation of an iron-amino acid chelate which increases the solubility of iron makes the iron available for absorption by the mucosa (Klavins et al., 1962; Kroe et al., 1966).

Influence of acidity on meat iron absorption

Polymerization plays an important role in the regulation of heme-iron absorption; monomeric heme compounds were absorbed in greater quantities than polymers of large molecular size (Conard et al., 1966a). Shack and Clark (1947) found that molecules of ferriprotoporphyrin in alkaline solution (pH ranged from 6.95 to 11.0) were in large aggregates or micellae. As pH decreased, the particle size was apparently reduced. Haurowitz (1938) reported that during the first seven hours the average particle weight of heme was 145,000 in 0.1 N NaOH at 15° C and over 4,000,000 at the end of four days.

It is well known that hemoglobin is split by acids into its protein constituent, globin and the prosthetic group heme. Gralén (1939) obtained globin and heme by splitting horse hemoglobin with 0.1 N HCl. Then he precipitated the globin by acetone and neutralized with 0.1 N NaOH, which precipitated denatured protein and the "native" globin was further freed from denatured globin. Alkaline heme solution were prepared by adding to the acetone solution 1 percent of its volume of 2N sodium acetate and dissolving the precipitate so obtained in a buffer solution. By adding this alkaline heme solution to a solution of neutral globin, "native" hemoglobin was recoupled with the same molecular weight (69,000). By a procedure involving the use of acid acetone, hemoglobin was rapidly separated into a precipitate of denatured globin

and a solution of heme (Anson and Mirsky, 1930). Thus, the condition of low pH tends to prevent heme aggregation.

Heme can combine with great variety of nitrogenous substances and forms hemochrome. The assumed reaction is $Fe + 2B \rightleftarrows FeB_2$, where Fe is in the protoporphyrin nucleus and B represents the coordinating base (Lemberg and Legge, 1949). Generally, the formation of hemochrome compound occurs at either neutral or alkaline pH but not acidic pH. Ferrohemochromes were more readily formed at high pH value (alkaline), while ferrihemochrome formation was favored by a neutral pH (Akoyunoglon et al., 1963). The influence of hemochrome formation on the hemoglobin iron absorption is unknown and there is no related research conducted. However, one reasonable expectation is that a possible increased molecular size of the hemochrome is less absorbable than heme in the small intestine. This might be another mechanism by which the absorption of meat iron is reduced in alkaline condition.

Influence of heating
on meat iron absorption

Heat treatment frees some iron from native hemoglobin. Jacobs and Greenman (1969) found that cooking increased the total amount of soluble iron released by peptic digest in five of seven meats tested. Nonheme iron is much less absorbable than heme iron because nonheme iron is easily chelated by inhibitors (Phytate, EDTA, etc.), which will decrease its absorption

(Sharpe et al., 1950). It has been shown that the mean absorption of uncooked hemoglobin by 10 subjects of mixed sex was 19 percent, and cooked hemoglobin 7 percent (Callender et al., 1957). They also reported that the mean iron absorption in 11 iron deficient subjects who consumed uncooked hemoglobin was 22 percent and was 12 percent for cooked hemoglobin. However, the study of Turnbull et al. (1962) indicated that cooking did not change the absorption of hemoglobin iron. His results showed that absorption of the uncooked hemoglobin was 15.7 percent and absorption of heated hemoglobin in a boiling water bath for 15 minutes was 14.4 percent.

Protein degradation products enhance the absorption of hemoglobin iron (Martinez-Torres and Layrisse, 1971). Heat has long been recognized as a factor lowering the nutritive value of meat protein. Thus, the effects of heating on meat protein may also affect the absorption of meat iron. Heating causes the destruction of many amino acids. Pork processed at 110°C for 24 hours lost 44 percent cysteine, 34 percent available lysine and up to 20 percent of other essential amino acids (Donoso et al., 1962). Dvorak and Vognarova (1965) also found that 3-hour heating at 70°C causes a 10 percent loss of available lysine, at 121°C the loss was 20 percent, at 140°C, 40 percent and 160°C, 50 percent. Donoso et al. (1962) reported a reduction in net protein utilization (NPU) value for pork protein from 76.2 to 40.8 upon heating meat for 24 hours at 110°C as protein fed at a level of 8 percent in the diet. Lysine supplementation raised the NPU to 44.0 and methionine

raised the NPU to 60.0. Hendricks et al. (1977a) demonstrated that cooking of desinewed beef shank decreased the protein efficiency ratio (PER) and nitrogen efficiency for growth (NEG) for rats. Using SDS-polyacrylamide gel electrophoresis, Cheng and Parrish (1979) found myosin is the first insoluble protein in the muscle during heating. Yamamoto et al. (1979) observed denaturation and insolubilization of sarcoplasmic protein after incubation meat at 40°C.

The cooking method and pH condition of the meat may also affect the damage of meat protein during heating. The classical experiments of Lea and Hannan (1949) established the destruction of meat protein caused by heating is most severe at 10-14 percent moisture (70 percent relative humidity). Dry materials are relatively resistant to heat and boiling in excess water usually causes no nutritional damage. Dry fish was heated for 24 hours at 105°C without nutritional damage. With 9 percent water added the NPU fell from 72 to 43 (Miller, 1956). Schroeder et al. (1961) indicated that autoclaving at a more alkaline pH causes a higher rate of amino nitrogen liberation. Heat decreases the nutritive value of meat protein only when the pH of the system is buffered above 6.0, but not at a more acid reaction.

The reduction of meat protein digestibility by heating may also decrease the bioavailability of the minerals (i.e. iron) in meat. As the digestibility of meat protein decreases the absorption rate of all ingredients (including heme compounds) would be expected to decrease. In this case, less heme would be available to be absorbed in the upper

small intestine (duodenum), where mucosal uptake of heme is greatest. Although more heme would become available in the jejunum or ileum, the heme iron absorption in this part of the intestine is not as rapid as in the duodenum. In addition, some heme may be trapped in the insolubilized protein in the cooked meat so that some iron becomes unavailable.

Fluoride

Fluoride in bone meal and MDM

Bone is a good source of fluoride as is bone meal and MDM. Approximately 99 percent of the fluoride retained in the body is stored in the bone and its quantity is proportional to the fluoride content in the diet (Underwood, 1971). Evans and Phillips (1938) found an average of 32 ppm of fluoride in veal bones and 190 ppm in bones of mature cows. According to Shupe et al. (1962) bones from animals receiving 10 ppm fluoride in hay for 588 days contained 328-528 ppm fluoride on a dry, fat-free basis.

Eighteen samples of bone meal analyzed for fluoride contained an average of 803 ppm expressed as total-dried basis (Blosser et al., 1954). The fluoride content from 16 samples of edible bone meal had an average of 572 ppm with 1.46 percent moisture (Bartlet et al., 1952). It is apparent that bones most suited for mechanical deboning are also the bones highest in fluoride content (Field, 1976a). Kolbye and Nelson (1977) reported that the mean fluoride content of

mechanically-deboned beef was 18.6 mg/g fresh tissue; and, pork contained 10.6 mg/g fresh tissue. MDM of broiler back and neck had an average fluoride content of 15 ppm, with a range from 8 to 21; values for the corresponding feeds average 34 ppm and ranged from 17 to 56 (Pool et al., 1965). MDM from flat bones of mature cows and bulls from three regions of the country and pork MDM from two regions were evaluated (Kruggel and Field, 1977). The average fluoride contents were 37.2, 47.6 and 30.3 ppm in the beef MDM from the West, Midwest and South region, respectively. The average fluoride content in pork MDM from the West and Midwest regions was 18.1 ppm on a dry matter basis.

Fluoride bioavailability in bone meal

The bioavailability of the fluoride in the bone meal was found to be the same as that of NaF (Ellis and Maynard, 1936). Pool and Thomas (1970) observed that weanling rats fed diets containing approximately 18 mg F /g diet for 3 weeks had about 170 mg F /g bone when either NaF or chicken bone meal was the source of fluoride. On the other hand, evidence has appeared to indicate that the bioavailability of fluoride in bone meal is relatively poor. Only between 37 and 54 percent of fluoride from bone meal will be absorbed by the adult human (Machle and Largent, 1943). McClure et al. (1945) found the absorption of the fluoride of bone meal was 64.5 percent as efficient as fluoride absorption from NaF mixed with the food. When fish protein

concentrate (150 - 300 ppm fluoride primarily from fish bone) was fed to growing rats, the bioavailability of its fluoride ranged from 25 to 52 percent that of NaF (Zipkin et al., 1970). Stillings et al. (1973) found that growing rats ingesting diets containing 32 to 70 ppm fluoride had fluoride retentions of 24 percent from fish protein concentrate and 50 percent from NaF. However, growing rats ingesting 7 ppm fluoride had retentions of 32 percent from both fish protein concentrate and NaF. Thus, the amount of fluoride ingested affects the amount of fluoride retained.

When rats were fed a level of 0.6 mg percent fluoride in the diet, the retention of ingested fluoride was the same when added as either NaF, pork bone or beef bone. The retention when added as veal bone was only one-fourth to one-third that of NaF (Jackson et al., 1950). Since beef and pork bones contained large concentrations of fluoride, the amount of bone required to add to 0.6 mg percent of fluoride to the diet was small (0.37 percent), so that the quantity of calcium added was also small. Fluoride content of veal bone was low, so that the diet containing veal bones had 1.78 percent calcium in order to meet the level of 0.6 mg percent of fluoride in the diet. These results indicated that the fluoride bioavailability of bone meal is dependent upon the fluoride and calcium content of the bone.

Absorption and excretion of fluoride

The absorption of fluoride from soluble fluoride compounds (e.g., NaF, HF, H_2SiF_6, Na_2SiF_6, Na_2PO_3F) is rapid and nearly complete. Fluoride absorption is passive and no active transport mechanism is involved. Zipkin and Likins (1957) found that 72 percent and 86 percent of a fluoride dose of 0.2 mg fluoride as NaF solution was absorbed in 60 and 90 minutes respectively. Observation with ^{18}F in man, Carlson et al. (1960) suggested that absorption of fluoride must occur from the stomach, in view of its rapid appearance in blood. According to Stookey et al. (1962) fluoride absorption from the stomach ranged from 26 to 36 percent from the first 5-cm segment of intestine between 20 and 49 percent, and from the second and third 5-cm intestinal segments between 36 and 49 percent. Besides ingestion absorption also occurs through the skin and lungs via HF.

The principal route of fluoride excretion is via the urine. Largent (1954) in balance studies in man, found that when extra fluoride is ingested about half is bound by bone and the remainder is excreted in the urine. Carlson et al. (1960) indicated that dogs that received carrier-free radiofluoride infusions excreted urine with a mean radiofluoride concentration 3.4 - 14.5 times that of the plasma. They also found that the radiofluoride clearances were always less than the creatinine clearances but were 7.8 - 179 times the chloride clearance. Chen et al. (1956) reported that dogs with a normal water load, the

average normal renal fluoride clearance was 2.7 ml/min; the fluoride: chloride clearance ratio, 19; and the fluoride: creatinine clearance ratio, 0.077.

Of the factors influencing renal fluoride excretion, the most prominent are the plasma concentration of fluoride and the urine flow rate. The tubular resorption of radiofluoride is best correlated with the urine flow rate indicating that the tubule is an important factor. The other possibility is the fluoride ion, because of its extensive hydration, is handled by the kidney in the same way as water. Rapid urinary excretion of fluoride is one of two major means by which the body prevents the accumulation of sufficient fluoride ion to reach toxic levels (Hodge and Smith, 1954).

Only about 10 percent of the total daily fluoride excretion is found in the feces. Part of the fluoride in feces is undissolved and unabsorbed fluoride. When diets contain relatively insoluble fluoride compounds, e.g., bone meal, cryolite, insoluble calcium salts, larger amounts of fluoride are excreted in the feces (Largent, 1961; Rich et al., 1964). Some fluoride is lost from the body sweat.

Calcium, fluoride and bone

As calcium is added to the diet, the fluoride absorption is markedly reduced to about 50 percent. In such a case the fluoride is bound in a less soluble form and fecal excretion increases (Hodge and Smith, 1965). Dietary calcium exerts a protective action in fluoride

intoxication and calcium deficient dietaries accentuate the symptoms of fluorosis (Bond, 1962). Compared with a 0.23 percent calcium diet, a 0.73 percent calcium diet depressed the total retention of fluoride by 10.5 percent for the smaller rats, and 12.8 percent for the larger rats (Lawrenz and Mitchell, 1941).

Studies using growing rabbits revealed that fluoride reduced the resorption of bone probably by producing a more stable system i.e. fluoroapatite (Faccini, 1967). Havivi and Guggenheim (1966) found that fluoride containing bone from mice had a reduced ^{45}Ca release, compared with control bone. A slower resorption of bone tissue also has been implicated as a factor in skeletal fluorosis (Havivi and Guggenheim, 1966).

A further important action of fluoride is the production of large amounts of osteoid. An increased amount of new bone has been described both in animals (Weidmann and Weatherell, 1962) and in man (Jowsey et al., 1968) when fed supplemented fluoride. From an in vitro study, Nichols et al. (1965) found bone collagen synthesis was increased in hyperparathyroidism and concluded that the stimulation of new bone formation in fluorosis was an effect of parathyroid hormone. They suggested that fluoride induces a special kind of secretory hyperparathyroidism in which increased bone resorption is blocked and only the stimulation of new bone formation is manifest. This finding is confirmed by Burkhart and Jowsey (1968) that thyroid glands in fluorotic animals caused inhibition of bone resportion. They concluded that it

is a compensatory phenomenon to maintain the serum calcium at a constant level.

Evidence for a possible role of fluoride in preventing osteoporosis has been obtained by Bernstein et al. (1966). In a radiological study, they found that the incidence of collapsed vertebrae in women was lower in a higher fluoride area (4.0 to 5.8 ppm fluoride in the water supply) than in a low fluoride area (0.2 to 0.3 ppm fluoride in the water supply). Rich and Ensinck (1961) gave six patients with osteoporosis 66 mg fluoride per day for 14 or more weeks. These patients changed from a negative calcium balance to a positive one. Rich et al. (1964) found that both calcium and phosphorus were retained to an increased degree after the tenth week of fluoride treatment.

As early as 1937, Roholm realized that an increase in bone growth and calcification resulted from a comparatively small dose of fluoride, while large doses produced an atrophic state with a reduction of calcification. De Senarclens (1941) observed resorption cavities in cortical bone of fluorotic goats. Pike and Brown (1975) indicated that high intakes of fluoride result in mottled enamel and fluorosis of bone has been reported in populations living for many years in areas of high fluoride water content (at least 8 ppm). The comparative resistance of rats to the effect of fluoride is important in view of their frequent use as experimental animals. Changes have been observed in rat bone. Rockert and Sunzel (1960) showed that rats fed 0.7 to 1 mg NaF per

day developed osteosclerosis in their vertebrae after nine months of administration.

METHODS AND PROCEDURES

Meat Preparation

Beef shank from a commercial packer was divided into two lots for the MDS and HDS preparation. Hand deboning was done at the Utah State University Meat Laboratory. Meat was manually cut from the bone and ground twice through a 0.31 cm plate. Mechanical deboning was done by Beehive Machinery, Inc., Salt Lake City, Utah. The whole shanks were put through a 1.27 cm plate of a bone cutter and then deboned with an AU 6173 Beehive deboner using an AU 0.457 mm head (0.018 inch perforation). The MDS extruded through the perforations was frozen with solid CO_2 and stored in plastic bags. Aliquots of the MDS and HDS were immediately lyophilized and stored at -20°C. The remaining MDS and HDS was processed by one of the following three methods: (1) Acid fermentation (Thuringer)--8.63 kg of MDS was mixed thoroughly with 65 g dextrose, 170 g salt, 0.67 g sodium nitrite, 10.77 g Pediococcus cervesiae and 0.68 kg water. Hand deboned shank meat (6.36 kg) was mixed thoroughly with 8.80 g $CaCO_3$, 48 g dextrose, 128 g salt, 0.50 g sodium nitrite, 7.94 g Pediococcus cervesiae and 0.50 kg water. Since the pH of the meat mixture did not decrease as expected, lactic acid was added until the pH reached about 5.0. The mixture was passed through a 0.3175 cm

plate and then stuffed into casings. The surface of the encased product was washed with a hot shower and hung at room temperature for two hours before transfered to a meat oven where it was heated until the internal temperature reached 160°F. (2) Dry-heat cookery (Bologna)--Samples of ground, fresh MDS and HDS were encased after mixing with only the salt and sodium nitrite and were then cooked as above. (3) Wet-heat cookery (Retort, Canned meat)--Fresh MDS and HDS samples were placed in glass jars with lids and rings and then heated in an autoclave for 90 minutes at 20 psi and 210°F. The HDS was mixed with $CaCO_3$ before processing to provide the same amount of calcium as was present in the MDS samples. After processing, this meat was also lophilized and stored.

Meat Analyses

Protein, fat, moisture, ash, calcium, phosphorus and iron levels were determined on fresh meat and lyophilized meat and meat products. Fluoride level was only determined on lyopilized meat and meat products. Demineralized water and acid rinsed equipment were used for all mineral analyses.

Three replicate samples (about 5.0 g for fresh meat and 2.5 g for lyophilized meat) of each meat were analyzed for moisture and ash using the vacuum oven at 70°C and 15 psi vacuum (A.O.A.C., 23.002) and muffle furnace at 575°C for 24 hours (A.O.A.C., 23.006), respectively. Fat content was determined by refluxing in a Soxhlet extractor for 48 hours with petroleum ether as the solvent (A.O.A.C.,

23.005, followed by a second extraction with 1:1 chloroform and methanol). The Macro Kjeldahl method for crude protein was used to determine nitrogen content (Appendix A). The pH values were determined for meat samples before lyophilizing. Fifty g of meat sample was homogenized with 50 ml demineralized water and the pH value was read from a pH meter.

The meat ash was solubilized by heating in 5 ml of 6N HCl for a few minutes and diluted to 25 ml with demineralized water. The dilution of the ashed solution and method used for mineral analyses are summarized in Table 1.

Diet Formulation

Eleven diets (Table 2) were prepared and assayed for calcium, phosphorus, iron, and fluoride bioavailability of the different processed MDS and HDS products. Control diets were prepared from raw HDS and different amounts of $CaCO_3$ to bring the calcium level of each diet to about 0, 2.5, 5.0 and 7.5 g Ca/kg. The calcium source was the microscopic bone particles in the MDS diets and $CaCO_3$ in the HDS diets. The diets used are shown in Table 2.

Diet Preparation

According to the amount of calcium desired and the experimental design, the amount of fresh MDS, processed MDS or processed HDS in each diet was calculated. Every diet was made to the same level of

Table 1. Meat mineral analyses methodology.

Mineral	Dilution of Ashed Solu.	Equipment	Wave Length (Å)	Method
Ca	MDS: 200 x in 10,000 ppm $SrCl_2$ solu. HDS: 40 x in 10,000 ppm $SrCl_2$ solu.	Varian Techtron AA.120 atomic absorption spectrophotometer	4226.7	Concentrations were calculated using regression equation developed from a standard curve.
Fe	2 x in demineralized water	Varian Techtron AA.120 atomic absorption spectrophotometer	2483.3	Concentrations were calculated using regression equation developed from a standard curve.
P	MDS: 40 x in demineralized water HDS: 20 x in demineralized water	photoelectric colorimeter	7000	Appendix B
F	no	microprocessor ionalyzer/901	--	Appendix C

Table 2. Composition of mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets.

Group No.	1	2	3	4	5	6	7	8	9	10	11
Item	MDS (Raw)	MDS (Ferment)	MDS (Dry-Heat)	MDS (Wet-Heat)	HDS (Raw)	HDS (Ferment)	HDS (Dry-Heat)	HDS (Wet-Heat)	HDS (Raw)	HDS (Raw)	HDS (Raw)
	5.01[a]	5.13	5.05	5.53	4.96	5.03	5.27	4.34	0.18	2.77	7.13
Ingredient (g/kg)											
Meat-total	523	585	526	517	434	507	493	482	434	434	434
MDS	301 (Raw)	441 (Ferment)	382 (Dry-Heat)	336 (Wet-Heat)	0	0	0	0	0	0	0
HDS							476 (Dry-Heat)	395 (Wet-Heat)			
	222 (Raw)	144 (Raw)	144 (Raw)	181 (Raw)	434 (Raw)	507 (Ferment)	17 (Raw)	87 (Raw)	434 (Raw)	434 (Raw)	434 (Raw)
Rendered beef kidney fat	42	--	59	43	118	63	101	108	118	118	118
Cellulose	50	50	50	50	50	50	50	50	50	50	50
Dextrose	353	332	332	359	343	339	311	326	355	349	337
Vitamin[b] Mix	20	20	20	20	20	20	20	20	20	20	20
Mineral[c] Mix	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6
$CaCO_3$	--	--	--	--	12.2	~~12.2~~	~~12.2~~	~~12.2~~	--	6.0	18.4
$NaH_2PO_4 \cdot H_2O$	6.8	8.3	6.9	5.6	16.1	14.0	16.8	15.6	16.7	18.0	16.4

[a] Level of dietary calcium (g/kg).

[b] Vitamin mixture composition is shown in Table 3.

[c] Mineral mixture composition is shown in Table 3.

protein (about 24 percent) by adding the appropriate quantity of fresh HDS. Beef kidney fat was added in appropriate amounts to bring all diets to the same level of fat (about 34 percent). Sodium acid phosphate ($NaH_2PO_4 \cdot H_2O$) was added in appropriate amounts to keep the phosphorus concentration the same in all diets (about 5.0 g/kg diet). Standard vitamin and mineral mixtures (Table 3) which provided all the essential vitamins and minerals except calcium, phosphorus and iron were added to each diet. The diets (Table 2) were individually prepared in batches of 3 kg in an electric mixer which had been epoxy coated on the bowl and paddle, and equipment was cleaned between the preparation of each diet. All diets were stored in a commercial refrigerator.

Diet Analyses

The 11 diets were analyzed in triplicate for protein, fat, calcium and phosphorus to ensure that they met the requirements of the experiment. Dietary moisture, ash, iron and fluoride content was also determined. Adjustments in the content of the diets were made if necessary and then reassayed. Protein, fat, moisture and ash were analyzed as previously described for meat samples.

The ash of each diet sample was solubilized by heating in 5 ml of 6 N HCl and diluted to 30 ml with demineralized water. The dilution of the ashed solution and method used for diet mineral analyses are summarized in Table 4.

Table 3. Mineral and vitamin mixtures[a] added to diets in determining calcium, phosphorus, iron and fluoride bioavailability of mechanically-deboned beef shank.

Salt	g salt/kg mixture	Vitamin	g/kg mixture[b]	mg/kg diet
KCl	296.7	Vitamin A concentrate (200,000 units/g)	4.5	90
$MgCO_3$	121.0	Vitamin D concentrate (400,000 units/g)	0.25	5
$MnSO_4$	12.7	Alpha-tocopherol	5.0	100
$CoCl_2 \cdot 6H_2O$	0.7	Ascorbic acid	45.0	900
$CuSO_4 \cdot H_2O$	1.6	Inositol	5.0	100
$Na_2MoO_4 \cdot 2H_2O$	0.1	Choline chloride	75.0	1500
$ZnSO_4 \cdot 7H_2O$	38.0	Menadione	2.25	45
KI	0.8	p-Aminobenzoic acid	5.0	100
Glucose	528.1	Niacin	4.5	90
		Riboflavin	1.0	20
		Pyridoxine hydrochloride	1.0	20
		Thiamine hydrochloride	1.0	20
		Calcium pantothenate	3.0	60
		Biotin	0.020	0.4
		Folic acid	0.090	1.8
		Vitamin B 12	0.00135	0.27

[a] Vitamin Diet Fortification Mixture supplied by Nutritional Biochemicals Corporation, Cleveland, Ohio.
[b] These amounts were made up to 1 kg with dextrose.

Table 4. Dietary mineral analyses methodology.

Mineral	Group No.	Dilution of Ashed Solu.	Equipment	Wave Length (Å)	Method
Ca	1-8, 11	100 x in 10,000 ppm $SrCl_2$ solu.	Atomic absorption spectrophotometer	4226.7	Concentrations were calculated using regression equation developed from a standard curve.
	9	2 x in 10,000 ppm $SrCl_2$ solu.			
	10	50 x in 10,000 ppm $SrCl_2$ solu.			
Fe	1-11	no	Atomic absorption spectrophotometer	2483.3	Concentrations were calculated using regression equation developed from a standard curve.
P	1-11	10 x in demineralized water	Photoelectric colorimeter	7000	Appendix B
F	1-11	no	Microprocessor ionalyzer/ 901	--	Appendix C

Animal Care and Sample Collection

One hundred and ten weanling, albino, male Sprague-Dawley rats were fed at two different time replications. Fifty-five rats were divided equally into 11 groups. Four rats in each group were housed in stainless steel Wahman rat metabolism cages and one rat in a glass

metabolism cage each replication. The animals were fed the assigned diet for about two days before the start of the experiment.

The animals were weighed at the beginning of the experiment and at the end of each week for three weeks. Diet and demineralized water were fed ad libitum. The amount of diet eaten by each rat was recorded by the difference of food consumed and food spilled. The urine and feces for each rat were collected separately at the end of each week. Before starting collection, approximately 5 ml of 2 percent boric acid solution and five to six drops of phenol were added to each urine collecting bottle to prevent bacterial growth. The volume of each urine sample was measured and then the total collection was frozen. Feces were separated from any spilled food and dried at 100°C for 24 hours. The dried weight of weekly feces samples was recorded. Dried feces were ground by mortar and pestle and stored at room temperature.

The day before sacrifice blood was drawn by heparinized capillary tube from the retro-ocular capillary bed. Hemoglobin was determined immediately. After decapitation blood was collected in small diameter test tubes. The blood was allowed to clot to a wooden application stick. The clot was removed and serum was harvested after centrifuging at 3500 rpm for 20 minutes. Serum samples were frozen. Femora and tails were removed and frozen. Livers were weighed and then charred and ashed for iron analysis. The femora and tails were later boiled and the bones cleaned of tissue and air-dried at room temperature for at least 2 weeks before analyses.

Analyses for Collected Samples

Blood

Blood hemoglobin (Hb) concentration was analyzed in duplicate using the cyanmethemoglobin method of Crosby, Munn and Furth (1954). Twenty µl of fresh blood was mixed well with 5 ml Drabkin reagent (1 g $NaHCO_3$, 52 mg KCN and 198 mg $K_3 Fe(CN)_6$ made to 1 liter with demineralized water), allowed to stand for 30 minutes and read at 540 mµ with a spectrophotometer. A standard of 15.9 g Hg/dl was analyzed similarly. The following formula was used for the calculation of the Hb concentration of the blood.

$$\frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times 15.9 \text{ g Hb/dl} = \text{g Hb/dl}$$

Serum

The serum collected at sacrifice was deproteinized by mixing 1 ml of serum with 4 ml of 12.5 percent trichloroacetic acid (TCA), waiting 10 minutes and centrifuging at 3000 rpm for 20 minutes. Two ml of the supernatant was mixed with 2 ml of double strength $SrCl_2$ solution (62 g $SrCl_2$ plus 20.66 g NaCl dissolved up to 1 liter), and the serum calcium content (mg Ca/dl) was calculated from the atomic absorption spectrophotometer (AA) reading using appropriate standards and a blank. Serum phosphorus was determined by mixing 0.5 ml of TCA supernate with 5 ml MS and 0.5 ml elon, and read at 700 mµ with a spectrophotometer after 45 minutes (Appendix B).

Liver

After weighing the fresh liver, it was charred and ashed at 575°C for 48 hours. The ash was solubilized in 5 ml of 6 N HCl and diluted to 50 ml with demineralized water. These ashed solutions were analyzed by AA for liver iron content.

Bone

Two air-dried caudal vertebrae were weighed, ashed at 575°C for 48 hours, boiled in 5 ml of 6 N HCl and diluted to 100 ml with demineralized water. Bone strength was determined by placing the femur across a 1.5 cm span and measuring the weight of the sand necessary to break the bone. The distance from the fulcrum to the blade on which the femur was placed was 5 cm, and from the fulcrum to the bucket of sand was 30 cm. The bone strength (kg) was calculated by the weight of sand (kg) times 6. The pieces of each femur were weighed, ashed, solubilized and diluted in the same manner as the caudal vertebra.

Feces

Approximately 2 g of dried, ground feces were ashed at 575°C for 24 hours. The ash was solubilized in 5 ml of 6 N HCl and diluted to 25 ml with demineralized water. (Samples from the 0.18 g Ca/kg and 2.77 g Ca/kg diet groups were diluted to 20 ml).

The dilution used for mineral analyses for the bones, feces and urine are summarized in Table 5. The equipment and methods used were the same as previously described.

Nutrient Balance Computation

Mineral balance data for calcium, phosphorus, iron and fluoride were calculated.

Apparent absorption for calcium, phosphorus, iron and fluoride were calculated using the following formula:

$$\% \text{ apparent absorption} = \frac{\text{Intake(mg,}\mu\text{g)} - \text{Fecal(mg,}\mu\text{g)}}{\text{Intake(mg,}\mu\text{g)}} \times 100$$

Retention of calcium, phosphorus and fluoride were calculated according to the following formula:

$$\% \text{ retention} = \frac{\text{Intake(mg,}\mu\text{g)} - [\text{Fecal(mg,}\mu\text{g)} + \text{Urinary(mg,}\mu\text{g)}]}{\text{Intake(mg,}\mu\text{g)}} \times 100$$

Retention in mg or μg was calculated for calcium, phosphorus and fluoride by subtracting fecal and urinary value from intake.

$$\text{Retention (mg,}\mu\text{g)} = \text{Intake} - (\text{Fecal} + \text{Urinary})$$

Dry matter absorption was calculated for each of the three weeks by the following formula:

$$\% \text{ dry matter absorption} = \frac{\text{Diet consumed (g)} - \text{Fecal wt. (g)}}{\text{Diet consumed (g)}} \times 100$$

Table 5. Dilutions used for mineral analyses of collected feces, urine, vertebra and femur.

Collected Sample	Mineral	Group No.	Dilution
Feces	Ca	1-8, 11	20 μl ashed solu. + 4 ml $SrCl_2$ solu.
		9	100 μl ashed solu. + 4 ml $SrCl_2$ solu.
		10	50 μl ashed solu. + 4 ml $SrCl_2$ solu.
	P	1-8, 11	20 μl ashed solu. + 5 ml MS + 0.5 ml elon
		9, 10	10 μl ashed solu. + 5 ml MS + 0.5 ml elon
	Fe	1-11	1 ml ashed solu. + 3 ml demineralized water
	F	1-11	5 ml of ashed solu. was used
Urine	Ca	1-8	1 ml urine + 3 ml $SrCl_2$ solu.
		9, 10	1 ml urine + 2 ml $SrCl_2$ solu.
		11	1 ml urine + 5 ml $SrCl_2$ solu.
	P	1-8, 11	0.5 ml urine was diluted with 2 ml demineralized water
			20 μl diluted urine + 5 ml MS + 0.5 ml elon
		9, 10	0.2 ml urine was diluted with 2 ml demineralized water
			20 μl diluted urine + 5 ml MS + 0.5 ml elon
	F	1-11	1 ml of urine was used
Vertebra	Ca	1-8, 10, 11	200 μl ashed solu. + 4 ml $SrCl_2$ solu.
		9	200 μl ashed solu. + 3 ml $SrCl_2$ solu.
	P	1-11	300 μl ashed solu. + 5 ml MS + 0.5 ml elon
	F	1-11	10 ml ashed solu. was used
Femur	Ca	1-11	50 μl ashed solu. + 4 ml $SrCl_2$ solu.
	P	1-11	50 μl ashed solu. + 5 ml MS + 0.5 ml elon
	F	1-11	5 ml ashed solu. was used

Statistical Analyses

After collecting the data, they were analyzed statistically by analyses of variance to determine if the two different feeding periods were statistically similar. Since there were no significant differences ($p < .05$) between the two feeding periods (Appendix E), data from the rats fed the same diet but different feeding periods were calculated as the same group. If the treatment F ratio was significant ($p < .05$), the least significant difference (LSD) was computed to determine the differences between dietary treatments (Steel and Torrie, 1960; Carmer and Swanson, 1973).

Data on the rats fed diets containing fresh HDS with 0.18, 2.77, 4.96 and 7.13 g Ca/kg diet from $CaCO_3$ respectively were used for regression analyses. The prediction equations were made between vertebral ash (or vertebral calcium) content and dietary calcium level, calcium consumed, or calcium retained. Relative biological values of the other seven diets containing fresh MDS, processed MDS or processed HDS were calculated relative to the control groups (groups 5, 9, 10 and 11).

RESULTS

Meat and Diet Composition

The composition of fresh MDS and HDS meat used in the diets is shown in Table 6. Analyses of the processed and lyophilized products are shown in Table 7. Mechanical deboning produced meat lower in water and protein than hand-deboning, and higher in fat, ash, calcium, phosphorus, iron and fluoride levels. The pH of raw MDS was slightly higher than that of raw HDS. Neither dry-heat nor wet-heat cookery changed meat pH much, while the addition of lactic acid in fermented meat decreased the pH from 6.62 to 5.17 in MDS and from 6.21 to 5.34 in HDS.

The composition and nutrient level of all 11 diets are shown in Table 8. The content of protein (234 - 256 g/kg), fat (327 - 364 g/kg) and phosphorus (4.77 - 5.75 g/kg) were similar among all 11 diets. Calcium levels in the four diets containing raw HDS were 0.18, 2.77, 4.96 and 7.13 g/kg. In accordance with the experimental design, calcium levels in the other seven diets were close to 5.0 g/kg (4.43 - 5.53 g/kg). Iron levels in the diets containing raw HDS ranged from 28.4 to 34.2 ppm. These values were slightly lower than the iron requirement (35 ppm) for growing rats (National Research Council, 1972). Iron levels in the four MDS diets ranged from 48.6 to 60.9 ppm

Table 6. Composition of mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meats.

	MDS		HDS	
	Fresh	Dried	Fresh	Dried
Protein (%)	17.4	38.5	18.3	52.0
Fat (%)	23.2	51.2	15.2	43.2
Moisture (%)	54.7	--	64.8	--
Ash (%)	2.7	6.0	0.8	2.3
Ca (mg/g)	7.4	16.3	0.1	0.3
P (mg/g)	4.5	9.9	1.4	4.0
Fe (μg/g)	45.7	100.9	24.8	70.4

Table 7. Composition and pH of different processed lyophilized mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meats.

Meat	MDS				HDS			
	Raw	Fer-ment	Dry-Heat	Wet-Heat	Raw	Fer-ment	Dry-Heat	Wet-Heat
Protein (%)	37.2	35.9	37.1	38.9	50.4	48.2	48.2	47.4
Fat(%)	52.4	52.2	50.8	51.6	41.8	41.1	39.3	40.5
Moisture (%)	1.9	2.6	3.0	0.6	2.2	3.8	2.3	2.9
Ash (%)	6.1	8.6	8.9	6.0	2.5	7.6	9.4	8.8
Ca (mg/g)	16.6	11.3	13.1	14.9	0.3	9.9	10.5	12.7
P (mg/g)	11.0	9.1	9.4	11.0	4.7	4.5	3.9	4.4
Fe (μg/g)	101.2	99.2	92.4	95.4	64.5	75.5	68.8	76.1
F (μg/g)	41.3	13.4	40.2	37.9	1.0	--	--	--
pH	6.62	5.17	6.25	6.57	6.21	5.34	6.20	6.47

Table 8. Nutrient level of mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets.

Group No.	1	2	3	4	5	6	7	8	9	10	11
Item	MDS (Raw)	MDS (Ferment)	MDS (Dry-Heat)	MDS (Wet-Heat)	HDS (Raw)	HDS (Ferment)	HDS (Dry-Heat)	HDS (Wet-Heat)	HDS (Raw)	HDS (Raw)	HDS Raw)
Protein (g/kg)	240	236	236	256	241	234	236	246	247	248	251
Fat (g/kg)	345	360	357	348	342	327	334	339	347	364	344
Moisture(g/kg)	61	66	69	56	58	70	56	108	62	66	64
Ash (g/kg)	28	45	39	29	30	50	56	24	23	26	34
Ca (g/kg)	5.01	5.13	5.05	5.53	4.96	5.03	5.27	4.34	0.18	2.77	7.13
P (g/kg)	5.75	4.98	5.20	5.37	5.08	4.77	5.10	5.21	5.29	5.13	5.05
Fe (mg/kg)	48.6	60.9	48.7	49.4	32.0	60.2	36.3	54.9	34.2	28.8	28.4
F (mg/kg)	11.1	5.4	15.4[a] 7.9[b]	11.1	2.3	--	--	--	--	--	--

[a] Expected value calculated from the fluoride content in dry-heated MDS meat.

[b] Analyzed value.

and in the three processed HDS diets 36.3 to 60.2 ppm. The reason for the lower iron level in the diets containing raw HDS was because they contained less meat (434 g/kg diet) than other treatments (482 - 585 g/kg diet). Fluoride level in the raw HDS diet containing 4.96 g Ca/kg was 2.3 ppm, while levels in the MDS diets were 5.4 to 15.4 ppm. The fluoride level of the diet containing dry-heated MDS used in this study was the expected value calculated from the fluoride content in dry-heated MDS meat. This value was used due to a large sampling error associated with this diet. This error may have occurred due to drippage or evaporation during cooking, or improper mixing.

Animal Responses

Body weight

There were no significant differences in body weight at the beginning of the experiment and also through the entire three week feeding period (Table 9). However, the rats fed the HDS diet with essentially no calcium reflected the lowest weight gain of all the treatment groups.

Liver weight

Liver weights (g) are presented in Table 9. The fermented HDS diets resulted in the highest liver weight and HDS diet containing 0.18 g Ca/kg caused the lowest liver weight among all 11 treatment groups. None of the differences were statistically significant.

Table 9. Animal and liver weight (g) of weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets for three weeks.

Group No.	Diet	Initial Wt.	Day 9 Wt.	Day 16 Wt.	Day 23 Wt.	Wt. Gain	Liver Wt.
1	MDS (Raw) 5.01[a]	68.3	105.3	141.3	180.5	112.2	4.91
2	MDS (Ferment) 5.13	67.0	101.7	136.9	176.2	109.2	4.95
3	MDS (Dry-Heat) 5..05	69.1	104.2	139.9	173.9	104.8	4.84
4	MDS (Wet-Heat) 5.53	71.6	107.2	140.8	177.4	105.8	4.77
5	HDS (Raw) 4.96	70.6	103.7	135.8	172.1	101.5	4.68
6	HDS (Ferment) 5.03	69.4	104.7	140.6	181.4	112.0	5.25
7	HDS (Dry-Heat) 5.27	69.0	101.1	134.5	171.8	102.8	4.68
8	HDS (Wet-Heat) 4.34	69.5	104.3	134.7	175.5	106.0	4.82
9	HDS (Raw) 0.18	66.7	100.8	130.4	144.3	77.6	4.22
10	HDS (Raw) 2.77	69.9	105.2	133.0	169.9	100.0	4.70
11	HDS (Raw) 7.13	66.8	104.9	135.8	168.9	102.1	4.27
F(10/98)[b]		0.60 N.S.	0.01 N.S.	0.47 N.S.	1.42 N.S.	1.65 N.S.	1.11 N.S.

[a] Level of dietary calcium (g/kg). [b] Treat. df/Error df.

Dry matter absorption

Dry matter absorption increased as dietary calcium level decreased for all three weeks (Table 10). Dry matter from the diet containing 7.13 g Ca/kg diet was less well absorbed than dry matter from diets containing calcium levels between 4.34 to 5.53 g Ca/kg. A further increase in dry matter absorption was observed with animals fed diets containing 2.77 g Ca/kg or less. Diets containing fermented and retorted MDS meats resulted in lower ($p < .05$) efficiency of dry matter absorption, but these processing methods did not affect dry matter absorption from HDS diets.

Calcium Bioavailability

Balance study

Calcium absorption from MDS and HDS diets is shown in Table 11. The lowest apparent calcium absorption was found in the group fed raw HDS containing 0.18 g Ca/kg during week one and in the group fed raw HDS containing 7.13 g Ca/kg during weeks two and three. For the entire three week balance period, percent calcium absorption decreased as dietary calcium increased, except that HDS diet containing 0.18 g Ca/kg resulted in a lower calcium absorption value (45.4 percent) than all other treatment groups.

Processing of MDS and HDS in this experiment did not affect calcium bioavailability. Calcium absorption from MDS was statistically similar to that from $CaCO_3$ in unprocessed, fermented or dry-heated

Table 10. Diet consumed (g) and apparent absorption of dry matter (g/kg) by weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets for three weeks.

Group No.	Diet	3 Wks Diet Consumed(g)	Apparent Absorption of Dry Matter (g/kg) Week 1	Week 2	Week 3	3 Week
1	MDS (Raw) 5.01[a]	203	895	898	903	900
2	MDS (Ferment) 5.13	221	882	888	884	886
3	MDS (Dry-Heat) 5.05	210	900	899	900	899
4	MDS (Wet-Heat) 5.53	206	887	888	890	888
5	HDS (Raw) 4.96	206	890	896	901	897
6	HDS (Ferment) 5.03	235	901	902	895	899
7	HDS (Dry-Heat) 5.27	202	896	887	898	894
8	HDS (Wet-Heat) 4.34	216	899	890	906	900
9	HDS (Raw) 0.18	170	921	920	925	923
10	HDS (Raw) 2.77	201	909	915	921	916
11	HDS (Raw) 7.13	207	873	865	873	870
LSD .05/.01		28/37	16/21	17/23	11/14	11/14
F		25.4	5.41	5.79	14.1	13.5
Treat. df/Error df		10/98	10/99	10/98	10/98	10/98

[a] Level of dietary calcium (g/kg).

Table 11. Apparent absorption of calcium by weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets for three weeks.

Group No.	Diet	Absorption (%)			
		Week 1	Week 2	Week 3	3 week
1	MDS (Raw) 5.01[a]	60.4	60.8	65.5	62.7
2	MDS (Ferment) 5.13	56.7	62.4	59.9	60.4
3	MDS (Dry-Heat) 5.05	59.4	57.4	63.3	60.4
4	MDS (Wet-Heat) 5.53	60.5	58.8	62.2	60.6
5	HDS (Raw) 4.96	63.8	63.5	67.6	65.3
6	HDS (Ferment) 5.03	64.7	66.7	65.5	65.6
7	HDS (Dry-Heat) 5.27	66.1	62.7	67.4	65.9
8	HDS (Wet-Heat) 4.34	68.5	67.4	69.4	69.0
9	HDS (Raw) 0.18	31.2	62.2	65.1	45.4
10	HDS (Raw) 2.77	79.6	83.9	85.6	83.5
11	HDS (Raw) 7.13	50.5	47.3	50.7	49.4
LSD .05/.01		7.7/10.1	8.2/10.8	9.4/12.4	7.8/10.3
F		17.1	9.19	6.21	12.8
Treat.df/Error df		10/96	10/97	10/98	10/98

[a] Level of dietary calcium (g/kg).

treatment groups. A significant difference ($p < .05$) of calcium absorption was found between the rats fed retorted MDS (60.6 percent) and retorted HDS (69.0 percent) diets. Retorting also tended to increase calcium absorption from the diets containing HDS above that of meat processed by fermentation or dry-heat. This may be due to a greater solubility of $CaCO_3$ used in this diet after processing by retorting, but this effect did not occur in the MDS diet. The results of calcium retention were almost identical to those for absorption because very small amount of calcium is excreted in the urine (Table 12).

Relative bioavailability of calcium in MDS and processed HDS

Six regression analyses were made to evaluate the relative biological value (RBV) of calcium contributed by the deboning process relative to the HDS meat diets supplemented with $CaCO_3$ and the influences of fermentation, dry-heat and retortion processing methods. Another prediction equation ($y = 4.49 x - 9.81$, $r = 0.96$) was calculated to determine the relationship between vertebral ash content and vertebral calcium concentration. A summary of these results is given in Table 13.

Results showed that the relative biological values of the diets containing raw MDS were consistently greater than 100 (103-113) (Table 13). Fermentation, dry-heating or wet-heating of MDS resulted in a lower RBV of calcium compared with raw MDS in all six regression analyses. However, processing improved the RBV of calcium in HDS

Table 12. Calcium retention by weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets for three weeks.

Group No.	Diet	3 Wks Ca Consumed (mg)	mg Ca Retention				% Ca Retention			
			Wk 1	Wk 2	Wk 3	3 Wks	Wk 1	Wk 2	Wk 3	3 Wk
1	MDS(Raw) 5.01[a]	1017	173	210	252	635	60.4	60.8	65.3	62.6
2	MDS(Ferment) 5.13	1132	172	265	245	682	56.6	62.3	59.8	60.3
3	MDS(Dry-Heat) 5.05	1061	179	210	251	640	59.3	57.7	63.2	60.3
4	MDS(Wet-Heat) 5.53	1140	199	226	266	691	60.4	58.8	62.0	60.5
5	HDS(Raw) 4.96	1022	190	223	253	666	63.6	63.4	67.3	65.2
6	HDS(Ferment) 5.03	1185	204	269	303	776	64.4	66.6	65.4	65.5
7	HDS(Dry-Heat) 5.27	1064	195	223	275	693	66.0	62.6	67.3	65.2
8	HDS(Wet-Heat) 4.34	938	186	209	251	646	68.4	67.3	69.3	68.8
9	HDS(Raw) 0.18	30	2	5	6	13	29.0	54.6	61.1	43.0
10	HDS(Raw) 2.77	556	127	152	184	463	79.4	83.8	85.3	83.2
11	HDS(Raw) 7.13	1476	224	228	274	726	50.4	47.3	50.6	49.3
LSD .05/.01		136/180	38/50	47/63	43/57	105/139	7.8/10.4	9.3/12.3	9.6/12.7	8.0/10.6
F		61.3	20.3	18.4	27.8	30.1	18.4	7.64	6.02	13.1
Treat.df/Error df		10/98	10/98	10/98	10/98	10/98	10/96	10/97	10/98	10/98

[a] Level of dietary calcium (g/kg).

Table 13. Effect of processing on relative biological value of calcium in diets containing mechanically-deboned shank (MDS) meat or hand-deboned shank (HDS) meat relative to unprocessed HDS meat supplemented with $CaCO_3$.

Linear Regression of:	Relative Biological Value						
	MDS (Raw)	MDS (Ferment)	MDS (Dry-Heat)	MDS (Wet-Heat)	HDS (Ferment)	HDS (Dry-Heat)	HDS (Wet-Heat)
	5.01[a]	5.13	5.05	5.53	5.03	5.27	4.34
Dietary Ca (g/kg) vs Vert. ash[b] (%)	109	101	100	102	111	108	116
Dietary Ca (g/kg) vs Vert. Ca[c] (mg/g)	107	100	101	103	118	106	116
Ca consumed (mg) vs Vert. ash[d] (%)	113	102	101	105	108	112	116
Ca consumed (mg) vs Vert. Ca[e] (mg/g)	103	94	96	99	104	103	110
Ca retained (mg) vs Vert. ash[f] (%)	106	96	96	99	98	103	105
Ca retained (mg) vs Vert. Ca[g] (mg/g)	104	95	97	100	104	101	106

[a] Dietary calcium level (g/kg).

prediction equation:

[b] $y = 16.34 + 2.30\ x\ (r = 0.86)$
[c] $y = 66.27 + 9.59\ x\ (r = 0.77)$
[d] $y = 16.73 + 0.01\ x\ (r = 0.86)$
[e] $y = 66.89 + 0.05\ x\ (r = 0.79)$
[f] $y = 15.98 + 0.02\ x\ (r = 0.86)$
[g] $y = 60.55 + 0.09\ x\ (r = 0.84)$

diets. The only exception is that fermented HDS has a lower RBV (98) than unprocessed HDS (100) in the regression analysis of calcium retained (mg) vs vertebral ash (%) (Table 13).

Bone ash, calcium and strength

A two blocked analyses of variance of vertebral and femoral data was made to evaluate the bone composition and breaking strength (Table 14). With an increase in dietary calcium there was a progressive rise in the bone ash, calcium concentration and the breaking strength of the femur. Maximal values of these were reached with the diet containing about 5.0 g Ca/kg and further increase of calcium in the diet (7.13 g Ca/kg) produced no further change in these values.

Processing methods used in this study did not affect bone calcium content or breaking strength. There was no difference in bone ash, calcium content or breaking strength of femur among four groups fed different processed MDS diets or among four groups given different processed HDS diets. Animals fed dry-heated MDS was lower ($p < .05$) in vertebral ash content than those fed fermented and dry-heated HDS diets. Both fermented and dry-heated MDS diets resulted in lower ($p < .01$) vertebral calcium concentration than fermented HDS diet.

Significantly higher ($p < .01$) ash and calcium values were found in the femurs consistently than those in the caudal vertebra. Generally speaking, femur ash and calcium concentration were about 1.5 times of the vertebral ash and calcium content.

Table 14. Composition of vertebrae and femora of weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets for three weeks.

Group No.	Diet	Bone Ash (%)		Bone Ca (mg/g)		Bone P (mg/g)		Bone F (μg/g)		Breaking Strength (kg)
		Vertebra	Femur	Vertebra	Femur	Vertebra	Femur	Vertebra	Femur	Femur
1	MDS(Raw) 5.01/5.75/11.1[a]	30.3	50.6	122.0	174.7	57.0	93.5	151.6	184.5	5.46
2	MDS(Ferment) 5.13/4.98/5.4	28.5	49.7	115.8	171.2	53.9	92.4	101.1	134.4	5.38
3	MDS(Dry-Heat) 5.05/5.20/15.4	27.8	48.9	115.3	167.7	53.5	91.0	175.3	212.5	5.12
4	MDS(Wet-Heat) 5.53/5.37/11.1	29.5	50.1	122.8	174.5	55.3	93.8	145.3	193.7	5.04
5	HDS(Raw) 4.96/5.08/2.3	29.0	49.4	123.8	170.8	55.7	92.4	75.3	53.2	5.29
6	HDS(Ferment) 5.03/4.77/--	30.9	51.6	135.3	180.0	59.6	97.4	--	--	5.64
7	HDS(Dry-Heat) 5.27/5.10/--	30.7	50.7	123.7	179.4	57.8	98.0	--	--	5.29
8	HDS(Wet-Heat) 4.34/5.21/--	30.4	51.1	125.6	180.8	56.5	96.1	--	--	5.57
9	HDS(Raw) 0.18/5.29/--	15.8	33.5	60.7	120.4	30.7	64.8	--	--	2.18
10	HDS(Raw) 2.77/5.13/--	23.6	43.1	99.5	149.9	45.2	83.1	--	--	3.37
11	HDS(Raw) 7.13/5.05/--	31.5	51.6	125.3	183.6	59.1	98.1	--	--	5.54
	LDS .05/.01	2.8/3.6		14.5/19.2		5.1/6.8		26.7/35.4		.79/1.04
	F(Total df, Error df)	F(215, 194)=14.0		F(211, 190)=4.94		F(215, 194)=14.1		F(94, 85)=3.85		--
	F(Treat. df, Error df)	F(10, 194)=53.2		F(10, 190)=27.3		F(10, 194)=50.5		F(4, 85)=60.2		F(10, 190) = 11.6
	F(Bone df, Error df)	F(1, 194)=234		F(1, 190)=599		F(1, 194)=2383		F(1, 85)=15.1		--

[a] Level of dietary calcium (g/kg)/phosphorus (g/kg)/fluoride (mg/kg).

Serum calcium

Changes in dietary calcium level from 2.77 g Ca/kg to 7.13 g Ca/kg did not produce detectable changes in serum calcium of rats (Table 16). This agrees with the finding of Kemm (1972) that there was no significant difference between the fasting serum calcium in the rats fed diets containing 0.2, 0.4, 0.8 and 1.6 percent calcium. The HDS diet containing 0.18 g Ca/kg led to a lower ($p < .01$) serum calcium than all other treatment groups. This is because that dietary calcium level of 0.18 g/kg was too low to maintain the normal serum calcium concentration.

Phosphorus Bioavailability

Balance study

Animals given HDS diets tended to have a higher efficiency of phosphorus absorption than those given MDS diets. Significantly higher absorption values were found with fermented HDS diet ($p < .05$), dry-heated HDS diet ($p < .01$) and retorted HDS diet ($p < .01$) compared with fermented, dry-heated and retorted MDS diets, respectively (Table 15).

Percent phosphorus retention from unprocessed MDS diet was significantly higher ($p < .05$) than that from unprocessed HDS diet. Processing of MDS by fermentation, dry-heat or wet-heat tended to decrease phosphorus retention. These processing procedures tended to increase phosphorus retention from HDS (Table 15). However, these

Table 15. Apparent absorption and retention of phosphorus by weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets for three weeks.

Group No.	Diet	2 Wks P Consumed (mg)	Absorption (%)			Retention (mg)			Retention (%)		
			Wk 2	Wk 3	2 Wk	Wk 2	Wk 3	2 Wks	Wk 2	Wk 3	2 Wk
1	MDS(Raw) 5.01[a]/5.75[b]	838	76.3	79.0	77.8	203	218	421	51.1	48.6	50.2
2	MDS(Ferment) 5.13/4.98	795	74.3	76.8	75.9	181	155	336	44.1	38.3	42.2
3	MDS(Dry-Heat) 5.05/5.20	784	77.7	76.7	77.2	176	167	343	47.0	40.4	43.7
4	MDS(Wet-Heat) 5.53/5.37	762	77.6	76.6	76.9	166	176	342	48.4	42.9	44.5
5	HDS(Raw) 4.96/5.08	755	82.2	81.2	81.7	149	172	321	43.6	41.6	42.4
6	HDS(Ferment) 5.03/4.77	822	84.0	80.3	82.0	208	222	430	52.3	50.3	52.0
7	HDS(Dry-Heat) 5.27/5.10	744	83.3	85.3	84.4	180	215	395	50.9	55.4	53.2
8	HDS(Wet-Heat) 4.43/5.21	821	82.0	86.7	84.6	181	228	409	47.8	51.3	49.8
9	HDS(Raw) 0.18/5.29	621	81.2	87.7	84.7	90	51	141	26.9	16.2	22.7
10	HDS(Raw) 2.77/5.13	768	81.1	83.1	82.3	118	104	222	34.3	23.8	28.9
11	HDS(Raw) 7.13/5.05	729	74.8	78.3	76.6	152	161	313	44.2	41.5	42.9
	LSD .05/.01	109/144	6.2/8.1	4.8/6.4	4.7/6.2	51/68	58/76	92/122	10.0/13.3	10.9/14.4	7.7/10.2
	F	2.96	2.46	5.67	4.36	3.63	7.22	7.11	4.49	9.28	11.7
	Treat. df/Error df	10/97	10/98	10/97	10/97	10/95	10/94	10/91	10/95	10/94	10/91

[a] Level of dietary calcium (g/kg). [b] Level of dietary phosphorus (g/kg).

processing methods did not affect the efficiency of phosphorus absorption from either MDS diets or HDS diets.

Animals given raw HDS diet containing 7.13 g Ca/kg showed a lower ($p < .05$) phosphorus absorption than all other treatment groups given raw HDS diets. There was no significant difference in phosphorus absorption in rats given raw HDS diet as dietary calcium level increased from 0.18 g Ca/kg to 4.96 g Ca/kg. Percent phosphorus retention increased linearly as dietary calcium level increased.

Bone phosphorus

Caudal vertebral and femoral phosphorus data are given in Table 14. The processing methods used in this experiment did not affect bone phosphorus content. Both vertebral and femoral phosphorus decreased as dietary calcium level decreased.

Serum phosphorus

There were no significant differences in serum phosphorus concentration among treatments (Table 16). These serum phosphorus levels (11.7 - 14.3 mg/dl) were much higher than the normal range. There is no apparent reason for these unusually high levels.

Iron Bioavailability

Balance study

Higher iron absorption was found among animals fed diets with the following characteristics: (1) fermentation of both MDS and HDS

diets (2) dry-heating of MDS diet (3) low dietary calcium levels. Retorting resulted in a significantly negative influence on iron absorption in both MDS ($p < .05$) and HDS ($p < .01$) diets (Table 17). Dry-heat caused a significant decrease ($p < .01$) in iron absorption from HDS but a significant increase ($p < .05$) in iron absorption from MDS diet. Generally, animals consuming HDS diets exhibited a higher efficiency of iron absorption than animals fed MDS diets except animals fed the dry-heated HDS diet exhibited a significantly lower ($p < .01$) iron absorption value compared with those fed dry-heated MDS diets.

Hemoglobin

Terminal hemoglobin (Hb) levels are shown in Table 18. A significantly higher hemoglobin concentration (g/dl) was observed in the rats fed (1) fermented MDS diet in comparison with raw MDS diet ($p < .01$) (2) fermented HDS diet in comparison with raw HDS diet ($p < .01$) or dry-heated HDS diet ($p < .01$) (3) raw HDS diet containing 4.96 g Ca/kg in comparison with raw HDS diet containing 7.13 g Ca/kg ($p < .05$). (4) raw HDS diet containing 4.96 g Ca/kg in comparison with dry-heated HDS diet ($p < .05$). Retorting the meat resulted in a significantly higher ($p < .01$) hemoglobin concentration in rats fed HDS diet.

Liver iron

Liver iron concentration (μg Fe/g liver) and total liver iron (μg) were consistent with each other due to similar liver weights of all 11

Table 16. Serum calcium and phosphorus of weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets for three weeks.

Group No.	Diet	Serum Ca mg/dl	Serum P mg/dl
1	MDS (Raw) 5.01[a]	10.5	14.3
2	MDS (Ferment) 5.13	10.0	13.8
3	MDS (Dry-Heat) 5.05	10.7	13.7
4	MDS (Wet-Heat) 5.53	10.3	13.2
5	HDS (Raw) 4.96	10.4	13.6
6	HDS (Ferment) 5.03	10.0	13.0
7	HDS (Dry-Heat) 5.27	10.6	13.6
8	HDS (Wet-Heat) 4.34	10.3	13.3
9	HDS (Raw) 0.18	7.8	11.7
10	HDS (Raw) 2.77	10.5	13.7
11	HDS (Raw) 7.13	10.6	12.8
	LSD .05/.01	1.0/1.3	N.S.
	F	4.76	1.93
	Treat. df/Error df	10/92	10/72

[a] Level of dietary calcium (g/kg).

Table 17. Iron consumed and apparent absorption by weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets for three weeks.

Group No.	Diet	Fe Consumed (mg)				% Fe Absorption			
		Wk 1	Wk 2	Wk 3	3 Wks	Wk 1	Wk 2	Wk 3	3 Wk
1	MDS(Raw) 5.01[a]/48.6[b]	2.8	3.4	3.8	10.0	31.0	35.0	37.1	35.0
2	MDS(Ferment) 5.13/60.9	3.7	5.0	5.0	13.7	36.2	42.5	37.1	39.3
3	MDS(Dry-Heat) 5.05/48.7	2.8	3.5	3.8	10.1	39.2	42.8	42.5	41.8
4	MDS(Wet-Heat) 5.53/49.4	2.9	3.4	3.8	10.1	25.7	30.2	32.0	29.7
5	HDS(Raw) 4.96/32.0	1.8	2.2	2.5	6.5	37.0	46.7	45.8	43.9
6	HDS(Ferment) 5.03/60.2	3.8	4.8	5.6	14.1	48.1	50.6	43.6	47.3
7	HDS(Dry-Heat) 5.27/36.3	2.0	2.5	2.8	7.3	29.9	28.0	35.2	31.2
8	HDS(Wet-Heat) 4.34/54.9	3.4	3.8	4.6	11.8	32.3	31.7	36.6	33.4
9	HDS(Raw) 0.18/34.2	2.0	1.9	1.9	5.8	48.4	52.0	55.1	51.7
10	HDS(Raw) 2.77/28.8	1.7	1.9	2.3	5.9	35.4	40.9	47.2	42.0
11	HDS(Raw) 7.13/28.4	1.8	1.9	2.2	5.9	28.1	28.6	30.8	29.3
	LSD .05/.01	.4/.6	.5/.7	.5/.7	1.6/2.4	8.2/12.6	7.0/10.8	6.5/10.0	5.3/8.2
	F	24.0	37.5	48.3	25.6	4.87	8.70	7.79	11.2
Treat.	df/Error df	10/99	10/97	10/96	10/98	10/94	10/92	10/95	10/98

[a]Level of dietary calcium (g/kg).
[b]Level of dietary iron (mg/kg).

Table 18. Hemoglobin (Hb) concentrations and liver iron in weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets for three weeks.

Group No.	Diet	Dietary Fe (mg/kg)	Terminal Hb (g/dl)	Liver Fe (μg Fe/g liver)	Total Liver Fe (μg)
1	MDS (Raw) 5.01[a]	48.6	12.5	51	249
2	MDS (Ferment) 5.13	60.9	14.4	63	322
3	MDS (Dry-Heat) 5.05	48.7	12.6	59	288
4	MDS (Wet-Heat) 5.53	49.4	11.6	49	236
5	HDS (Raw) 4.96	32.0	11.6	53	241
6	HDS (Ferment) 5.03	60.2	14.7	89	469
7	HDS (Dry-Heat) 5.27	36.3	10.3	53	253
8	HDS (Wet-Heat) 4.34	54.9	13.7	58	286
9	HDS (Raw) 0.18	34.2	11.4	65	271
10	HDS (Raw) 2.77	28.8	10.5	50	232
11	HDS (Raw) 7.13	28.4	10.2	45	192
LSD .05/.01			1.3/1.7	11/18	68/105
F			11.6	6.98	6.85
Treat. df/Error df			10/98	10/95	10/95

[a] Level of dietary calcium (g/kg).

treatment groups (Table 18). As with terminal Hb concentration and percentage of iron absorption, both fermented MDS and HDS diets exhibited higher iron storage in the liver. Dry-heat and wet-heat caused no significant difference on the liver iron. Among four groups of rats given raw HDS diets, the rats receiving an essentially zero calcium diet showed the greatest iron storage, whereas rats on 7.13 g Ca/kg diet showed the lowest liver iron storage.

Fluoride Bioavailability

Balance study

There was no significant difference in apparent fluoride absorption among rats given four MDS diets in the first two weeks. In the third week, rats fed fermented and dry-heated MDS diets had a significantly lower ($p < .05$) fluoride absorption in comparison with those fed raw MDS diet. For the three week balance period, only the animals fed the fermented MDS diet exhibited a significantly lower fluoride absorption efficiency than those fed the raw MDS diet (Table 19).

Percent fluoride retained tended to be lower in animals fed all processed MDS diets compared with raw MDS diet (Table 20). Significantly lower fluoride retention values were found with rats given fermented MDS ($p < .01$) and dry-heated MDS ($p < .05$) diets than with rats given raw MDS diet.

Bone fluoride

Both vertebral and femoral fluoride contents were directly related to dietary fluoride level (Table 14). Statistical analyses revealed that significant differences are more related to dietary fluoride levels than to processing methods. Significant differences (at least $p < .05$) were found between all treatment groups except the groups fed raw MDS diet containing 11.1 ppm fluoride and those fed wet-heated MDS diet containing 11.1 ppm fluoride. Femora contained a significantly higher (at least $p < .05$) level of fluoride than vertebrae for all treatment groups except the group given raw HDS diet containing 4.96 g Ca/kg and 2.3 ppm fluoride. The bone ash content was more closely related to dietary calcium level than to dietary fluoride level, since rats fed diets containing 4.96 g Ca/kg and only 2.3 ppm fluoride did not exhibit a decrease in their bone ash content. Bone breaking strength (kg) revealed the same pattern as bone ash. Breaking strength decreased as dietary calcium level decreased but did not appear to be related to dietary fluoride level. The decreased fluoride level in the HDS diet did not result in a weaker bone as long as dietary calcium level stayed optimum (about 5.0 g Ca/kg).

Table 19. Apparent absorption of fluoride by weanling male rats fed mechanically-deboned shank (MDS) meat diets for three weeks.

Group No.	Diet	Dietary F (mg/kg)	Absorption (%) Wk 1	Wk 2	Wk 3	3 Wk
1	MDS (Raw) 5.01[a]	11.1	65.6	67.3	72.8	69.5
2	MDS (Ferment) 5.13	5.4	57.7	56.2	60.9	56.9
3	MDS (Dry-Heat) 5.05	15.4[b]	63.8	62.5	57.4	59.3
4	MDS (Wet-Heat) 5.53	11.1	64.7	59.7	67.8	64.4
	LSD .05/.01		N.S.	N.S.	13.0/17.5	11.2/15.1
	F		0.87	1.20	7.36	7.68
	Treat.df/Error df		3/34	3/35	3/33	3/33

[a] Level of dietary calcium (g/kg).

[b] Expected value calculated from the fluoride content in dry-heated MDS meat.

Table 20. Fluoride retention by weanling male rats fed mechanically-deboned shank (MDS) meat diets for three weeks.

Group No.	Diet	Dietary F (mg/kg)	3 Wks F Consumed (μg)	μg F Retention				% F Retention			
				Wk 1	Wk 2	Wk 3	3 Wks	Wk 1	Wk 2	Wk 3	3 Wk
1	MDS (Raw) 5.01[a]	11.1	2253	314	370	490	1174	53.0	49.1	56.3	52.1
2	MDS (Ferment) 5.13	5.4	1192	129	144	135	408	37.1	37.7	29.0	34.2
3	MDS (Dry-Heat) 5.05	15.4[b]	3234	369	488	462	1319	45.2	43.3	37.0	40.8
4	MDS (Wet-Heat) 5.53	11.1	2286	307	332	374	1013	49.5	42.6	44.0	44.3
	LSD .05		242	62	119	137	278	8.5	N.S.	12.0	9.5
	LSD .01		324	84	159	184	374	11.4		16.1	12.8
	F		269	82.8	29.3	33.0	20.6	6.03	1.40	7.69	15.8
	Treat. df/Error df		3/35	3/30	3/32	3/31	3/29	3/31	3/32	3/32	3/29

[a] Level of dietary calcium (g/kg).

[b] Expected value calculated from the fluoride content in dry-heated MDS meat.

DISCUSSION

Meat Composition

The MDS beef used in this study contained 7.4 mg Ca/g on a fresh weight basis (Table 6), which is higher than the average value (5.0 mg/g) reported for mechanically-deboned red meat (MDRM) (Field et al., 1974b). However, this calcium level was within the range of the USDA's proposed rules, which regulate the maximum calcium level to 0.75 percent for MDM and of 1.0 percent for MDM for processing (Field, 1976b). Mechanically-deboned beef has been reported to contain 2.17 g Ca/kg from beef shank and 3.19 g Ca/kg from beef plate (Hendricks et al., 1977b). Field et al. (1976) found that mechanically-deboned beef contained 10.6 to 15.5 g Ca/kg. Chat et al. (1977) reported that mechanically-deboned meat obtained from flat bone contained 17.2 g Ca/kg. Thus, the calcium content of the MDS used in this study falls within the range of reported values. The calcium content of HDS (0.1 mg/g fresh meat) in this study was just the same as that reported by Watt and Merrill (1963).

Iron levels in this fresh MDS (45.7 μg/g) and fresh HDS (24.8 μg/g) are in the same range as reported by others for mechanically-deboned beef (43 - 63 μg/g) and hand-deboned beef (26 - 31 μg/g) (Field, 1976b). The results that MDS contained nearly twice as much iron as

HDS agree with those reported by Field (1976b). However, MDS had a higher moisture level than HDS; thus, MDS only contains 30 percent more iron than HDS on a dry weight basis (Table 6). This result supports the finding of Farmer (1977) that MDS contains 33 percent more metabolizable iron than HDS.

The fluoride level of MDS meat in this study was 13.4, 37.9, 40.2 and 41.3 μg/g in fermented, wet-heated, dry-heated and raw MDS meat respectively based on lyophilized weight. Kruggel and Field (1977) reported that the mean fluoride content of mechanically-deboned beef ranged from 30.3 to 47.6 μg/g on a dry weight basis. Kolbye and Nelson (1977) reported that the mean fluoride content of mechanically-deboned beef was 18.6 μg/g fresh tissue. The fluoride levels of MDS meats in this study were within the range described above except the fermented MDS meat. However, the MDS meat processed by fermentation in this study was from a different lot of cattle.

Animal Responses

Body weight

Bernhart et al. (1969) estimated that the daily calcium requirement of young male rats was 22 mg (about 2.2 g/kg diet) for maximal growth. This is in agreement with the data reported here since there were no statistical differences in body weight gain for the animals fed diet containing 2.77 g Ca/kg or more (Table 9). The lower weight of the rats fed HDS diet with 0.18 g Ca/kg may be explained only partly

by the lesser weight of their bones and livers. The possible influence of calcium-deficient diet on other tissue was not examined in this study. Neither fermentation, dry-heat nor wet-heat cookery affect the body weight gain of rats fed MDS or HDS diets. This finding agrees with that of Hendricks et al. (1977a) that rats fed cooked desinewed beef shank or cooked control beef shank grew the same as those fed raw meat diets.

It is known that fluoride is required for growth. Schwarz and Milne (1972) concluded that 2.5 ppm fluoride is required for an optimal growth response for rats. Our results support their conclusion, since there is no depression in weight gain for the rats given HDS diet which contained 2.3 ppm fluoride compared with those given MDS diets (5.4 - 15.4 ppm fluoride).

McLaughlin (1979) observed a significantly higher body weight gain for rats receiving a HDS diet containing 7.18 g Ca/kg than those receiving a HDS diet containing 4.88 g Ca/kg. The data in this study do not support that finding, since the rats fed HDS with 7.13 g Ca/kg grew the same as the other groups. However, the rats in McLaughlin's study grew faster than those in this study. Bell et al. (1941) reported that variation of the calcium intake from 0.75 to 13.9 g Ca/kg did not affect the appetite nor the body weight if the amino acids and vitamins were adequate. Toothill and Hosking (1968) observed that at 10 weeks of age the weight of rats fed a high calcium diet (0.74 percent) was significantly lower ($p < .01$) than that of rats fed a low calcium diet

(0.13 percent); however, the level of calcium had no effect on net body-weight when the diets were fed for 48 or 60 weeks.

Dry matter absorption

Many researchers have reported that heating may decrease the digestibility and net protein utilization of meat protein (Donoso et al., 1962; Dvorak and Vognarova, 1965; McNab, 1975). Both fermentation and retorting of the MDS meat resulted in lower dry matter absorption in this study. However, dry-heating MDS meat or any processing of HDS meat did not cause any depression of dry matter absorption.

Results show a lower dry matter absorption as dietary calcium level increased (Table 10). It has been suggested that a high level of dietary calcium, usually in the form of $CaCO_3$, will reduce the digestibility of food nutrients. Keener (1953) observed a consistent depression of protein digestibility when diets had approximately 1.0 percent of $CaCO_3$ (0.4 percent calcium) added to them. It was also found that increasing dietary calcium level (up to 0.6 percent) resulted in a sharp depression (over 50 percent) in the apparent digestibility of high melting point fat (Cheng et al., 1949). The mechanism by which calcium may have reduced the digestibility of other nutrients has been but poorly evaluated, perhaps the formation of insoluble complexes may be involved.

Calcium Bioavailability

Balance study

Drake et al. (1949) reported that calcium from bone meal was readily utilized by rats. This study demonstrated that the calcium in MDS is also well utilized (Table 11). The apparent absorption of calcium from the bone in MDS (62.7 percent) was equivalent to the apparent absorption of calcium from $CaCO_3$ (65.3 percent). Similarly, McLaughlin (1979) found the calcium absorption from MDS was 66.3 percent compared with 68.7 percent from $CaCO_3$. Skrypec et al. (1978) observed that the calcium absorption from mechanically-deboned beef was 88.1 percent compared with 87.7 percent from $CaCO_3$. These higher calcium absorption values observed by Skrypec et al. (1978) were probably due to differences in diet composition and length of feeding period. The major differences between the diet composition in the study of Skrypec et al. (1978) and this study were the protein and fat content. The dietary protein levels in the study of Skrypec et al. (1978) were 160 to 162 g/kg compared with 234 to 256 g/kg in this study. The dietary fat levels in the study of Skrypec et al. (1978) were 65 to 97 g/kg compared with 327 to 364 g/kg in this study. The experimental period was 4 weeks in the study of Skrypec et al. (1978) while this study was only 3 weeks in duration.

Processing by fermentation, dry-heat or wet-heat did not affect calcium absorption from MDS. This agrees with the finding of McLaughlin (1979) that autoclaving MDS at 121°C for 90 minutes did not change

calcium absorption. Heat was expected to cause a greater solubility of bone particles in MDS and consequently increase the absorption of calcium. However, it has to be noted that foods are complex mixtures of chemicals and reactions between chemicals are dependent on many factors, such as oxidation/reduction potential, pH, temperature, moisture content and presence of other constituents (Lund, 1979). Differences may be caused by the changes of protein in meat caused by heating. As previously cited, cooking caused denaturation of proteins. The lower digestibility of protein in cooked MDS may decrease calcium absorption and possibly offset the beneficial influence of greater solubility of bone particles caused by heating.

Since bone particles are readily solubilized in weak acid (Johnson et al., 1970; Lawire, 1974; Field et al., 1977), increased acidity caused by fermentation was expected to be an important dietary factor in calcium utilization from MDS. However, the fermented meat was not sufficiently acid to affect solubility significantly. Bone particles are reported to be readily solubilized at pH 0.82 to 2.15 (Johnson et al., 1970; Field et al., 1977), whereas the pH of the fermented meat were 5.17 and 5.34 in this study. The gastric juice is generally considered to favor the absorption of calcium, since the acid of the gastric juice renders dietary calcium salts more soluble (Bills, 1935; Mahoney and Hendricks, 1974). Field et al. (1977) reported that bone particles solubilized in 0.15 M HCl, which is similar to the concentration of HCl in gastric juice (Lehninger, 1970). Mahoney and Hendricks (1974)

found that hypochlordria (fasting pH 6.0 or higher) resulted in decreased absorption of calcium from dibasic calcium phosphate compared with sham-operated, pair-fed controls. These findings probably can explain the results of this study in which conditions practically all the bone particles in MDS would be expected to be dissolved in the stomach. Acidification of MDS by fermentation do not give any further favorable effect on the calcium absorption from MDS.

Calcium consumption was inversely related to absorption (Table 11), which was also shown by Cohn et al. (1968) and Sammon et al. (1970). This may be viewed as a consequence of a controlled calcium absorption mechanism such as that mediated by calcium-binding protein (Wasserman et al., 1968). Kemm (1972) reported that there is an inverse correlation between calcium absorption and the calcium pool in the skeleton. Mammals, including man, adapt to a low calcium diet by more efficient absorption of calcium from the intestine. Shaw and Draper (1966) strongly indicated that the influence of the parathyroid hormone (PTH) on intestinal calcium transport increases as the level of calcium in the diet reduced. Furthermore, PTH has been shown to enhance 1,25-dihydroxychlecalciferol production by the kidney (Fraser and Kodicek, 1973).

Relative bioavailability

Calcium in raw MDS had higher relative biological values (103 - 113) than calcium in raw HDS supplemented with $CaCO_3$ (100) (Table 13).

Calcium bioavailability from MDS is, thus, comparable to that from $CaCO_3$. This confirms that calcium from bone can be well absorbed and retained (Drake et al., 1949; Udall and McCay, 1953). Processing by fermentation, dry-heat and wet-heat resulted in a slight decrease of relative biological value (RBV) of MDS (94 - 102) (Table 13). However, this does not cause any practical problem, since the RBV is at least 94.

The correlation coefficients of all six regression analyses which were produced to evaluate the calcium bioavailabilities were not very high (0.77 - 0.86). As previously cited, there is an inverse relationship between dietary calcium level and the efficiency of calcium absorption. However, the same regression analyses were evaluated by McLaughlin (1979), and found that the correlation coefficients were as high as 0.94 to 0.98 in that feeding trial.

The correlation coefficient was as high as 0.96 when the regression analysis of caudal vertebral ash (%) vs caudal vertebral calcium (mg/g) was determined. Thus, bone (at least caudal vertebra) ash content is a good indicator of the concentration of bone calcium. This study indicated that the use of RBV to determine the bioavailability of calcium from different sources is an easier and less expensive method to use than balance study. Among six parameters the relative biological values of dietary calcium (g/kg) vs vertebral ash (%) were most consistent with the percent calcium absorption data (Table 11). Thus, the

use of the prediction equation from dietary calcium level vs bone ash content measured the calcium bioavailability more reliable than other parameters used here.

Bone ash, calcium and strength

About 99 percent of the body calcium is concentrated in the hard structures of bones and teeth (Pike and Brown, 1975). Thus, bone calcium and ash content can be good indicators of dietary calcium bioavailability. Processing of MDS and HDS by fermentation, dry-heat or wet-heat did not influence calcium and ash content in caudal vertebrae and femora (Table 13). The changes in femur composition caused by processing of the dietary meat were not so obvious as those in the vertebra. This may be due to a more rapid turn over rate of vertebra compared with the femur.

The results indicate a direct relationahip between dietary calcium intake and bone calcium, ash content and mechanical strength of the femora. Toothill and Hosking (1968) showed that the higher level of dietary calcium (0.75 percent calcium, 0.80 percent phosphorus) led to a highly significant increase in the bone weight and percent ash content than a lower level of calcium (0.13 percent calcium, 0.36 percent phosphorus) after 7 weeks. Similarly, Williams et al. (1964) using an X-ray technique, found differences in density of the caudal vertebra of rats given diets containing 0.1 or 0.5 percent calcium and 0.39 percent phosphorus from 74 days of age to death. The positive

relationship between dietary calcium level and bone breaking strength found in this study had also been demonstrated by others (Bell et al., 1941; Salmon and Volpin, 1972). This is because of the bone resorption caused by low calcium intake. The loss of bone calcium and organic matrix weaken the bone and the bone can no longer withstand a pressure stress (Salmon and Volpin, 1972).

Although a progressive rise of bone constituents was found as dietary calcium increased, there was no difference in bone ash, calcium content or breaking strength of femora between rats given about 5.0 g Ca/kg and 7.13 g Ca/kg. This may be because of the saturation of the skeletal calcium pool in the animals fed high level of calcium (7.13 g/kg) which may prevent further calcium absorption from the intestine. Bell et al. (1941) also reported that above an intake of 0.36 percent calcium, there was no increase in the size, calcium content or strength of the bone.

Phosphorus Bioavailability

Phosphorus from the raw MDS diet was retained better (50.2 percent) than phosphorus from the raw HDS diet (42.4 percent). Phosphorus retention was lower from diets containing fermented, dry-heated and wet-heated MDS meat than from HDS meat diets processed by the same methods (Table 15). In this study, the phosphorus in the diet was the combination of phosphorus from meat, bone particles and sodium acid phosphate (NaH_2PO_4). The diet containing MDS had a higher

proportion of bone phosphorus and a lower proportion of NaH_2PO_4 (5.6 - 8.3 g/kg), while diet containing HDS had higher proportion of NaH_2PO_4 (14.0 - 18.0 g/kg).

Using the percentage of bone ash as a criterion, many studies have found that the phosphorus in bone meal was highly available (Miller and Joukovsky, 1953; Johnson et al., 1953). Gillis et al. (1954) using a biological assay technique rated the bioavailability of tricalcium phosphate 100, sodium acid phosphate 101, domestic bone meal 70 to 100 and an imported bone meal 87. They also found that phosphorus from steamed bone meal was less available than monobasic calcium phosphate and defluorinated phosphate and the same as tribasic calcium phosphate and commercial dicalcium phosphate. Motzok and Branion (1956) added phosphorus supplements to 0.4 percent with the calcium: phosphorus ratio maintained at 2:1. They found that the bone ash of chicks was 45.5 percent for steamed bone meal, an average of 46.0 percent for three feed grade bone meals and 46.8 percent for commercial grade NaH_2PO_4. Combining the results of this study and others, it appears that phosphorus from MDS and bone meal are at least as good as that from NaH_2PO_4, Ca_3PO_4 and Ca_2HPO_4.

The values for phosphorus absorption in this study confirmed the findings of Clark (1969) that phosphorus absorption is inversely related to calcium intake. The adverse influence of dietary calcium upon phosphorus absorption is probably due to the precipitation in the gut of insoluble calcium phosphate (Davis, 1963), and to the absence

of a specific control mechanism for phosphorus absorption. The increase in fecal phosphorus with increasing dietary calcium level (Apfelbaum and Brigant, 1963) was presumably due to the salts being rendered insoluble on passing into the more alkaline medium in the intestine.

The excretion of urinary phosphorus reflects the level of absorbed phosphorus relative to that of calcium, and the rate of urinary excretion is a function of the difference between the absorption rates of these two elements. High dietary calcium level (7.13 g Ca/kg) resulted in a reduction of phosphorus absorption (76.6 percent). The need to conserve absorbed phosphorus by a reduction in urinary phosphorus (42.9 percent retention) is well illustrated here. With low intake of calcium relative to phosphorus (0.18 g Ca/kg, 5.29 g P/kg) the removal of dietary calcium interference resulted in a high absorption of phosphorus (84.7 percent absorption) which was excreted almost totally in the urine (only 22.7 percent retention). It is apparent that, as a result of limited control over phosphorus absorption, the kidney constitutes the principal organ for the mediation of phosphorus homoeostasis.

Iron Bioavailability

The higher iron level in MDS in comparison with HDS is due to the presence of bone marrow iron and the iron from the wear on the deboning machinery. Iron in the MDS contributed by the abrasive

activity within the grinder and deboner would likely be in the form of elemental iron. The bone marrow iron is combined with protein apoferritin to form hemosiderin (Guyton, 1976). Most organically bound iron (about 95 percent) in meat is found in heme compounds (Layrisse and Martinez-Torres, 1972). Thus, iron present in the MDS would be both protein (heme or apoferritin) bound iron from the meat or bone marrow and elemental iron from the wear of the equipment. Heme iron is much better absorbed than non-heme iron (Layrisse et al., 1968; Layrisse et al., 1969; Monson et al., 1978). Therefore, the lesser extent of iron absorption from MDS than from HDS in this study might be due to the presence of non-heme iron in the MDS.

Rats fed dry-heated MDS diet had a significantly higher ($p < .05$) iron absorption value than those fed raw MDS diet (Table 17). This might be because of the beneficial influence of heating on the absorption of non-heme iron present in MDS. Wood et al. (1979) indicated that heat and pressure processing resulted in a significant and positive enhancement of the relative biological value of several ferric compounds. Theuer et al. (1971, 1973) found that heat processing improved iron bioavailability of various iron salts added to infant soy isolate formulas and milk-based formulas. However, rats fed dry-heated HDS diet had significantly lower ($p < .01$) iron absorption value compared with those fed raw HDS diet (Table 17). This finding agrees with the result of Callender et al. (1957) that cooking depressed iron absorption from hemoglobin in iron-deficient subjects. On the other hand, Turnbull et al.

(1962) indicated that cooking did not change the absorption of hemoglobin iron. From the results of this study and some others, it might be concluded that the effects of dry-heating on the absorption of heme iron and non-heme iron are different. Dry-heating results in an increase in non-heme iron absorption, whereas it results in a decrease of heme iron absorption.

Retorting resulted in a significant decrease in iron absorption from both MDS diet ($p < .05$) and HDS diet ($p < .01$). Lea and Hunnan (1949) found that the reduction of meat protein digestibility by heating is most severe in moist-heat at 70 percent relative humidity. The depression of meat protein utilization by retorting might decrease the iron absorption from meat, since meat degradation products enhance the absorption of iron (Martinez-Torres and Layrisse, 1971; Kroe et al., 1966). It was also found a reduced dry matter absorption ($p < .05$) on rats fed retorted MDS diets; however, this did not occur on the rats fed retorted HDS diet (Table 10).

Fermentation processing in this study was the combination of acidity and dry-heat. This study indicated that iron from fermented MDS or HDS was more efficiently utilized than the iron from raw MDS or HDS by increasing iron absorption efficiency, terminal hemoglobin level and liver iron content. Both heme and non-heme iron are better absorbed at a lower pH; however, the mechanisms involved are different.

Several studies have demonstrated that ascorbic acid increases the iron absorption from vegetable (Sayers et al., 1973; Layrisse et al., 1974) but does not affect the absorption of heme iron (Turnbull et al., 1962; Martinez-Torres and Layrisse, 1971). Acidity increases non-heme iron absorption by maintaining iron in a reduced, more soluble form which is then ready to be absorbed in the small intestine. Increased acidity favors the heme iron absorption by preventing heme aggregation (Anson and Mirsky 1930; Gralén, 1939).

It would appear that increasing the intake of calcium will inhibit, whereas decreasing the dietary calcium level will enhance iron absorption. This research shows that iron bioavailability of the HDS diet with 7.13 g Ca/kg was depressed as evidenced by iron absorption ($p < .01$) and terminal hemoglobin concentration ($p < .05$) when compared with that of the HDS diet with 4.96 g Ca/kg (Table 17, 18). An essentially zero calcium diet had a significantly higher iron bioavailability than diets containing about 5.0 g Ca/kg. Dietary calcium affects iron utilization, though the mechanism is not well understood. Chapman and Cambell (1957) have suggested that the mucosal cells of animals fed a high calcium intake became blocked with calcium and thus interfere with iron absorption. David (1959) indicated that it had been possible to meet the needs for iron by stepping up the dietary iron level under conditions of high dietary calcium intake.

Generally, hemoglobin generation is used as a mean of measuring iron utilization. Liver iron storage is also an indicator of iron

bioavailability because 26 percent of the total body iron is stored in the liver, spleen and bone in the form of ferritin and hemosiderin (Pike and Brown, 1975). The dietary iron levels were different among groups in this study; thus, the iron absorption values are more reliable, since it takes into account both iron consumption and the efficiency of its absorption.

Fluoride Bioavailability

Balance study

Jackson et al. (1950) observed that fluoride retention of growing rats fed different bone meals varied from 17 to 43 percent. Stillings et al. (1973) found that growing rats ingesting diets containing 32 - 70 ppm fluoride had fluoride retention of 24 percent from fish protein concentrate (containing 150 - 300 ppm fluoride primarily from fish bone) and about 50 percent from NaF. However, growing rats ingesting diets containing 7 ppm fluoride had retentions of about 32 percent from both fish protein concentrate and NaF. These retention values are somewhat lower than the fluoride retention values (34.2 - 52.1 percent) from MDS by growing rats (Table 20).

Very little information exists on the effects of food processing on the absorption and retention of fluoride. Rats fed a diet based on raw MDS or retorted MDS diet had the same fluoride level in their diets (11.1 ppm). The percentage of fluoride absorption and retention tended to be lower for the rats given the retorted MDS diet. These results do

not support the findings of Evans and Phillips (1938). They found in rats fed a basal ration plus 4.0 percent bone meal or 4.4 percent steamed bone meal to make the total fluoride of 26 ppm in rations, that the average fluoride content of femora was 288 and 336 μg/g bone respectively in the rats fed normal and steamed bone meal. Their data showed that heating of the bone meal increased the fluoride bioavailability. The bone data in this study do not agree with their conclusions, since rats given either raw MDS or retorted MDS had the same fluoride content in their vertebrae and femora. Fermentation or dry-heat of MDS resulted in lower fluoride absorption and retention values compared with unprocessed MDS. However, the dietary fluoride level varied from 5.4 to 15.4 ppm in these 3 groups and the amount of fluoride ingested might influence the amount of fluoride retained.

Bone fluoride

Growing rats fed MDS diets containing 5.4 to 15.4 ppm fluoride for 3 weeks had 101.1 to 175.3 μg F /g in their vertebrae and 134.4 to 212.5 μg F /g in their femora (Table 14). Pool and Thomas (1970) found that weanling rats fed diets containing 18 μg F /g diet had about 179 μg F /g bone (ground sample from femur, tibia, fibula, pelvis, humerus, radius, ulna, scapula and mandibles) when chicken bone meal was the source of fluoride. Thus, the fluoride in MDS and chicken bone meal appear to be retained with similar efficiency.

Bone fluoride content is probably a more reliable measure to evaluate fluoride bioavailability than the percent absorption and retention obtained from balance studies. This is because that ingested fluoride accumulates in bone. Furthermore, the fluoride lost in the sweat is not accounted for in balance studies. McClure et al. (1945) conducted extensive balance experiments on 5 young men. They found that under "comfortable" conditions, 24 percent of the absorbed fluoride was lost in the sweat. Under "hot humid" conditions, 44 percent of the absorbed fluoride was excreted in the sweat. Retorting of MDS tended to cause a lower percentage of fluoride retention for growing rats, but there is no difference in the vertebral and femoral fluoride content between rats fed retorted MDS and raw MDS diets (Table 20). The evidence that dry-heating of MDS led to an increased bone fluoride level and fermentation of MDS led to a decreased bone fluoride level is more related to the different fluoride intakes rather than to the processing methods.

Fluoride produces changes in the bone crystals by replacing the hydroxyl ion of the apatites. Fluoride substitution results in a more stable bone mineral and more resistant than hydroxyapatite to parathyroid hormone (Heaney, 1965; Posner, 1969). It has also been reported that fluoride increases the density of the skeleton and stimulates bone formation (Heaney, 1965; Jowsey et al., 1968). However, the breaking strength of the femora in this study was not different between rats fed MDS diets containing 5.4 to 15.4 ppm fluoride and

rats fed HDS diets containing only 2.3 ppm fluoride. This study indicated that the ash content and mechanical strength of the bone are more related to dietary calcium levels than to dietary fluoride levels.

CONCLUSIONS

The differences in mineral between mechanically-deboned meat (MDM) and hand-deboned meat (HDM) include calcium, iron and fluoride, which are due to the incorporation of fine bone particles and bone marrow. The major objectives of this research were to evaluate the bioavailability of calcium, iron and fluoride in mechanically-deboned beef shank (MDS) and the influences of food processing by acid fermentation, dry-heat and wet-heat.

Under the conditions of this experiment mechanically deboning increases the meat levels of fat, ash, calcium, phosphorus, iron and fluoride, and decreases the levels of protein and moisture. This study demonstrated that calcium in the MDS is as well utilized as calcium carbonate. Processing by acid fermentation, dry-heat or wet-heat did not cause any practical impact on calcium bioavailability from MDS. Therefore, the marginal intakes of calcium in humans would indicate the intake of MDM with its increased calcium to be nutritionally beneficial. The relative biological value (RBV) of calcium in MDS indicates that the parameter of dietary calcium level vs vertebral ash content gave the most consistent data among six regression analyses. The use of RBV to determine the bioavailability of calcium from different sources is a less tedious and expensive method than balance study.

Iron in MDS is less efficiently utilized than the iron in hand-deboned shank (HDS) due to the presence of elemental iron. However, the higher iron content makes MDS a significantly better source of iron than HDS. Thus, the consumption of MDM might relieve the frequency of iron deficiency anemia in many populations. Especially, the product of acid fermentation (thuringer) causes increased iron bioavailability due to the function of acidity.

The fluoride retention values (34.2 - 52.1 percent) in this study were somewhat higher than the retention values from various bone meals reported by others. Among the concerns about the safety of MDM is the amount and bioavailability of the fluoride that it contains. This research indicates that the consumption of fluoride in MDS would be far below the range (20 - 80 mg/day) usually required to produce toxicity in humans. However, it has been recommended that mechanically-deboned meat not be used in baby and junior foods.

Overall, mechanically-deboned meat is a clean, safe product of considerable nutritional worth. Calcium and iron are of special interest because dietary surveys of people in the United States indicate that these minerals are often the ones in short supply. It is recommended to expand the use of mechanically-deboned meat in the future.

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APPENDIXES

Appendix A

Macro Kjeldahl Procedure

Reagents:

(a) Indicator solution--0.1% methyl red and 0.2% bromocresol green in alcohol.

(b) Standardized sulfuric acid solution, 0.1N--standardize acid against tris (hydroxymethyl) aminomethane, also known as 2-amino-2-(hydroxymethyl)-1, 3-propanediol, which has an equivalent wt. of 121.14 g. A 0.3029 g of sample dissolved in water is equivalent to 25 ml of 0.10N H_2SO_4. Use the indicator solution (a) and make at least three determinations. Use the average normality of the three.

(c) Catalyst mixture--mix 7% reagent grade fine crystalline $CuSO_4$ with reagent grade K_2SO_4.

(d) Zinc, Mossy

(e) Boric acid solution, 4%. Dissolve 40 g boric acid reagent per liter and add 5 ml indicator solution.

Procedure:

(a) Weigh by difference a sample to contain approximately 25 to 50 mg N into 650 ml Kjeldahl flask, and run two reagent blanks (with filter paper) through all the steps of the procedure and subtract the blank titration from the sample titrations.

(b) Add 10 g of catalyst mixture followed by 25 ml of concentrated sulfuric acid.

(c) Digest sample until the solution clears and all carbon has been oxidized. Cool on the heaters until fuming ceases.

(d) Add 250 ml cold tap water while cooling the flask under running cold water.

(e) Add 50 ml of the boric acid solution to the 500 ml Erlenmeyer flask and set under the condenser with the tip beneath the surface of the solution.

(f) Carefully add 110 ml of NaOH of flask containing digested sample. Add a few pieces of mossy zinc (1-2 g) and quickly attach the flask to the distillation condenser.

(g) Distill until about two-thirds of the liquid in the flask is contained in the receiving flask. Lower the receiving flask and allow the condenser to drain for 5 minutes.

(h) Titrate the ammonia with standardized 0.1 N H_2SO_4 to a faint purple or colorless endpoint.

Calculations: Nitrogen % of sample =

$$\frac{(\text{ml acid titration} - \text{ml blank titration})\ (\text{acid N})}{\text{wt. of sample in g}} \times 0.014 \times 100.$$

References: Seales, F. M. and Harrison, A. P. 1920. Boric acid modification of the Kjeldahl method for crop and soil analysis. J. Ind. Eng. Chem. 12:350.

Official Methods of Analysis of the Association of Official Agricultural Chemist. 1965. 10th ed., Published by the Association of Official Agricultural Chemists, P. O. Box 540, Benjamin Franklin Station, Washington, D. C.

Appendix B

Inorganic Phosphorus Procedure

Reagents:

1. "MS" solution--disolve 10 g $MgCl_2$ (or 21.33 g $MgCl_2 \cdot 6H_2O$ and 10 g $(NH_4)_2MoO_4 \cdot H_2O$ in about 500 ml water. Add 28 ml concentrated H_2SO_4 which has previously been diluted to about 200 ml with demineralized water. Bring to 1 liter total volume with demineralized water.

2. Elon solution--dissolve 1 g elon (p-methylaminophenol sulfate) in 100 ml of 3% sodium bisulfite ($NaHSO_3$). Prepare fresh monthly and store in refrigerator.

3. Standard phosphorus solution--make standards of 10, 25, 50, 100, 150, 200 and 250 ppm phosphorus from a 1000 ppm solution of $NaH_2PO_4 \cdot H_2O$.

Procedure: Prepare blank and standards by mixing 250 ml of each phosphorus standard with 5 ml MS and 0.5 ml elon. Prepare appropriate dilutions of samples using same amounts of MS and elon. Mix well. Allow the tubes to stand 45 minutes from the time of elon addition and read the absorbance at 700 mμ wavelength on a spectrophotometer. Calculate the phosphorus concentration (ppm) of sample using the prediction equation from the standards.

Reference: Gömöri, G. 1953. Standard Method of Clinical Chemistry. pp. 84-87. Academic Press, New York. Vol. I.

Appendix C

Fluoride Procedure

Preparation of TISAB:

Prepare a total ionic strength adjustor (TISAB) to provide a constant background ionic strength, decomplex fluoride and adjust solution pH. Place about 500 ml demineralized water in a 1 liter beaker. Add 57 ml glacial acetic acid, 58 g NaCl and 4 g CDTA (cyclohexylene dinitrilo tatraacetic acid or 2, 2-diaminocyclohexane N, N, N', N'-tetraacetic acid). Stir with magnetic stirrer and add approximately 5 M NaOH until pH is between 5.0 and 5.5. Cool to room temperature and dilute to 1 liter with demineralized water.

Procedure: Prepare standards of 0.1, 0.5, 1.0 and 10.0 ppm of fluoride solutions in plastic bottles. (All samples and standards must be prepared in plastic since fluoride reacts with glass.)

Neutralize appropriate amount of ashed solution or urine with 10 ml of 0.125 M NaOH. Add 5 ml of 0.05 M NaOAC buffer, 5 ml demineralized water and 25 ml TISAB. The final pH of the mixture has to be between 5.0 and 7.0. If the pH has been adjusted by the addition of acid or base, add an equal volume of TISAB, carefully noting the total volume.

Using a magnetic stirrer, read standards and samples using a fluoride specific ion electrode with a digital pH/mv meter with the function switch set to REV MV. Wait for a stable reading (about 5 minutes) and record. Rinse electrode with demineralized water and blot dry between readings. Calculate ppm fluoride in sample using the logarithmic prediction equation from the standards.

Reference: Orion Research Inc., 1977. Instruction Manual. Form IM 94, 96-09/7721. U.S.A.

Appendix D

Urinary Calcium Procedure

Principle: Calcium is precipitated from urine as oxalate and the precipitate is dissolved in acid.

Reagents: Methyl red solution--dissolve 1 g of methyl red in sufficient alcohol to make 1 liter.

Ammonium oxalate solution--dissolve 1 g of ammonium oxalate in sufficient demineralized water to make 100 ml.

Procedure: Add 2 drops of methyl red and 2 ml of $(NH_4)_2C_2O_4$ solution to 1 ml urine, and adjust to pH 4.5 with 1 N HCl or 2.5 percent ammonium hydroxide solution. Let stand for 24 hours at room temperature. Centrifuge and pour off the supernatant liquid. Wipe the inside surface of the centrifuge tube with liquid. (This step is required to remove excess oxalate present in the supernatant or any dissolved magnesium oxalate.) Dissolve the precipitate in 1 ml of 1 N HCl and place in a boiling water bath. Mix frequently to facilitate complete solution. Dilute with proper amount of 10,000 ppm $SrCl_2$ solution. Mix well and read with atomic absorption spectrophotometer at 4226.7 Å. The urinary calcium concentration is calculated using regression equation developed from standard data.

References: Clark, E. P. and Collip, J. B. 1925. A study of the tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem. 63:461.

Oser, B. L. 1965. Hawk's Physiological Chemistry. 14th ed., p 1263. The Blackiston Division. McGraw-Hill Book Company, New York, Toronto, Sydney, London.

Appendix E

The Feeding Period F Ratio for Collected Data

Item	Period df/Error df	F ratio
Weight gain (g)	1/107	3.97
Liver weight (g)	1/107	2.06
3 Wk dry matter absorption (%)	1/106	1.13
3 Wk Ca absorption (%)	1/106	2.69
3 Wk Ca retention (%)	1/106	2.68
3 Wks Ca retention (mg)	1/106	3.98
Vertebral ash (%)	1/107	1.22
Vertebral Ca (mg/g)	1/106	2.12
Vertebral P (mg/g)	1/107	1.33
Femur ash (%)	1/105	2.02
Femur Ca (mg/g)	1/104	0.12
Femur P (mg/g)	1/105	3.30
Bone strength (kg)	1/199	0.46
Serum Ca	1/101	1.87
Serum P	1/81	0.02
2 Wk P absorption (%)	1/106	2.69
2 Wk P retention (%)	1/100	0.36
2 Wks P retention (mg)	1/100	0.65
3 Wk Fe absorption (%)	1/107	0.04
Terminal hemoglobin (g/dl)	1/107	2.32
Liver Fe (μg/g)	1/104	3.46
Total liver Fe (μg)	1/104	0.52
3 Wk Fl absorption (%)	1/36	0.46
3 Wk Fl retention (%)	1/33	0.03
3 Wks Fl retention (μg)	1/33	0.66
Vertebral Fl (μg/g)	1/47	0.43
Femur Fl (μg/g)	1/46	3.12