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Bioavailability of Calcium, Fluoride and Iron in Mechanically-Deboned Beef Fed to Growing Rats

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BIOAVAILABILITY OF CALCIUM, FLUORIDE AND IRON IN MECHANICALLY-DEBONED BEEF FED TO GROWING RATS

by

Kathryn McLaughlin

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This work is dedicated to Mary Jane Reynolds Boyt, who gave her best.

Kathryn McLaughlin

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ABSTRACT

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by

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Weanling rats were fed diets containing raw lyophylized hand-deboned shank beef (HDS) supplemented with $CaCO_3$, retorted lyophylized mechanically deboned shank beef (MDS), or raw lyophylized MDS. Ca in the latter two diets originated from bone during the mechanical deboning process. Ca absorption, Ca retention, bone weight, and bone breaking strength were similar for comparative dietary levels of Ca regardless of the source. Bone ash and Ca content was significantly higher ($P < .05$) in the MDS 393 (3.93 g Ca/kg diet) diet and in the retorted MDS 460 diet compared with the HDS 488 diet. Relative biological values for the MDS diets calculated relative to the HDS diets ranged from 102 to 132 when the linear regression of Ca consumed, Ca retained, or dietary Ca level vs bone ash or bone Ca content was obtained. The retorted MDS 460 diet contained the highest Fe level and exhibited a lower percent absorption, terminal hemoglobin level, and liver Fe storage. Humerus F content increased as dietary F (also present from bone) level increased. Dietary F level did not determine humerus breaking strength. (74 pages)

INTRODUCTION

A process which mechanically retrieves meat from hand-deboned carcasses can contribute additional nutrients to the world's inadequate food supply. Mechanically-deboned red meats (MDRM) were approved for human consumption in November, 1974, but a court injunction in 1976 (Bryant, 1976) prevented their production in the U.S. because this food product had not yet been evaluated to determine its nutritional quality, composition and safety.

Ca levels in mechanically-deboned meat (MDM) vary according to the cut of meat and the mechanical adjustment of the machine, but are generally higher than Ca levels in hand-deboned meat (HDM). Ca in MDM has been reported in the range of 0.02 to 1.55 percent, though most samples of MDRM average 0.5% Ca (Farmer et al, 1977; Hendricks et al, 1977; Kolbye and Nelson, 1977b; Kruggel and Field, 1977; Field, 1976). Hand-deboned beef (HDB) contains between 0.008 and 0.013% Ca (American Meat Institute Foundation, 1960; Composition of Foods, 1963). Fluoride (F) concentration in mechanically-deboned beef (MDB) averages 5 ppm (Kolbye and Nelson, 1977a), in contrast with 0.42 ppm in raw ground beef (Osis et al, 1974). MDB contains 24-38 ppm Fe compared with 15-18 ppm Fe in HDB (Hendricks et al, 1977). Calcium, fluoride, and iron are three nutrients of particular importance relative to mechanical deboning.

Mechanical deboning is a process designed to recover edible meat that is currently discarded as a by-product. Meat or fish retrieved by mechanical deboning could increase U.S. production by 25-92%, depending

on the physical condition of the flesh and the type of equipment involved. Whole or partial carcasses, such as (1) poultry necks and backs; (2) pork, beef and lamb necks and backs; (3) hand-deboned shanks; and (4) fish frames can be utilized in the mechanical process (Noble, 1974). Mechanical deboning of lamb breasts and mutton has been described as feasible and nutritionally sound (Anderson and Gillet, 1974; Field and Riley, 1974). An automatic deboning system developed by the Japanese gives a 35% yield from whole turkey carcasses (Food Engineering, 1970). The Bibun machine uses a meat separator with a rubber belt to force meat through a screen, and then a meat strainer to remove fine bone particles. The Prince machine uses a stationary screen with internal rotating scrapers in place of the rubber belt (Froning, 1976). After the whole or partial carcass is first coarsely ground, the Beehive deboner separates edible tissue from a residue high in bone and connective tissue. The separation is accomplished by compressing a ground mixture of meat, marrow and bone into a conical sieve with holes approximately 0.5 mm in diameter. MDM is extruded through these perforations as a viscous paste. This technique results in 13-16 more pounds of meat from the average beef carcass (Hendricks, 1977; Field, 1976), with a potential total yield of 737 million pounds annually from beef bones (Field and Riley, 1975; Field et al., 1976).

Mechanical separation of meat from bone results in additional minerals, water, fat and other materials being added to the meat. The yield of MDM increased (yield is the weight of MDM divided by the weight of bone plus meat) concurrent with the percent fat and calcium in the product (Field and Riley, 1974). The proximate composition of MDM from a Beehive machine averages 0.2% Ca, 19% protein, 9.7% fat, 69.3% moisture,

and 1.1% ash (Meiburg et al, 1976). The essential amino acid content (except for proline) of MDM is similar to that of the meat on the bone before mechanical deboning (Field, 1976). If the meat (e.g., beef shank) has a heavy concentration of connective tissue, mechanical deboning can enhance protein quality by removing 40-50% of the connective tissue (Hendricks et al, 1977; Field and Riley, 1974). Frankfurters made from 40% MDM were acceptable on the basis of flavor and color (Meiburg et al., 1976).

Mechanically-deboned turkey has been used for over ten years in turkey franks and bologna with 1% bone in the finished product (Hendricks et al, 1977). On April 27, 1976, the U.S.D.A. set definitions and standards, on an interim basis, for MDRM and for MDRM for processing, which was limited to 20% of the meat in the product. A maximum of 0.75% Ca and 30% fat and a minimum of 14% protein were proposed for MDRM. The interim standards were temporarily adopted until additional data were developed to further evaluate the safety and quality of the product. However, a coalition of consumer-oriented organizations and the Attorney General of Maryland were successful in having the interim regulation legally repealed until the potential health hazards of MDM were evaluated (Bryant, 1976). The public expressed concern about the health and safety aspects of calcium and bone particles, toxic and essential trace minerals, pesticide residues, lipid quality and quantities and microbiological hazards of MDM (Kolbye and Nelson, 1977b). In general, the consumer tended to regard bone as a diluent. A panel of experts was formed to develop and evaluate the available data on the kinds and amounts of nutrients and substances present in MDM. The panel concluded that MDM was wholesome and safe and should be permitted in all foods

except baby, junior and toddler foods due to the F content (Kolbye and Nelson, 1977a). The exception was justified on the basis that (1) the concentration of F in MDM varies and may be higher than the average 5 ppm, (2) a prediction of dietary F intake from meat and other foods based on quantity is difficult, and (3) infants' diets are typically supplemented with vitamins containing F (Kolbye and Nelson, 1977a). U.S.D.A. (Federal Register, June, 1978) ruled that a MDRM product was legal, and must contain a minimum of 14% protein, a maximum of 30% fat and 0.75%Ca and a PER of 2.5 calculated on the amino acid content. MDRM may also constitute up to 20% of the meat portion of any product, but may not be used in baby, junior or toddler foods, hamburger, corned beef, roast beef, etc.

Recommended Dietary Allowances (Food and Nutrition Board, 1974) suggests that the minimum requirement for Ca normally be 800 mg/day, or 1200 mg/day for lactating and pregnant women, and teenagers, Heaney et al. (1977) suggest that the Ca requirement for middle-aged women may be 1241 mg daily. Since Ca is frequently low in the American dietary (Center for Disease Control, 1968-70) and some people cannot tolerate milk well (Albanese et al, 1973), MDM could be beneficial as a source of this essential mineral. Setting a requirement is difficult, since different populations have demonstrated an ability to adapt to various levels of dietary Ca (Walker, 1972). Experiments (Drake et al, 1949; Blosser et al, 1954) have shown that Ca from bone is retained well. A recent U.S.D.A. report on MDM indicates that Ca levels in most MDRM samples approaches 0.5% (Kolbye and Nelson, 1977b).

Field (1976) stated that commercial samples of MDM contain approximately twice as much Fe as HDM. Data from the Ten-State Nutritional

Survey (1968-70) and the First Health and Nutrition Examination Survey (1971-72) show that the American diet does not adequately meet the RDA for Fe, especially for women aged 12-44 (Guthrie, 1975). Recommended Dietary Allowances (Food and Nutrition Board, 1974) recommends an intake of 18 mg of Fe a day for women of child-bearing age, noting that the typical American diet doesn't meet this requirement, and suggesting that other dietary factors could be used to enhance Fe absorption. Not only does the source of Fe affect its utilization (Waddell, 1974; Callendar, 1971), but other nutrients consumed with the same meal, e.g., ascorbic acid, enhance its absorption in the human (Cook and Monsen, 1976; Cook, 1977).

The microscopic particles of bone added to MDM during the deboning process will also add F to the product, depending on the amount of F naturally occurring in the water, vegetation and feed consumed by the meat animal before slaughter (Kruggel and Field, 1977; Kolbye and Nelson, 1977b). Estimates of F concentration in MDRM range from 7-19 ppm when selected from specific geographical regions (Kruggel and Field, 1977). The Food and Nutrition Board (1974) recommends adding 1 ppm F to drinking water, and considers fluorine an essential element. In humans, chronic fluoride toxicity occurs after years of consuming 20-80 mg of fluorine daily. However, mottling of teeth in children can occur at F concentrations in the diet and drinking water of 2-8 ppm. Kruggel and Field (1977) conclude that F intake from MDM and other foods in the diet would be much less than the toxic amount.

The purpose of this research is to assess the bioavailability of Ca and the absorption of F and Fe from MDB shank, using rats as experimental animals.

REVIEW OF LITERATURE

Calcium

Physiology

In the human, calcium represents 1.5 - 2% of the total body weight. Ninety-nine percent of the calcium is in the skeletal tissue and the rest is in body fluids. Calcium has other physiological functions in addition to structural support, including an essential role in 1) converting prothrombin to thrombin in blood clotting, 2) activation of enzymes such as lipase and ATPase, 3) normal transmission of nerve impulses and 4) controlling cell wall permeability (Amens, 1973). Bone calcium is important in adults (after growth) because the processes of apposition and resorption continue throughout life in order to maintain mineral homeostatis. The recommended dietary allowance for Ca is 800 mg/day, except for teenagers and pregnant or lactating women, for whom the RDA is 1200 mg/day (Food and Nutrition Board, 1974). Older previous work on the minimum Ca requirements of adult men concludes that 100-200 mg of Ca was a reasonable requirement (Hegsted et al., 1952). Even though the majority of the population can adjust to levels of 400-500 mg Ca/day, a significant number cannot (Rodahl et al., 1960, pp. 46-66). No definite acute Ca deficiency syndrome has been described, and different populations have demonstrated an ability to adapt to various levels of dietary Ca (Walker, 1972). Therefore, a precise Ca requirement has been difficult to establish.

Absorption

Ca absorption in the human is approximately proportional to its

ionic concentration in the intestine (Cantaro and Schepartz, 1962). The intestinal mucosa plays a homeostatic role in controlling Ca absorption, since high levels of Ca excreted in the urine could cause renal calcinosis (Harrison, 1959).

Ca absorption results from the following two types of movement: peristalsis movement which limits the amount of time that Ca is in contact with absorptive surfaces, and transmural movement which carries Ca across the intestinal epithelium. There are two types of concentration-dependent transport across the epthelium. At a low concentration of Ca, an active (or saturable) process requires energy and a vitamin D dependent calcium binding protein (CaBP), and moves Ca against an electrochemical gradient. The second type of transport is termed nonsaturable (or passive). It is predominant when the diet is high in Ca, and relies on passive, diffusion-dependent inward processes in the duodenum to accomplish Ca absorption (Bronner et al., 1976). When dietary levels are below 300 mg Ca/day, a negative Ca balance may result, i.e. more Ca will be lost via intestinal secretions than will be absorbed (Harrison, 1959).

When dietary Ca levels were altered, an adaptation to the previous dietary level was reflected in the absorption pattern. If the previous diet was low in Ca, an increase in percent absorption was observed. A previous diet high in Ca decreased the percent absorption. This adaptation of the absorptive mechanism has resulted from a change in enzyme activity or membrane permeability. Some researchers believe that the parathyroids are necessary for adaptation to a low Ca diet (Winter et al, 1972), while another has postulated a hormone may be essential. The rate of this theoretical hormone's secretion was

determined by the degree of bone mineralization and was shown to influence intestinal Ca absorption (Kemm, 1973; Kemm, 1972).

Since Ca must be in an ionic form to be absorbed, the solubility of a particular Ca compound also affects the rate of absorption. Calcium lactate, calcium gluconate, calcium chloride, hexacalcium inosite hexaphosphate, dicalcium phosphate and calcium glycerophosphate are absorbed at their respective rates beginning with lactate which is the fastest (Bliss and Morrison, 1935). The lactate ion increases Ca solubility by reducing the alkalinity in the intestine. Another study showed that the percentage of Ca retained was essentially the same when Ca sources were calcium carbonate, whole milk powder, calcium chloride, dicalcium phosphate, calcium gluconate, pablum, or calcium lactate (Tisdall and Drake, 1938). Calcium carbonate is absorbed at the same rate as calcium gluconate and should therefore give satisfactory results in a Ca balance study (Mahoney et al, 1975; Ivanovich et al, 1967).

Factors other than the physical properties of the Ca salt affect absorption and retention. High protein levels in the diet seem to reduce Ca retention, probably due to higher Ca excretions through the urine (Bell et al, 1975; Chander and Linkswiler, 1974). Chander and Linkswiler (1974) conclude that a high protein level increased Ca absorption in man, but not retention. A sodium deficiency imposed for one week on growing rats decreased Ca absorption, suggesting that sodium is required for Ca transport across the intestine (Thomasset et al, 1976). Ca absorption was enhanced by many sugars (xylose, glucose, lactose etc.), when the sugar concentration induced an osmotic pressure higher than 1,000 mOsm/l. The sugar increased permeability of the epithelium to Ca due to mechanisms involved in the regulation of

osmolarity (Pansu et al, 1976). Anions, e.g., oxylate, phytate, or phosphate, which precipitate Ca and decrease the amount of ionic Ca in the intestine, decrease the rate of absorption (Amens, 1973). The optimum ratio of Ca:P for absorption is 1:1 to 1:2. An excess of phosphorus decreases the amount of ionic Ca by forming insoluble calcium phosphate (Cantarow and Schepartz, 1962).

Assessment of Ca Bioavailability

Calcium bioavailability has been measured by 1) assessing bone growth or strength, 2) determining the rate of exchange between dietary Ca and serum Ca, and 3) calculating Ca balance by subtracting total excretion from total intake. An understanding of bone structure and formation has led to numerous quantitative methods of measuring bone growth. Staining techniques can be used to reveal changes in bone ground substance related to the amount of growth (Baylink et al, 1970; Frost, 1969).

The fact that endosteal formation increased where resorption (induced by a previous lower Ca diet) had occurred, while no generalized bone increase at the perioteum or epiphyses occurred, is evidence for a local factor which stimulates bone formation (Stauffer et al, 1972). Phosphate esters which occur in plasma inhibit the formation of new bone mineral. Alkaline phosphatase, secreted in large quantities by osteoblasts when actively depositing bone mineral, hydrolyzes this polyphosphate fraction and is usually a good indicator of rate of bone formation (Fleisch and Newman, 1961).

The percent ash and calcium in the bone and body is also an indication of mineralization and dietary Ca level. Williams et al. (1957) reported that bone density on the ninth caudal vertebra, and total body

Ca both increased with dietary levels of 0.1, 0.3 and 0.5% Ca. They also found an increase in body Ca if the 0.5% Ca diet was fed ad libitum instead of pair-fed. Highly significant increases in percentage ash contents of bones were found when dietary Ca was increased from 0.13% to 0.74%. Greater differences in treatment responses to dietary Ca levels were evident when a poorly mineralized bone, such as a caudal vertebra, was analyzed (Toothill and Hosking, 1968). Slopes of a plot of carcass Ca content vs Ca intake leveled off when Ca intake increased to 4 mg Ca/g diet (Bernhart et al, 1969).

A direct correlation between dietary Ca content and breaking strength of the bones has been shown (Rowland et al, 1967). The difference in breaking strength between normal and low Ca bones increases with time, with the effect occurring after receiving the diet for 2 to 4 weeks (Solomon and Volpin, 1972). When dietary Ca level increased from 0.075 to 0.36%, there was a progressive rise in Ca retention, weight of bone, cortical thickness of bone shell, and bending and torsional strengths of bone (Bell et al, 1941).

As mentioned above, Ca bioavailability can also be assessed by the balance method (intake-fecal output - urinary output). When interpreting Ca balance data, the previous dietary Ca level must be considered for six days before the start of the experiment. If the experiment uses a low Ca diet, the fecal Ca may initially be higher than Ca intake. Even though fecal balance data are imperfect due to a small fraction of unabsorbed, endogenous Ca from digestive juices, they are a more accurate reflection of dietary Ca bioavailability than are urinary Ca balance data. Urinary Ca is usually low, and depends on renal function, acid-base balance, deposition of Ca in bone, rate of bone resorption, intestinal

absorption of Ca and previous Ca intake (Zipkin, 1973). Lentner et al. (1975) reported satisfactory results from a metabolic balance study on humans when conducted for 20 days if the patient's regular Ca intake is maintained and a liquid formula diet is given. Whittemore et al. (1973) found that the Ca retained by rats increased from 17.2 mg/d to 20.6 mg/d when Ca levels were increased form .8 - 1.5%.

Bone Meal

Bone meal is a fine dry powder obtained by boiling bones to remove fat and organic material, drying with hot air, and grinding. Sixteen samples of edible bone meal from three processors contained an average of 33.0% Ca, 15.4% P, and 572 ppm F (Bartlet et al, 1952). Bone meal has been used as a Ca supplement in some enriched foods, and is widely used as a mineral supplement in livestock feeding. Blosser et al. (1954) concluded that bone meal was a good calcium and phosphorus supplement for animal feed based on its content of essential nutrients. Interpretation of the analyses indicated that bone meal is a good source of Ca, P, Fe, Mn and Zn but a poor source of Cu and Co. If bone meal were added to a grain ration completely lacking in Ca, the ration would contain 0.3% Ca (more than adequate) based on average composition data. Drake et al. (1949), using human subjects, attempted to compare Ca balance when Ca was supplied from either milk or bone meal. The results were a net negative Ca balance for both groups, possibly due to the fact that this study was not designed to compensate for the test subject's adaptation to a previous dietary level of Ca. The researchers evaluated the results using only those subjects who exhibited a better Ca balance when consuming either milk or bone meal, and concluded that the availability of Ca in bone meal is approximately

that in milk. From a similar experiment using rats, it was concluded that the retention of Ca from bone is approximately 90% of that from milk (Drake et al, 1949).

Fluoride

Bone Meal

Both bone meal and MDM contain F in addition to Ca. Dog litters fed 5 mg F/kg body weight as it occurs in purified bone powder (up to a period of about one year) produced superior teeth compared with litters fed the same F level provided as sodium fluoride (Greenwood et al, 1946). Eighteen samples of bone meal assayed for fluorine contained an average of 803 ppm, expressed as ppm of rehydrated ash (Blosser et al. 1954). When several different brands of bone meal tablets were analyzed in the Utah State University laboratory, their F content ranged from 1.08 to 2.77 mg F ingested along with the recommended daily allowance of Ca.

Physiology

Dietary F readily accumulates preferentially in bone tissue and probably is incorporated into surface layers of existing hydroxyapatite crystals by replacing OH ions (Vaughan, 1970; Costeas et al, 1971; Faccini, 1969). As the concentration of F in drinking water is increased to 4 ppm, the concentration of F also increases in various bones, where 95% of body F is found (Zipkin, 1973). Fluorhydroxyapatite crystals are larger than calcium apatite crystals, which may be responsible for the more stable mineral systems noted with fluoride treatment in dentistry and osteoporosis. Fluoride stimulates resorption over formation on the endosteal surface (bone layer surrounding marrow cavity) but produces an

excess of bone formation on the periosteal surface (outside layer of bone) with little resportion (Spencer et al, 1974; Faccini, 1967). These effects are influenced by the dose, the solubility of fluoride, the duration of intake and the quantity of Ca and P in the diet.

Fluoride absorption occurs by simple diffusion, and is most rapid for soluble salts such as NaF, HF, H_2SiF_6, Na_2SiF_6 and Na_2PO_3F. Not only is the entire GI tract a site of absorption, but ingestion also occurs through the skin and lungs via hydrogen fluoride. The major differences in the absorption of fluorides evidenced in humans are (1) 86-97% of the F in drinking water is absorbed, 2) 80% of the F in a normal diet is absorbed, and 3) 37-54% of the F in bone meal is absorbed. Approximately 50% of the fluoride absorbed is retained. Fluoride is excreted through urine, shedding skin, sweat and feces. Infants excrete 32-50% of the F ingested daily, since they are actively laying down bone mineral (WHO, 1970). Plasma F concentration is fairly stable as determined by the rate of absorption from the gut, excretion by the kidney and incorporation into calcified tissues (Faccini, 1969). About 99% of F stored in the body is retained in the bone (Kruggel and Field, 1977). At low concentrations in the drinking water (.1-.2 ppm), bone contains approximately 500 ppm F on a dry, fat-free basis in an adult human (Zipkin, 1973).

Ca-F Interaction

A complex Ca-F interaction exists, in which F has an effect on Ca metabolism, and the concentration of dietary Ca has an effect on F metabolism. Ca impedes the absorption of F (Faccini, 1969). The percent retention of F by rats fed low Ca diets was 2-3 times the

amount of F absorbed by rats fed high Ca diets (Havivi, 1972). An increase in concentration of dietary Ca (in rats) from 0.23 to 0.73% depressed the total retention of F by 10-13%. An even greater depressed retention of F was evident in teeth and soft tissue (Lawrenz and Mitchell, 1941). Burhart and Jowsey (1968) reported that higher levels of Ca protect against the osteomalacic effects of high levels of F. When a low Ca diet (0.4%) was supplemented with 0.075% F, Ca retention, growth rates, and bone ash increased in Japanese quail. From a second experiment, in which quail were fed a 1.2% Ca diet for 10 days, and then a diet containing 0.4% Ca supplemented with 0.075% F for 35 days, adequate mineralization of new bone, but a 30% decrease in bone torsional strengths was found. The researchers concluded that F increases Ca retention, but at this level did not increase bone strength or integrity (Chan et al, 1973). Spencer et al. (1974) report that F with a mineral-deficient diet reduces mineralizing surfaces, whereas F with a diet adequate in Ca and P increased these surfaces. Other researchers found an increased fluoride concentration in bone ash when the diet contained normal levels of Ca and P. Increased bone ash fluoride was accompanied by gradual decreases of cortical thickness, maximal load, breaking strength and modulus elasticity (Guggenheim, 1976). To illustrate the complex effect of F, 77 ppm F (much higher than is present in MDM) added to a low Ca diet decreases ash concentration and mechanical strength, but 150 ppm F did not affect these parameters.

F and Bone Strength

As noted above, very large quantities of F generally decrease bone strength. Nordenberg et al. (1971) reported that NaF and Na_2PO_3F

decreased the breaking strain in mobile tibiae. Strength of osteopenic bone was measured when a diet low in Ca (0.1% Ca, 0.6% P) was supplemented with NaF (100 ppm). Bone strength was significantly reduced in all F supplemented rats, with no difference in cortical thickness (Riggins et al., 1975). Other researchers (Yamamoto et al, 1974) measured bone hardness in F-treated rats, with the supposition that an increase in microhardness is related to an increase in mineral concentration. Bone hardness was measured at different F levels (1, 30, 90, or 120 ppm) and the following observations were made. Bone microhardness was increased in bone formed during F treatment of 30 ppm in drinking water. Toxic doses of F delay, but do not prevent achievement of normal maximum microhardness. Changes in microhardness are seen only in bone formed during F treatment. Spencer et al. (1974), using electron microscopy techniques, concluded that more Ca and P were needed to achieve greater mineralization as the amount of F in the diet increased. Microscopy indicated that F reduces mineralization at osteoid seams unless adequate Ca and P is available, even though F stimulates a larger surface of osteoid seams. Chan et al. (1973) also reported greater formation of osteons with F treatment and state that this leads to a greater chance of bone fracture since there are more cement lines (sites for fractures).

Chan et al. (1973) also found no body growth effect as the result of consuming F. However, other research reported that adding 1, 2.5 and 7.5 ppm F to a basal diet increased growth rates 17, 30.8 and 27.9% respectively (Schwarz and Milne, 1972). As mentioned above (Chan et al, 1973), F can increase Ca retention and bone ash. A balance trail was conducted using two groups of rats, with each group receiving either 0.2 ppm or 20 ppm F in the diet for 4 weeks and then an injection of labeled

$CaCl_2$ to determine the effect of F. No significant differences in intake, total fecal excretion, endogenous fecal excretion, balance, % retention and true absorption of Ca din the two groups were observed. Humeri of the higher F group contained more water, and the breaking loads of femur shafts were 17% less than for controls. The Ca content of femurs of high F animals was less ($P < 0.05$) on a fresh weight basis than those of low F animals, but not on a dry weight basis. These researchers (Deshmukh et al, 1970) concluded that reports of increased ash or Ca in bone due to administration of F was related to a low Ca diet and that there was no F effect when dietary Ca is adequate.

F Requirement

The Food and Nutrition Board considers F an essential element due to experiments with rats showing a growth effect. The Board also advises fluoridation of the water supply, since this preventative measure results in a 50% decrease in tooth decay in children. Mottling of dental enamel can occur when 3 mg of fluoride per day are ingested, or when the drinking water contains greater than 1.4 - 1.6 ppm F. An intake of 1.5 - 2.5 mg F/day or a 1 ppm F level in the drinking water is beneficial (Kolbye and Nelson, 1977a). Chronic toxicity, e.g., fluorosis of the skeleton, results with years of consuming 20 to 80 mg F/day, and there is evidence of possible kidney damage when the kidney is excreting F in excess of the requirement (Faccini, 1969). Dietary F ranged from 1.6 - 1.9 mg/day, when the intake was measured exclusive of the drinking water (Osis et al, 1974). Dietary F (including drinking water) in twelve cities fluoridating their water supplies ranged from 1.7 - 3.44 mg/day. In four cities where the water supply was not fluoridated, dietary F was approximately 1 mg/day

(Kramer et al, 1974). An analysis of F in infant diets, where the recommended intake is 0.5 mg/day, resulted in a gradually increasing F intake from 0.32 mg/day at one week to 1.23 mg/day at 6 months (Wiatrowski et al, 1975). The revised proposal for standards and labeling requirements for MDM states a maximum limit of 20% MDM in any product (Federal Register, 1978). The expert panel (Kolbye and Nelson, 1977a) studying the safety of MDM has estimated the dietary effect of F from this source, and considers there to be no danger for adults. Assuming an intake of 2 franks and 2 oz. of bologna per day and 5 ppm F in MDM, 0.15 mg F would be added to the diet. Kruggel and Field (1977) conclude that F intake from MDM and other foods in the diet would be much less than the 20 to 80 mg F/day that is toxic. However, the panel did recommend that MDM not be incorporated into infant and junior foods due to the variability of F in MDM and other foods.

Iron

Requirement

Disagreement exists concerning the human requirement for iron, since a greater need prompts a larger percent absorption. Typically, two to ten percent of the iron content of food is absorbed, though individuals having a low hemoglobin concentration may absorb up to 60% of the iron consumed. The Food and Nutrition Board (1974) set the RDA at 10 mg for men, and 18 mg for women. Good sources of iron include liver, meat, leguminous plants, potatoes, green stalks and leaves (Guthrie, 1975). Data from the Ten State Nutrition Survey (1968-70) have been used to show that the American diet does not adequately meet the RDA for iron, especially for women aged 12-44. In fact, the 18 mg requirement is impossible to ingest in a

typical American diet. Meeting this requirement can be enhanced or hindered by other dietary factors.

Iron Availability

Questions exist concerning what foods should be enriched with iron, and which iron compounds should be used (Bing, 1972). A comparison of the availability of different iron supplements in bread, expressed as a mean absorption ratio relative to ferrous sulfate (Waddell, 1974), was 5% for sodium iron pyrophosphate, 31% for ferric orthosphosphate and 95% for elemental iron. In 1970, phosphate salts comprised 1/3 of the iron used in food enrichment. Hussain et al. (1965) noted that the availability of food iron is lower when the source was from a diet high in vegetables compared to an experimental diet containing iron salts. Other research (Cowan et al, 1967) utilizing hemoglobin regeneration as a measurement of absorption indicated that iron in plant foods was much less available than that in ferrous sulfate.

Callendar (1971) concludes that there is a "great superiority" of iron absorption from muscle and hemoglobin compared with vegetables and eggs. Heme iron is absorbed as a heme molecule, in which case iron is released by xanthine oxidase within the mucosal cell. Non-heme iron is absorbed to receptors in the brush border of mucosal cells, passing into the cell by an energy-dependent process. Iron absorption is regulated by individual iron status, erythropoietic rate, and other factors (Linder and Munro, 1977). A number of researchers have suggested that hemoglobin iron is absorbed via a different mechanism than is soluble food iron, and that this explains the greater efficiency of meat iron (Cook and Monsen, 1976; Turnbull et al, 1962). The relatively small

amount of heme iron, compared with nonheme iron, in a meat diet may comprise up to 1/3 the total iron absorbed (Cook, 1977). In addition, animal proteins enhance the absorption of dietary nonheme iron (Cook, 1977; Cook and Monsen, 1976; Cook and Monsen, 1975).

Ca and Fe Interaction

Dietary Ca affects Fe utilization, though the mechanism is not understood. Research was conducted to study the effect of high vs low Ca:P diets on bone and liver mineral composition. There was a significant decrease in iron content of the radius-ulna of pigs with a 1.2% Ca and 1.0% P, compared to a 0.5% Ca and 0.4% P diet (Pond et al., 1975). When Chapman and Campbell (1957a) fed diets made up of 80% bread, and containing Ca in the form of bone meal, calcium carbonate, calcium lactate, calcium chloride, disodium phosphate, or commercial sodium hexametaphosphate, it was found that each of these calcium salts interferred with iron utilization. The researchers suggested that the mucosal cells become blocked with Ca, interferring with iron absorption. However, this diet contained a relatively large amount of bone meal and a small amount of iron. Results of another experiment (Chapman and Campbell, 1957b) showed that the addition of bone meal had no significant effect on the iron content of livers or on hemoglobin level. Bing (1972) points out some contradictions in the research concerning the effect of Ca and P on iron utilization. Generally, increasing the Ca content of the diet reduces iron retention, though the effect is not consistent or quantitatively predictable.

Iron in MDM

The USDA Select Panel (Kolbye and Nelson, 1977b) considers the iron in MDM to be "nutritionally advantageous," and also that Ca does not interfere with its utilization. The Panel and Field (1976) both state that commercial samples of MDM contain approximately twice as much iron as do HDM. By comparing iron levels in bone, marrow, MDM and HDM, Farmer et al. (1977) conclude that some of this iron must come from the deboning machinery. Relative biological values (relative to ferrous sulfate) of reduced (or elemental) iron have been reported as 70, 32 and 25 (Coccodrilli et al, 1976), and between 20 and 70 (Pla and Fritz, 1970). Shah and Belonje (1973) state that the biological availability of reduced iron depends on its particle size, and that at least 95% of the particles should be less than 10μ in diameter. Allred (1976) concluded that iron in mechanically-deboned turkey (18.8 ppm Fe) and in hand-deboned turkey (10.8 ppm Fe) was equally utilized when tested by hemoglobin regeneration. Therefore, the higher content of mechanically-deboned turkey makes it a better source of iron. Farmer et al. (1977) determined that mechanically-deboned shank contained 33% more metabolizable iron (as measured by hemoglobin regeneration) than hand-deboned shank, since there was 93.1 mg Fe/kg dry matter of mechanically deboned shank compared with 52 mg Fe/kg dry matter of hand-deboned shank.

METHODS AND PROCEDURES

Experimental Design

Seven diets were formulated for the purpose of comparing the absorption of Ca from mechanically-deboned shank (MDS) with that from Ca supplemented hand-deboned shank (HDS) diets (Table 1). Control diets were prepared by adding different levels of $CaCO_3$ to each of four HDS diets. The amount of MDS in the diet was determined according to the amount of Ca desired. The percent protein was maintained at the same level in all seven diets by the addition of the appropriate quantity of HDS. Na_2HPO_4 was added in appropriate amounts to keep phosphorus levels constant. Beef kidney fat was rendered and added in appropriate amounts to balance the fat content in the seven diets. A mineral mix provided all the essential minerals except Ca, P and Fe. A standard vitamin mix was added.

Animal weight gains and dry matter absorption were calculated during a three-week feeding trial. Ca bioavailability was assessed by comparing percent Ca retention, percent Ca apparent absorption, Ca retention, Ca consumed, Ca and P in blood, Ca, P and ash of bone, and bone strength. Information on Fe utilization was gathered from percent Fe absorption, terminal hemoglobin levels, liver Fe content, Fe per gram of liver and liver weight. F bioavailability was assessed by comparing percent F absorption, percent F retention and F content of humeri.

Table 1. Composition of mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets.

	MDS 393[a]	MDS 221	MDS 460[b]	HDS 016	HDS 264	HDS 488	HDS 718
Ingredient (g/kg)							
Meat - total	758	660	758	562	562	562	562
MDS	571	285	571	-	-	-	-
HDS	187	375	187	562	562	562	562
Rendered Beef Kidney Fat	81	162	81	243	243	243	243
Dextrose	76	90	76	103	97	91	85
Cellulose	50	50	50	50	50	50	50
Mineral Mix[c]	11.6	11.6	11.6	11.6	11.6	11.6	11.6
Na_2HPO_4	6.6	5.9	6.6	10.0	10.0	10.0	10.0
$CaCO_3$	-	-	-	-	6.0	12.2	18.5
Vitamin Mix[d]	20	20	20	20	20	20	20
Nutrient Level (g/kg)							
Fat	420	419	417	414	416	414	417
Protein	352	365	317	348	332	347	350
Ash	40	35	42	31	35	40	44
Moisture	50	50	24	46	44	42	42
Ca	3.93	2.21	4.60	0.16	2.46	4.88	7.18
P	4.60	4.32	4.73	4.27	4.14	4.02	4.02
Fe (mg/kg)	53.50	48.50	60.40	39.00	42.90	46.30	47.70
F (mg/kg)	10.70	6.28	15.10	-	-	0.66	-

[a]Level of dietary calcium (3.93 g/kg)

[b]Processed at 121°C for 90 minutes in quart glass home canning jars.

[c]The mineral mixture contained (g/kg): KCl, 296.7; $MgCO_3$, 121.0; $MnSO_4$, 12.7; $CoCl_2.6H_2O$, 0.7; $ZnSO_4.7H_2O$, 38.0; $CuSO_4.H_4O$, 1.6; KI, 0.8; $Na_2MoO_4.2H_2O$, 0.1; and glucose 5281.1.

[d]Vitamin Diet Fortification Mixture, Nutrition Biochemicals Corp., Cleveland, Ohio. The mixture contained (in g/kg of mixture); Vitamin A concentrate (200,000 IU retinyl acetate per gram), 4.5; Vitamin D concentrate (400,000 IU calciferol per gram), 0.25; α-tocopherol, 5.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; menadione, 2.2; p-aminobenzoic acid, 5.0; niacin 4.5, riboflavin, 1.0; pyridoxine hydrochloride, 1.0; thiamin hydrochloride, 1.0; calcium pantothenate, 3.0; and in mg/kg: biotin, 20; folic acid, 90; and vitamin B_{12}, 1.4.

Preparation of Diets

Meat Preparation

Beef shank (femora and humeri) from a commercial packing house was utilized for the MDS and HDS diets. Hand-deboning was done by the U.S.U. Meat Laboratory. Meat tissues were ground twice through a 1/8 inch plate and stored at -20°C. Mechanical deboning was done by Beehive Machinery, Inc. (9100 South 500 West, Salt Lake City, Utah) by putting the whole shanks through a bone cutter having a cutting plate with 1/2 inch perforations and then deboning with an AU6173 Beehive machine using an AU0.018 inch head (0.457 mm perforations). The MDS extruded through the perforations was immediately quick-frozen and stored at -20°C. Both the MDS and HDS were cut into 1/2 inch thick sections, deleted by lyophilized to a moisture content of 2-5%, reground, and stored in plastic bags at -20°C. Some of the MDS was thawed, reconstituted with deionized water, processed at 121°C. for 90 minutes, relyophilized and stored similarly.

Analyses of Meat

Moisture, fat, protein, ash, Ca, P and Fe content were determined on the fresh meats before lyophilization. Five replicate samples of meat were analyzed (AOAC, 1970) for moisture, fat, protein and ash using the vacuum oven at 60°C. and 15 inches vacuum (A.O.A.C., 7.003), the Soxhlet apparatus for 24 hours with petroleum ether (A.O.A.C., 7.045), the Kjeldahl method (A.O.A.C., 2.049), and the muffle furnace at 575°C. for 16 hours (A.O.A.C., 7.010) respectively.

The ash from each sample was boiled in 5 ml of 6 N HCl acid for a few minutes and diluted to 25 ml with deionized water. Deionized water

and acid-rinsed equipment were always used for mineral analyses. Fifty µl aliquots (five replications for each meat) of these ash solutions were mixed with 2 ml of a $SrCl_2$ solution (31 g of $SrCl_2$ plus 10.33 g of NaCl dissolved up to one liter with deionized water). The samples, along with Ca standards, were quantitated using a Varian Techtron AA 120 atomic absorption (AA) spectrophotometer at 4226.7Å. The Ca concentrations were calculated using regression equations developed from standard curve data obtained at the same time as the samples were assayed.

The method of Gömöri (1942), with some modification in the reagents, was used to determine P in the meat (Appendix A). Twenty-five µl of the ashed solution was mixed with 5 ml of MS and .5 ml of elon.

One ml of the ashed solution was mixed with 2 ml of deionized water and assayed on the AA spectrophotometer at 2483.3 Å to determine Fe concentration.

F in the lyophilized meats was analyzed by the method described by Orion Research, Inc. (1977) on 2-2.5 g samples (Appendix B).

Diet Analysis

The diets were analyzed in triplicate for nutrient content to ensure that they conformed to the specifications of the experimental design. Adjustments in the content of the diets were made where necessary and reassayed for nutrient content. Resampling was used to confirm the adjustments in nutrient content. Dietary moisture, fat, ash and protein content were determined as previously described for the meat except that only three samples of approximately 4 g diet each were analyzed.

Fifty µl aliquots of each ashed solution, excepting the ashed

solution of one HDS diet, were mixed with 5 ml of $SrCl_2$ and Ca was quantitated on the AA spectrophotometer. In the case of the one HDS diet, a 0.5 ml aliquot was taken because of the low Ca content (0.16 g/Kg). Ca, P, F and Fe content were determined as described above for meat analysis. P was determined using 10 µl aliquots of these ashed solutions. F in the diets was measured after adjusting an aliquot of these ashed solutions to the appropriate pH. Iron was determined as described for meat analysis on the ashed solutions without dilution.

Animal Care and Sample Collection

Seven groups of ten weanling, Sprague-Dawley, male rats were each fed one of the seven diets. The animals were fed the assigned diet for 24 hours before the start of the experiment. Forty-eight rats were housed in stainless steel Wahman rat metabolism cages and twenty-two in glass metabolism cages. The rats were weighed at the start of the experiment and at the end of each week of the three week metabolism study. Food and deionized water were provided ad libitum. Records were kept of food consumed with corrections for spillage.

Urine and feces for each rat were collected separately at the end of each week. Each urine collection flask contained approximately 2 ml of a 2% boric acid solution and approximately 2 ml of toluene to prevent bacterial growth and evaporation. After recording urine volume, the weekly collection was frozen. Spillage and feces were separated, dried and weighed. Weekly feces samples were dried overnight at 105°C., ground by mortar and pestle, and stored at room temperature.

Hemoglobin was determined the day before sacrifice by collecting

Table 2. Animal and liver weights (g) of weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) diets.

Diet	Initial Wt.	Day 8 Wt.	Day 15 Wt.	Day 22 Wt.	Wt. Gain	Liver Wt.
MDS393[a]	52	103	149[d]	204	152	8.94
MDS221[b]	58	106	145	196	139	8.80
MDS460[b]	59	110	143	201	143	8.65
HDS016	56	101	117	124	68	4.24
HDS246	56	101	145	182	126	8.59
HDS488	54	102	137	184	130	8.70
HDS718	54	101	145	206	152	8.93
LSD .05[c]	N.S.	N.S.	12.7	15.9	16.8	0.99
.01			16.8	21.2	22.3	1.32
F (6/63)[d]	2.09	1.81	5.81	26.06	23.73	24.03

[a]Level of dietary calcium (3.93 g/kg).

[b]Processed at 121°C. for 90 minutes in glass quart canning jars.

[c]Mean differences must exceed or equal the least significant difference values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

[d]Treat df/Error df

blood in a haparinized capillary tube from the retro-ocular capillary bed. At sacrifice blood was collected in test tubes containing an applicator stick. The stick was removed later along with the adhering blood clot, and the remaining blood centrifuged at 5000 rpm for 15 minutes. Serum was collected and frozen.

At sacrifice, humeri, tail and liver were dissected from the body and frozen. The humeri and tails were later boiled (for about 20 minutes) and the bones cleaned of tissue and air-dried at room temperature. The second through fourth caudal vertebrae were retained, one of them being analyzed for Ca, P and ash content.

Analytical Procedures for Collected Samples

Approximately 2 g of the feces collected each week were ashed at 550°C. for 16 hours and solubilized in 5 ml of 6 N HCl before diluting to 25 ml with deionized water. (Some samples selected on the basis of low Ca content or small fecal collections were diluted to 20 ml). Aliquots of the ashed solutions were added to a $SrCl_2$ solution and Ca content was determined as previously described. Fe was measured in the fecal ashed solutions as described for diet analysis. An aliquot of the weekly urine output of each rat was diluted with a $SrCl_2$ solution and analyzed for Ca.

One of the caudal vertebrae was air-dried at room temperature for at least two weeks, weighed, ashed at 550°C. for 16 hours, solubilized in 5 ml of 6 N HCl, and diluted to 100 ml with deionized water. Ca content of the vertebra was determined from a mixture of 0.5 ml ashed solution plus 4.5 ml $SrCl_2$ solution. Phosphorus was analyzed using a 200 µl aliquot of the ash solution.

Bone strength was determined by placing each humerus across a 1.5 cm span and measuring the weight of sand necessary to break the bone. The distance from the fulcrum to the bucket of sand was 39.5 cm, and from the fulcrum to the blade was 5.0 cm. The following formula was used to calculate bone strength in Kg.

$$\frac{\text{Long Lever (39.5 cm)}}{\text{Short Lever (5.0 cm)}} \times \frac{\text{Wt. of sand (g)}}{1000 \text{ g/kg}} = \text{kg to break}$$

The pieces of each broken humerus were weighed, ashed at 550°C. for 16 hours, solubilized in approximately 5 ml of 6 N HCl, and diluted up to 25 ml with deionized water. A 15 ml aliquot of the ashed solution was adjusted to a pH range of 4.5 to 5.5 in order to analyze F content (Appendix B).

Hemoglobin was analyzed according to the cyanmethemoglobin method of Crosby, Munn and Furth (1954). The 20 ml sample of fresh blood was well mixed with Drabkin's reagent (1 gm $NaHCO_3$, 52 mg KCN and 198 mg $K_3Fe(CN)_6$ made to 1 liter volume with deionized water), allowed to stand for 30 minutes and quantitated at 540 wavelength with a spectrophotometer. A 5 ml standard of 15.8 g Hb/100 ml was analyzed similarly. The following formula was used to calculate the Hb concentration of the fresh blood.

$$\frac{15.8 \text{ g Hb/100 ml}}{\text{O.D. standard}} \times \text{O.D. sample} = \text{g Hb/100 ml}$$

The serum collected at sacrifice was used to determine serum Ca and P. The serum was deproteinized by mixing 1 ml serum with 4 ml of 12.5% trichloracetic acid, waiting 10 minutes and centrifuging at 5000 rpm for 15 minutes. Two ml of the supernatant were mixed with 2 ml $SrCl_2$

(31 g $SrCl_2$ plus 10.33 g NaCl dissolved up to 0.5 liters), and the readings from the AA were used to calculate mg Ca/100 ml of serum. Appropriate standards were prepared using double strength $SrCl_2$ solution also. Serum phosphorus (Appendix A) was analyzed using 0.5 ml of the TCA supernate plus 0.2 ml of elon and 5 ml of MS.

After recording the fresh liver weight, each liver was charred in a porcelain crucible and then ashed at 575°C. for 16 hours. The ash was solubilized in approximately 5 ml of 6 N HCl and diluted to 25 ml with deionized water. The solutions and appropriate standards were quantitated using the AA spectrophotometer for Fe as before and the data used to compute liver Fe content.

Nutrient Balance Computation

Balance data were calculated for Ca, F and Fe. Retention in milligrams was calculated by subtracting fecal plus urinary Ca from Ca intake (I-[F+U] = Retention). Apparent absorption for Ca, Fe and F were calculated according to the following formula:

$$\frac{\text{Intake-Fecal}}{\text{Intake}} \times 100 = \% \text{ apparent absorption}$$

Apparent retention was calculated for Ca and F according to the following formula:

$$\frac{\text{Intake - (Fecal + Urinary)}}{\text{Intake}} \times 100 = \% \text{ apparent retention}$$

Dry matter absorption was calculated for each of the 3 weeks by computing total dry diet ingested and using the following formula:

$$\frac{\text{Diet Wt. - Fecal Wt.}}{\text{Diet Wt.}} \times 100 = \% \text{ dry matter absorption}$$

Statistical Analysis

Analyses of variance were used to determine whether significant differences existed among the groups. If the treatment F ratio was significant ($P < .05$), post hoc comparisons using Least Significant Differences (LSD) were computed to determine which treatment groups were significantly different (Steel and Torrie, 1960).

Regression analyses (Mendenhall, 1971) were used on the diets containing HDS to obtain a prediction equation for the parameters measured. The test was used to determine if a linear relationship existed between bone ash (or bone Ca) content and dietary Ca level, Ca consumed, or Ca retained. Where the correlation coefficient indicated a "good fit," relative biological values of the three diets containing MDS were calculated relative to the $CaCO_3$ supplemented diets by the following steps:

1. The independent variable, i.e., dietary Ca level, for each of the test diets was substituted into the prediction equation to get an expected dependent variable, i.e., bone ash.

2. The actual experimental dependent variable was divided by the expected value and multiplied by 100 to give relative biological value.

RESULTS

Meat and Diet Analyses

The results of analyses of HDS and MDS used in the diets are in the order of moisture (g/Kg), fat (g/Kg), protein (g/Kg), ash (g/Kg), Ca (mg/g), P (mg/g), Fe (μg/g), and F (μg/g) for MDS/HDS: 620/698, 208/110, 163/193, 16/9, 3.4/0.1, 2.3/1.4, 36.8/32.7 and 11.43/0.27. Water and protein content were higher for HDS than for MDS, while fat and ash content were higher for MDS than for HDS. All the minerals assayed (Ca, F, Fe and P) were present in larger quantities in MDS than in HDS.

The compositions of MDS and HDS diets are found in Table 2. According to the experimental design, fat, protein and phosphorus contents of the diets were found by analysis to be similar, e.g., 414-420 g fat/kg diet, 332-371 g protein/kg diet, and 4.02-4.73 g P/kg diet. Ca levels in the four diets containing HDS only were 0.16, 2.46, 4.88 and 7.18 g/kg. Ca level in the diets containing MDS were 2.21, 3.93 and 4.60 g/kg. The ratio of MDS:HDS is about 3:4 for the diet labeled MDS221 while the other two diets containing MDS have a ratio of 3:1. F level in the HDS diet analyzed was 0.66 mg/kg, while levels in the MDS diets were 6.28, 10.74 and 15.12 mg/kg. Fe levels in the diets containing only HDS ranged from 39.03 to 47.69 mg/kg with a mean of 43.97 mg/kg. Fe levels in diets containing MDS were 48.53, 53.64 and 60.44 mg/kg.

Animal Responses

Body Weight

The body weights of the animals fed the HDS016 diet with essentially no Ca reflected a very poor weight gain in comparison with all other treatment groups (Table 2). The two diets resulting in the greatest weight gains were the MDS393 and the HDS718 diets.

There were no significant differences in mean body weight among the treatments at the start of the feeding period, or one week after. On day 15, body weight of the animals receiving the HDS016 diet was significantly lower ($P < .01$) than all other treatment groups. On Day 22, this difference ($P < .01$) was again noted. Also lower ($P < .01$) body weight (g) was revealed in the rats fed 1) HDS 246 diet in comparison with those fed the MDS393 diet or the HDS 718 and 2) HDS488 diet in comparison with the HDS718 diet. Lower ($P < .05$) body weights (g) were revealed in the 1) HDS246 diet in comparison with the MDS460 diet and 2) the HDS488 diet in comparison with the processed MDS diet. and 2) the HDS488 diet in comparison with the processed MDS diet containing 4.60 g Ca/kg diet or the MDS diet containing 3.93 g Ca/kg diet.

Statistical analyses of weight gain over the 3-week period again was used to show a significantly lower ($P < .01$) value for the HDS016 diet. A lower ($P < .01$) weight gain (g) was noted for the HDS diets containing either 2.46 or 4.88 g Ca/kg diet in comparison with the HDS diet containing 7.18 g Ca/kg diet or the MDS diet containing 3.93 g Ca/kg diet.

Liver Weight

Liver weight also reflected the inadequacy of the low Ca diet (Table 2), since the HDS016 diet had a lower ($P < .01$) liver weight (g)

than all other treatment groups. As seen in comparisons of weight gain, the highest liver weights were found in rats fed the HDS718 diet and the MDS393 diet.

Dry Matter Absorption

Dry matter absorption (g/kg) generally increased as dietary Ca level decreased (Table 8). For each of the three weeks, rats fed the HDS718 diet had a lower ($P < .01$) efficiency of dry matter absorption (g/kg) than all other treatment groups. For weeks 2 and 3, dry matter absorption was lower for the HDS488 and MDS(P)460 groups.

Calcium Bioavailability

Balance

Ca absorption and retention from MDS and HDS diets are given in Table 4. Apparent absorption of Ca from the HDS016 diet was always lowest among the groups ($P < .05$). The general trend over the three-week balance period was for those diets containing from 2.21 - 4.88 g Ca/kg to yield the highest percent absorption. Rats fed the HDS718 diet had a lower apparent absorption ($P < .05$). When considering the total three week period, animals displayed the same significant differences among groups for apparent retention as for absorption of Ca.

Statistical analyses of the three-week means for Ca consumed (mg) and Ca retained (mg) yielded similar results (Table 4). In both cases, all groups were different ($P < .05$) from each other except for the groups fed the MDS221 and the HDS246 diets. With the exception of the HDS016 diet, Ca retained or consumed was inversely related to absorption.

Table 3. Dry matter absorption (g/kg) consumed by weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets.

Diet	Week 1	Week 2	Week 3
MDS393[a]	871	880	902
MDS221	883	892	908
MDS(P)460[b]	876	861	882
HDS016	887	892	884
HDS246	881	892	896
HDS488	846	856	886
HDS718	807	835	828
LSD .05/.01[c]	14/18	16/21	20/27
F	35.62	16.76	13.37
Treat df/Error df	6/63	6/63	6/62

[a]Dietary calcium level (3.93 g/Kg)

[b]Processed at 121°C for 90 minutes in glass quartz canning jars.

[c]Mean differences must exceed or equal the Least Significant Difference values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

Table 4. Calcium absorption and retention by weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meat diets.

	Absorption (g/kg consumed)			Three Week Means			
	Wk 1	Wk 2	Wk 3	Ca Consumed (mg)	Ca Retained (mg)	Absorption (g/kg consumed)	Retention (g/kg consumed)
MDS393[a]	504	688	752	726[3]	482[3]	663	662
MDS212	560	763	864	390	285	730	729
MDS(P)460[b]	664	706	662	884	567	674	672
HDS016	-1700	149	597	18	-10	-526	-546
HDS246	639	818	833	416	324	781	779
HDS488	584	714	732	915	629	687	686
HDS718	449	591	593	1549	858	557	556
LSD .05/.01[c]	208/276	190/253	199/265	65/87	56/80	128/170	128/171
F	134	11	2	430	16	95	97
Treat df/ Error df	6/63	6/63	6/62	6/62	6/62	6/62	6/62

[a]Dietary calcium level (3.93 g/kg)

[b]Processed by autoclaving MDS at 121°C for 90 minutes

[c]Mean differences must exceed or equal the Least Significant Difference values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

Relative Bioavailability of Ca in MDS

The relative biological values of the diets containing MDS were always greater than 100, i.e., the Ca contributed by the deboning process was as biologically available as calcium from calcium carbonate (Table 5). Regression analyses were performed for various measures of Ca intake vs a bone response (Table 5). Graphs of bone ash and bone Ca levels vs dietary Ca level illustrate the correlation between dietary treatment and these bone parameters (Figures 1 and 2).

Serum Ca and P

Animals fed the HDS016 diet had a lower ($P < .01$) serum Ca and P value than all other treatment groups. Dietary Ca did not appear to be related to serum Ca or P levels (Table 6).

Bone Parameters

Statistical analyses of bone ash, Ca and P were used to indicate differences ($P < .05$) paralleling dietary Ca level. However, the MDS393 diet was higher ($P < .05$) in bone Ca concentration than the HDS488 diet. In addition, rats fed the MDS(P)460 diet showed a higher ($P < .05$) bone P content, than those fed the HDS488 diet (Table 7).

The weight of the humerus appears to be directly related to dietary Ca level, as does breaking strength of the humerus (Table 8). Dietary Ca levels of 0.16 - 2.46 g Ca/kg revealed significant differences in strength ($P < .05$) when compared with levels of 3.93 to 4.88 g Ca/kg. A comparison of breaking strength per gram bone for the seven diets reveals increasing strength with increasing dietary calcium level.

Table 5. Relative biological values for mechanically-deboned shank (MDS) diets calculated relative to the hand-deboned shank (HDS) diets supplemented with $CaCO_3$.

Linear Regression of:		Relative Biological Value		
		MDS(P)460	MDS393	MDS221
Ca consumed(mg)vs	Vert Ca[b](g/kg)[a]	132	132	104
	Hum Ca	116	138	111
Ca retained(mg)vs	Vert Ca[c]	127	129	102
	Hum Ca[d]	113	136	109
Dietary Ca vs (g/kg)	Vert Ca[e]	127	130	106
	Hum Ca[f]	113	135	110
Ca consumed vs (mg)	Vert Ash[g]	128	114	108
	Hum Ash[h]	111	105	104
Ca retained vs (mg)	Vert Ash[i]	121	110	104
	Hum Ash[j]	110	106	105
Dietary Ca vs (g/kg)	Vert Ash[k]	121	109	107
	Hum Ash[l]	108	104	104

[a]Prediction Equation (developed using data from caudal vertebrae or humeri measured in g/kg Ca or ash: $y = 0.04x + 51.94$ ($r=0.96$)

[b]$y=0.05x + 139.26$ ($r = 0.80$)

[c]$y=0.07x + 49.01$ ($r = 0.97$)

[d]$y=0.09x + 135.8$ ($r = 0.82$)

[e]$y=9.47x + 45.35$ ($r = 0.98$)

[f]$y=10.92x + 134.60$ ($r = 0.84$)

[g]$y=0.12x + 105.61$ ($r = 0.95$)

[h]$y=0.14x + 343.59$ ($r = 0.81$)

[i]$y=0.21x + 98.05$ ($r = 0.94$)

[j]$y=0.25x + 322.34$ ($r = 0.70$)

[k]$y=27.08x + 94.06$ ($r = 0.95$)

[l]$y=31.56x + 330.60$ ($r = 0.82$)

Figure 1. Graph of linear regression analysis of dietary Ca level vs bone ash content for hand-deboned shank (HDS) meat diets containing $CaCO_3$, and biological values of mechanically-deboned shank (MDS) meat diets calculated relative to the HDS meat diets. MDS (P) was prepared by autoclaving MDS at 121°C for 90 minutes.

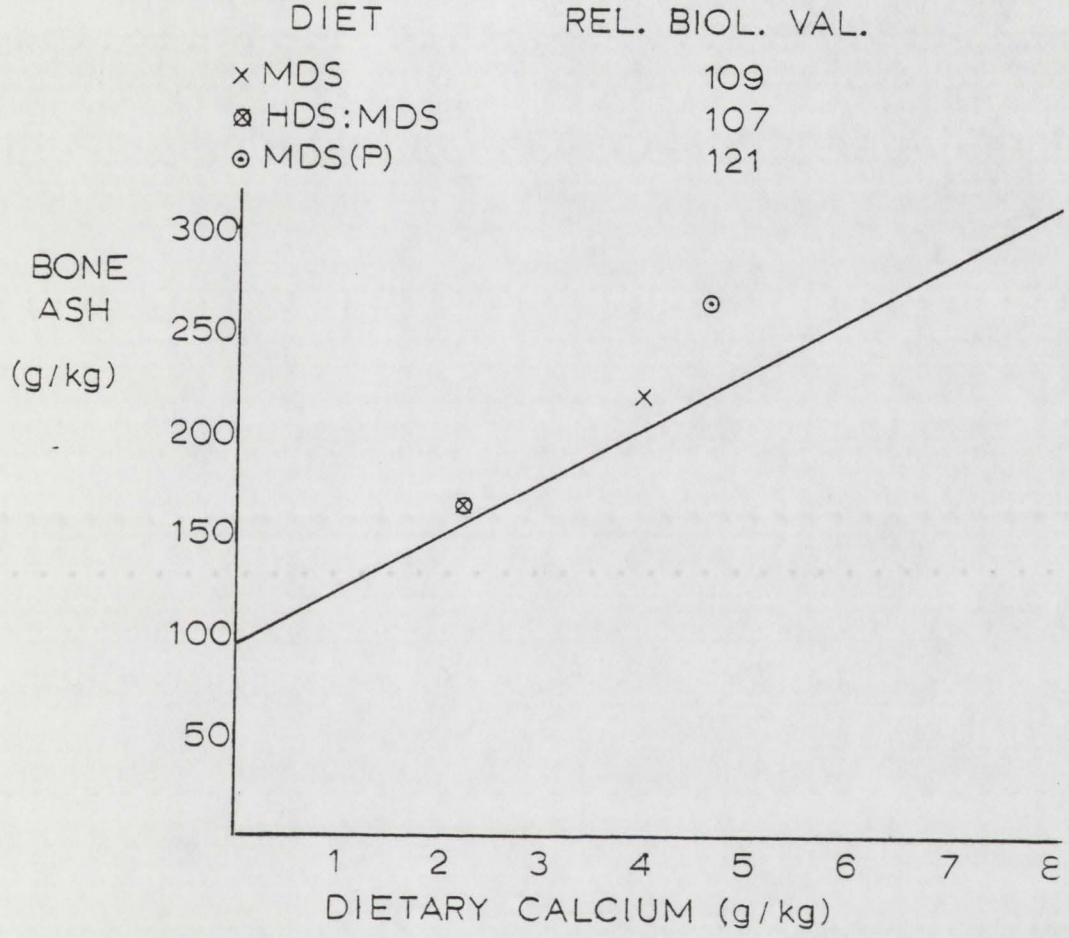
DIET
REL. BIOL. VAL.
× MDS
109
⊗ HDS:MDS
107
⊙ MDS(P)
121
BONE ASH (g/kg)
300
250
200
150
100
50
1
2
3
4
5
6
7
8
DIETARY CALCIUM (g/kg)

Figure 2. Graph of linear regression analysis of dietary Ca level vs bone Ca concentration for hand-deboned shank (MDS) meat diets containing $CaCO_3$, and biological values of mechanically-deboned shank (MDS) meat diets calculated relative to the HDS meat diets. MDS (P) was prepared by autoclaving MDS at 121°C for 90 minutes.

DIET	REL. BIOL. VAL.
MDS	130
HDS:MDS	106
MDS(P)	127

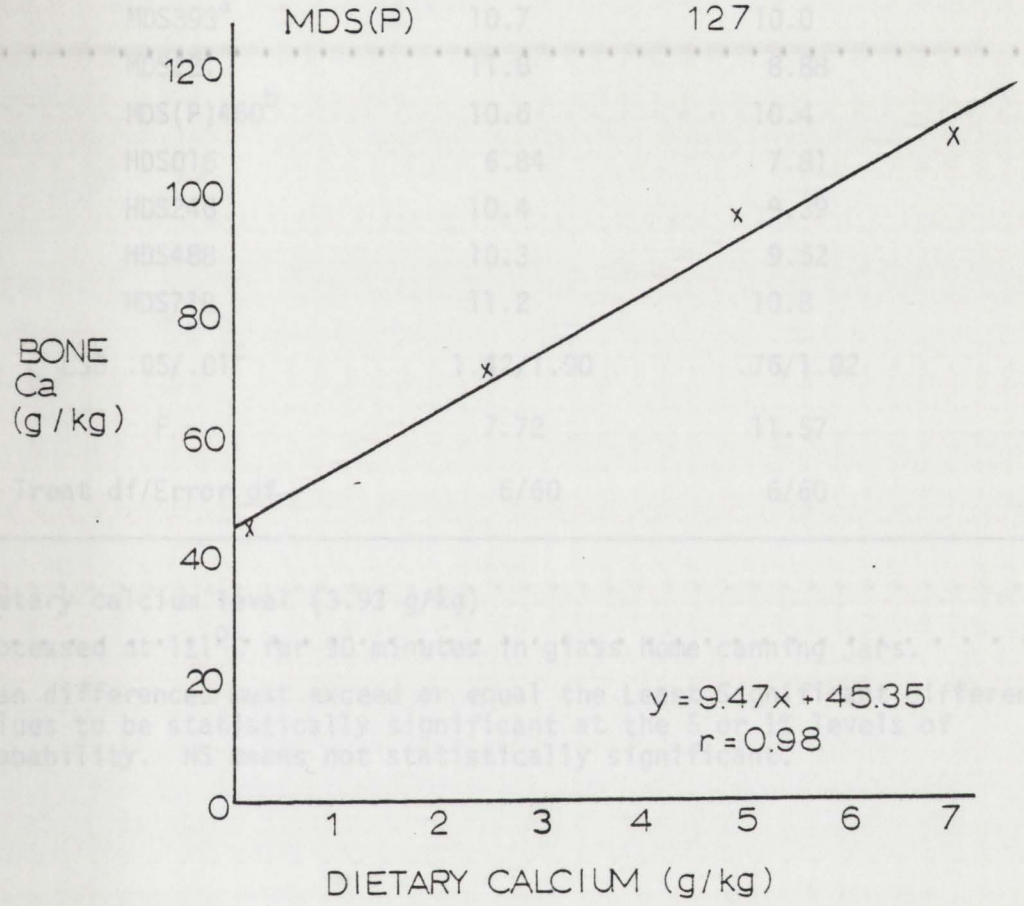

Table 6. Serum calcium and phosphorus in weanling rats fed MDS and HDS diets.

Diet	Serum Ca mg/dl	Serum P mg/dl
MDS393[a]	10.7	10.0
MDS221	11.8	8.88
MDS(P)460[b]	10.6	10.4
HDS016	6.84	7.81
HDS246	10.4	9.39
HDS488	10.3	9.52
HDS718	11.2	10.8
LSD .05/.01[c]	1.42/1.90	.76/1.02
F	7.72	11.57
Treat df/Error df	6/60	6/60

[a]Dietary calcium level (3.93 g/kg)

[b]Processed at 121°C for 90 minutes in glass home canning jars.

[c]Mean differences must exceed or equal the Least Significant Difference values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

Table 7. Ash, calcium and phosphorus content of caudal vertebra and humerus of weanling rats fed MDS and HDS diets.

Diet	Vertebra			Humerus	
	Ash (g/kg)	Ca (g/kg)	P (g/kg)	Ash (g/kg)	Ca (g/kg)
MDS393[a]	219[3]	107[4]	43.5[3]	471[3]	240[5]
MDS221	165[2]	70.0[2]	33.7[2]	416[2]	175[2,3]
MDS(P)460[b]	246[4]	113[4]	49.9[4]	512[4]	209[4]
HDS016	94[1]	46.7[1]	28.0[1]	326[1]	133[1]
HDS246	169[2]	70.8[2]	31.0[2]	418[2]	165[2]
HDS488	222[3]	96.2[3]	43.0[3]	487[3,4]	190[2,3,4]
HDS718	289[4]	110[4]	52.4[4]	553[4]	210[4]
LSD .05/.01[c]	23/31	6.5/8.6	3.4/4.6	42/55	28/37
F	64.22	122.8	60.48	153.1	28/71.7
Treat df/ Error df	6/63	6/63	6/63	6/60	6/60

[a]Dietary calcium level (3.93 g/kg).

[b]Processed at 121°C for 90 minutes in glass quart canning jars.

[c]Mean differences must exceed or equal the Least Significant Difference values to be statistically significant at the 5 ot 1% levels of probability. NS means not statistically significant.

[1-5]Groups with the same superscript are not different ($P < .05$).

Table 8. Humerus weight and breaking strength of weanling rats fed MDS and HDS diets.

Diet	Weight (mg)	Strength (kg)	Breaking Strength g Bone
MDS393[a]	104.1[3]	2.3[2]	19.3[2,3]
MDS221	91.0[2]	1.53[1]	16.9[1,2]
MDS(P)460[b]	115.0[4]	2.53[3]	21.8[3,4]
HDS016	65.6[1]	1.14[1]	15.3[1]
HDS246	91.6[2]	1.44[1]	15.6[1]
HDS488	111.5[4]	2.52[3]	22.4[3,4]
HDS718	128.5[5]	3.21[4]	25.0[4]
LSD .05/.01[c]	6.8/9.0	0.46/0.62	3.5/4.6
F	69.1	19.99	52.3
Treat df/Error df	6/61	6/62	6/61

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

[b]Processed at 121°C for 90 minutes in glass quart canning jars.

[c]Mean differences must exceed or equal the Least Significant Difference values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

[1-5]Groups with the same superscript are not different ($P < .05$).

Fluoride

Balance

No significant differences were found between the three MDS diets and the HDS diet containing 0.66 mg F/kg for F absorption for week 1 (Table 9). For the three week balance period, the MDS diet containing 2.21 g Ca and 6.28 mg F/kg revealed the higher ($P < .05$) absorption efficiencies, and the MDS diet containing 3.93 g Ca and 10.74 mg F/kg revealed the lowest ($P < .05$) absorptions except for week 3.

Whereas F absorptions (g/kg) were highest for the MDS diet containing 6.28 mg F/kg, F retention (g/kg consumed) tended to be higher for the processed MDS diet containing 15.12 mg F/kg (ignoring data for the diet with 0.66 ppm F) (Table 10). The MDS diet containing 10.7 mg F/kg was always one of the significantly lower treatment groups for F absorption, and was also lowest for F retention (except for week 2). No significant differences were found among the three-week means, because shifts throughout the balance period cancelled out the differences.

Bone Parameters

Table 11 contains data relating F level and bone parameters. Statistical analysis of humerus ash reveals that significant differences are related to an increase in dietary Ca level rather than dietary F level. The exception is that the MDS(P)460 diet containing 15.12 mg F/kg is higher ($P < .01$) than the HDS488 diet containing 0.66 mg F.

Breaking strength measured in kg force necessary to break the humerus reflected the same pattern as humerus ash. Breaking strength increased as dietary Ca level increased, except that the HDS diet containing 4.88 g Ca and 0.66 mg F/kg was not higher ($P < .05$) than

Table 9. Fluoride absorption by weanling male rats fed diets containing mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meats.

Diet	F ppm	Gm F Absorbed/kg Ingested			
		Wk 1	Wk 2	Wk 3	3 Wks
HDS488[a]	0.66	707	692[1,2]	810[1,2,3]	744[1,2]
MDS221	6.28	802	875[3]	905[3]	872[3]
MDS393	10.74	611	654[1]	752[1]	685[1]
MDS(P)460[b]	15.12	776	763[1,2,3]	725[1,2]	762[1,2]
LSD .05/.01[c]		ns	128/172	101/136	81/120
F		2.88	4.70	5.16	5.74
Treat df/Error df		3/32	3/36	3/36	3/32

[a]Dietary Ca level (4.88 g/kg).

[b]Processed by autoclaving MDS at 121°C for 90 minutes in glass quart canning jars.

[c]Mean differences must exceed or equal the Least Significant Difference values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

[1-3]Groups with same superscript are not different ($P < .05$).

Table 10. Fluoride retention by weanling male rats fed diets containing mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meats.

Diet	F ppm	Gm F Retained/kg Ingested			
		Wk 1	Wk 2	Wk 3	3 Wks
HDS488[a]	0.66	421[1,2]	262[1]	376[3]	328
MDS221	6.28	552[2]	419[2,3]	235[1,2]	385
MDS393	10.74	384[1]	286[1,2]	176[1]	303
MDS(P)460[b]	15.12	583[2]	431[3]	292[2,3]	436
LSD .05/.01		158/ns	142/191	111/149	ns
F		3.32	3.13	4.50	2.50
Treat df/Error df		3/30	3/35	3/36	3/31

[a]Dietary Ca level (4.88 g/kg).

[b]Processed by autoclaving MDS at 121°C for 90 minutes in glass quart canning jars.

[c]Mean differences must exceed or equal the Least Significant Difference values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

[1-3]Groups with the same superscript are not different ($P < .05$).

Table 11. Dietary fluoride; body weight at sacrifice; humerus ash, fluoride and breaking strength of bones from weanling male rats fed MDS and HDS diets.

Diet	Dietary F ppm	Terminal Body Wt.	Humerus Ash (g/kg)	Humerus F ppm	Breaking Strength (kg)	Strength kg/g Bone
HDS488	0.66	184.4	187.4[3]	19[1]	2.52[2]	22.4[2]
MDS221	6.28	196.5	415.7[1]	156[2]	1.53[1]	19.3[1,2]
MDS393	10.70	204.5	470.5[2]	190[2]	2.03[1,2]	16.8[1]
MDS(P)460[b]	15.10	201.2	512.3[4]	313[3]	2.53[2]	21.8[2]
LSD .05/.01[c]		ns	14.3/19.3	37.2/50.1	0.58/0.78	4.22/ns
F		2.51	63.74	93.07	5.29	3.02
Treat df/Error df		3/36	3/34	3/33	3/35	3/35

[a]Dietary Ca level (4.88 g/kg).

[b]Processed by autoclaving MDS at 121°C for 90 minutes in glass quart canning jars.

[c]Mean differences must exceed or equal the Least Significant Difference values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

[1-4]Groups with same superscipt are not different ($P < .05$).

the MDS diet containing 3.93 g Ca and 10.74 mg F. These similar strengths probably are not related to dietary F, because the processed MDS diet containing 4.60 g Ca and 15.12 mg F/kg also was not different ($P < .05$) from the MDS diet with only 3.93 g Ca and 10.74 mg F.

Humerus F (mg/kg) increased as dietary F level increased (Table 11). Significant differences ($P < .01$) were found among all treatment groups except the MDS diet containing 6.28 mg F/kg diet and the MDS diet containing 10.74 mg F/kg diet.

Fe Bioavailability

Balance

The lowest Fe absorption was generally exhibited by animals fed diets with characteristics such as 1) being processed, 2) containing the highest Fe level (60.4 mg/kg), and 3) containing a relatively low dietary Ca level (Table 12). Although no dietary Fe level appeared to exhibit a high Fe absorption, generally the diets containing 2.21 or 2.46 g Ca/kg diet, levels below the recommended amount for the rat (National Research Council, 1972) exhibited the highest Fe absorptions. The processed MDS diet was lower ($P < .05$) than all other groups for Fe absorption in week 2 and over the entire three weeks.

Hemoglobin

Terminal hemoglobin (Hb) concentrations were similar to the pattern exhibited by Fe absorption, in that the processed MDS diet containing 60.44 and 4.60 g Ca/kg diet had a significantly lower ($P <.05$) concentration than all other treatment groups excepting the HDS016 diet (Table 12). The other two MDS diets exhibited relatively high terminal Hb concentrations when compared with the HDS diets.

Table 12. Iron absorption, hemoglobin concentrations and liver iron in weanling male rats fed mechanically-deboned shank (MDS) and hand-deboned shank (HDS) meats.

Diet	Dietary Fe ppm	Fe Absorption (g/kg)				Terminal Hb (g/dl)	Liver Fe µg	Liver Fe µg Fe/g Liver
		Wk 1	Wk 2	Wk 3	3 Wks			
MDS393[a]	53.54	235	398	467	381	11.74	549	60
MDS:HDS221	48.53	335	456	517	447	11.22	536	61
MDS (P)460[b]	60.44	165	156	262	218	9.94	369	43
HDS016	39.03	161	423	382	315	10.59	354	84
HDS246	42.90	412	534	367	441	11.04	560	64
HDS488	46.27	224	406	503	426	11.46	512	59
HDS718	47.69	241	409	229	320	11.30	450	50
LSD .05/.01		ns	118/157	179/ns	125/166	0.94/1.24	149/ns	19/25
F		1.34	7.73	2.49	3.63	3.29	2.62	3.66
Treat df/ Error f		6/63	6/63	6/62	6/62	6/63	6/61	6/61

[a]Dietary Ca level (3.93 g/kg).

[b]Processed by autoclaving at 121°C. for 90 minutes in glass quart canning jars.

[c]Mean differences must exceed or equal the Least Significant Difference values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

Liver Fe

When the quantity of Fe in the liver was measured the processed MDS diet containing the highest Fe level exhibited the next to lowest Fe storage (Table 12). The low liver Fe exhibited by animals receiving the HDS016 diet could be a reflection of the significantly lower weight gain and liver weight of this group (see Table 3). Though significant differences among groups exhibited no particular pattern that could be linked with dietary Fe level, those diets with the greatest Fe storage also contained dietary Ca levels in the mid-range.

When compared on the basis of liver iron concentration, the animals that consumed the HDS016 diet exhibited the best storage due to their very low liver weight (Table 12). However, the processed MDS diet containing the highest dietary Fe level again revealed the lowest Fe storage. The same pattern of high Fe storage occurring in diets with 3.93, 2.21 or 2.46 g Ca/kg diet was again seen.

DISCUSSION

Composition of Meat

Nutrient contents of hand-deboned shank (HDS) and mechanically-deboned shank (MDS) fall within the normal ranges (Hendricks et al, 1977; Meiburg et al, 1976; Field 1976; American Meat Institute, 1960). The MDS in this study contained 0.34% calcium on a fresh weight basis, which is slightly less than the average value for mechanically-deboned red meat (MDRM) (Kolbye and Nelson, 1977b). A fluoride level of 11.4 ppm is higher than the average value of 5 ppm reported by Kolbye and Nelson (1977b) but within the 7-19 ppm range reported by Kruggel and Field (1977). Iron levels in this HDS (32.7 ppm) and MDS (36.8 ppm) do not agree with reports of MDRM containing nearly twice as much iron as hand-deboned red meat (HDRM) (Field, 1976). Kolbye and Nelson (1977b) reported a 54 ppm iron level in MDM which is slightly less than twice that in lean pork and beef.

Animal Responses

Body Weight

Generally, body weight was positively influenced by increasing dietary calcium levels. There are two specific exceptions to the pattern of dietary calcium level resulting in a higher weight gain. Weight gain over the three week period for animals receiving the MDS393 diet was higher ($P < .0.$) than for those receiving the HDS488 diet. Body weight at sacrifice (Day 22) was higher ($P < .05$) for animals receiving the MDS393 than for those receiving the HDS488 diet. Allred (1976) suggested that the significantly lower weight gains noted for

animals fed meat diets was due to oxidation lowering the digestibility of meat diets. These data do not support this theory, since animals fed the MDS(P)460 diet, which would be expected to be more rancid than the HDS488 that was kept frozen more consistently, did not have depressed weight gains.

Solomon and Volpin (1972) established that animals receiving a diet deficient in calcium exhibited a lower body weight than animals receiving sufficient calcium. In their study, animals receiving 0.43% calcium in the diet gained more weight than those receiving 0.02 - 0.03% calcium. It can be seen from this study that weight gain should increase up to a dietary level of 0.393%calcium if the calcium source is MDS, and up to a dietary level of 0.718 calcium if the calcium source is $CaCO_3$. In contrast, Bernhart et al (1969) concluded from a graph of weight gain vs calcium intake, that a dietary level of 0.342% calcium was sufficient for maximal growth. However, their experimental design did not include a dietary level above 0.362% calcium. The National Research Council (1972) has set 0.5% calcium as the requirement for the growing rat.

Dry Matter Absorption

Dry matter absorption decreased as calcium concentration increased; e.g., animals fed the HDS718 diet exhibited a lower dry matter absorption than the other animals for each of the three weeks (Table 3). This is expected, since the formation of insoluble complexes, possibly calcium complexes of either organic or inorganic matter, lowers absorption (Pike and Brown, 1975). The pattern of an inverse relationship between dietary Ca level and dry matter absorption is consistent. In week 1,

the animals receiving the HDS488 diet had a lower ($P < .01$) dry matter absorption than did all other diets with a lower dietary calcium level. Even the animals receiving the MDS(P)460 diet had a lower ($P < .05$) dry matter absorption than those receiving the MDS393 diet for week 3. This latter difference may be due to the effects of processing and not to dietary calcium level, however.

Serum Calcium and Phosphorus

Serum calcium and phosphorus levels were within the normal range, except for the animals fed the HDS016 diet containing essentially no Ca (Table 6). Kemm (1972) reported no significant differences in fasting serum calcium among animals fed 1.6, 0.8, 0.4 or 0.2% calcium in the diet. Animals receiving a dietary level of 0.64% calcium had a serum calcium of 10.82 mg%, while those receiving a dietary level of 0.099% calcium had a serum calcium of 9.35 mg%. In this study, animals receiving a dietary level of 0.016% calcium had serum calcium and phosphorus levels lower ($P < .01$) than all other treatment groups. Analyses of the serum for this low calcium group was questionable, since the volume of blood obtained at sacrifice was small. Occasionally, the aliquot was halved, or data for an animal were dropped.

Calcium Bioavailability

Balance

As previously cited, lower dietary calcium levels lead to higher percent calcium absorption. Calcium from the MDS221 and HDS246 diets were absorbed more efficiently ($P < .01$) than the HDS718 or HDS016 diets (Table 4). Obviously, the dietary calcium level in this latter diet is

too low for meaningful comparison. During the third week, calcium from the MDS 221 diet was absorbed more efficiently than from the MDS(P)460 diet. Apparent absorption of calcium during the entire three-week period supports the conclusion of others that lower dietary levels of calcium are absorbed more efficiently (Bronner et al, 1976; Harrison, 1959; Bell et al, 1941). Results for apparent retention of calcium were almost identical to those for absorption, since very little calcium is lost in the urine.

Calcium absorption from the bone in MDM is similar to that from $CaCO_3$. Absorptions of animals fed MDS393, MDS221, HDS246 and HDS488 diets were similar ($P < .05$) at the end of the three-week balance study when rats are adapted to their respective dietary calcium levels. Rats fed the MDS(P)460 diet have a somewhat lower absorption. When Drake et al (1949) measured retention by rats of calcium in $CaCO_3$ and in various bone sources, they found that calcium from beef bones had a relative biological value of 99% when compared with that of $CaCO_3$ (100%). The biological availability of calcium relative to that for $CaCO_3$ was 133-138% for bonemeal fed to cattle and 109% for bone meal fed to chicks (Peeler, 1972). Therefore, bone material from MDM is as good a source of dietary calcium as is $CaCO_3$.

Prediction Equations

Relative biological values, calculated relative to the HDS diets supplemented with $CaCO_3$, were consistently greater than 100 for diets containing MDS (Table 5). Thus, calcium bioavailability from MDS is at least as good as that from $CaCO_3$. The correlation coefficients were higher when caudal vertebral, rather than humeral, response was

the dependent variable. When caudal vertebral ash rather than bone Ca is the dependent variable, the MDS(P)460 diet has a higher relative biological value than the unprocessed MDS393 diet. The two MDS diets differed in fluoride level as well as processing. This study supports the conclusion of others that the retention of calcium from bone material is good (Blosser et al, 1954; Drake et al, 1949).

The use of prediction equations to evaluate calcium bioavailability is valuable since control and experimental dietary levels do not have to be identical to obtain meaningful data. In addition, these data can be used to maintain that only bone ash content and dietary calcium levels are necessary for valid comparisons of calcium sources ($r = 0.95$). This procedure would be much less tedious and expensive to excute than a balance study using metabolism cages.

Bone Parameters

In general, caudal vertebral ash (g/kg), caudal vertebral calcium (g/kg), humerus weight (g), and breaking strength of humerus (kg) was relatively high in animals fed a high calcium level (Table 13). These bone responses are good indicators of biological availability of ingested bone calcium in the MDM diets. A direct relationship between dietary calcium level and bone weight has been demonstrated (Solomon and Volpin, 1972; Toothill and Hosking, 1968; Bell et al, 1941). Toothill and Hosking (1968) report a highly significant increase in percent ash of bone when dietary calcium level is increased from 0.13% to 0.74%. Solomon and Volpin (1972) report a decrease of from 180g to 85g in metatarsal bone strength with dietary calcium levels of 0.43% and 0.02 - 0.03% respectively. This study can be used to show a direct relation-

Table 13. Bone parameters of humeri and vertebrae as a reflection of dietary Ca and F levels. MDS and HDS diets fed weanling male rats.

Diet	Dietary F (mg/kg)	Vertebra Ash (g/kg)	Vertebra Ca (g/kg)	Humerus Wt. (mg)	Humerus Ash (g/kg)	Humerus Ca (g/kg)	Humerus F (mg/kg)	Humerus Breaking Strength (kg)	Humerus Breaking Strength (kg/g bone)
MDS221[a]	6.28	165^{2}	70.0^{2}	91.0^{2}	416^{2}	$174^{2,3}$	156^{2}	1.53^{1}	$16.88^{1,2}$
MDS393	10.74	219^{3}	107^{4}	104.1^{3}	471^{3}	240^{5}	190^{2}	2.03^{2}	$19.32^{2,3}$
MDS(P)460[b]	15.12	264^{4}	113^{4}	115.0^{4}	512^{4}	208^{4}	313^{3}	2.53^{3}	$21.81^{3,4}$
HDS016	-	94^{1}	46.7^{1}	65.6^{1}	326^{1}	133^{1}	-	1.14^{1}	15.29^{1}
HDS246	-	169^{2}	70.8^{2}	91.6^{2}	418^{2}	165^{2}	-	1.44^{1}	15.63^{1}
HDS488	0.66	222^{3}	96.2^{3}	111.5^{4}	$487^{3,4}$	$190^{2,3,4}$	19^{1}	2.52^{3}	$22.44^{3,4}$
HDS718	-	289^{4}	110^{4}	128.5^{5}	552^{4}	210^{4}	-	3.21^{4}	24.99^{4}
LDS .05/.01[c]		23/31	6.5/8.6	6.8/9.0	41.6/55.4	27.9/37.2	37.2/50.1	.46/.62	3.49/4.64
F		64.22	122.8	69.07	153.09	71.72	93.07	19.99	52.27
Treat df/ Error df		6/63	6/63	6/61	6/60	6/60	3/33	6/62	6/60

[a]Dietary calcium level (2.21 g/kg).

[b]Processed at 121°C for 90 minutes in glass quart canning jars.

[c]Mean differences must exceed or equal the Least Significant Differences values to be statistically significant at the 5 or 1% levels of probability. NS means not statistically significant.

[1-5]Groups with the same superscript are not different ($P < .05$).

ship when comparing bone ash, bone calcium, bone weight and breaking strength of bone with dietary calcium levels up to 0.718%. However, the reliability among experiments of relating these bone parameters to dietary calcium levels as high as 0.718% is questioned. Recall that Bernhart et al (1969) set a calcium level of 0.342% as maximal for growth. In conjunction, Bell et al (1941) report no increase in size, calcium content or strength of bone above an intake of 0.36% calcium when comparing data from diets ranging from 0.075 to 1.390% calcium. However, a maximal effective Ca level of 0.36% is lower than that of 0.5% Ca or greater already cited as increasing total body Ca in rats (Williams et al, 1957).

Similar to the relative biological values, bone parameters can be used to indicate that calcium from MDS is at least as biologically available as calcium from $CaCO_3$. The calcium from the MDS(P)460 diet is especially available to the bone, and must be due to a processing effect, rather than dietary Ca level. Bone Ca (g/kg) is higher ($P < .05$) in animals fed MDS393 or MDS(P)460 than in those fed HDS488. In addition, bone ash (g/kg) is higher ($P < .05$) in animals fed MDS(P)460 than in those fed HDS488.

Fluoride Bioavailability

Dietary fluoride in the MDS diets was examined for its possible role in increasing bone response to dietary calcium levels higher than might be expected. Schwarz and Milne (1972) report an enhanced growth rate of 17, 30.8 and 27.9% with dietary levels of 1, 2.5 and 7.5 ppm fluoride fed to animals that were fluoride-deficient. In this study, recall that weight gain was not different ($P < .05$) for the three MDS

diets in comparison with the HDS718 diet containing substantially more calcium, but were somewhat higher than the HDS diets containing comparable dietary Ca levels and no F (Table 2). Lawrenz and Mitchell (1941) reported heavier bones with significantly higher percentages of ash and calcium when animals were fed a high calcium (0.73%) diet with 9.4 ppm F than a low calcium diet (0.23% Ca) with 9.4 ppm fluoride.

In general, fluoride retentions (g/kg consumed), though not absorptions, were higher ($P < .05$) for the animals fed the MDS(P)460 diet. However, retentions over the entire three weeks were not different ($P < .05$). Jackson et al (1950) reported that the retention of fluoride from bone meal varied from 17-43% for young rats. Stillings et al (1973) found that fluoride retention is 32% for rats fed a diet of fish protein concentrate containing 7 µg fluoride/g. These reported data compare well with the 30-44% fluoride retention from MDS diets in this study.

In conclusion, 20% of the MDS used in this study would add 0.13 mg fluoride to two ounces of bologna, or 0.26 mg to two frankfurters. Even in cities where the water contains no fluoride, this would increase consumption by only 25%, and would be far less than the 3 mg fluoride/day limit for mottling of children's teeth (Kramer et al, 1974).

Iron Bioavailability

Iron absorption (g/kg), terminal Hb (g/dl), and liver iron storage were generally lowest ($P < .05$) in animals fed the MDS(P)460 diet (Table 12). Since this diet contained the highest dietary iron level, these responses are not a result of dietary iron level. The greatest responses to iron occurred in animals fed diets containing 2-3 g

calcium/kg diet. This agrees with the theory cited earlier that dietary calcium interferes with iron utilization. Even though Chapman and Campbell (1957b) reported no significant effect of bone meal on liver iron content or on hemoglobin level, they did note a tendency for increasing amounts of dietary bone meal to be correlated with decreasing amounts of iron in the liver. A dietary level of 7.23 g calcium from bone meal/kg diet and 43.3 ppm Fe resulted in animals with 69 µg Fe/g liver, while 3.20 g calcium from bone meal and 36 ppm Fe resulted in animals with 116 µg Fe/g liver (Chapman and Campbell, 1957b)

Other reasons why iron bioavailability does not reflect its dietary level have been suggested. Iron from MDS may be less biologically available due to the form that the iron is in. The conclusion of Farmer et al (1977) that some of the iron in MDS must come from the machinery (stainless steel) means that part of the iron is elemental iron or possibly ferric oxide, both of which are poorly absorbed (Ammerman and Miller, 1972). A comparison of data from this study (a dietary level of 7.18 g calcium from bone material/kg diet and 47.7 ppm iron resulted in 50 µg Fe/g liver) and that from Chapman and Campbell (1957b) (a dietary level of 7.23 g calcium from bone meal/kg diet and 43.3 ppm Fe resulted in 69 µg Fe/g liver) indicates a superior utilization of iron from $FeSO_4$ in the latter case.

CONCLUSIONS

Determinations were made of the biological availability of calcium from mechanically-deboned shank diets with 2.21 - 4.60 g calcium from bone particles in relation to hand-deboned shank diets with 0.16-7.18 g calcium from $CaCO_3$/kg. Percent absorption, relative biological values, bone ash content, bone Ca content, bone weight, and breaking strength of bone support the conclusion that the calcium naturally occurring in MDS as a result of the deboning process is as biologically available as the calcium added to HDS as calcium carbonate. This study indicates that a correlation of dietary Ca level vs caudal vertebral bone ash is a valid measure of the biological availability of a calcium source when compared with standard diets containing $CaCO_3$. Calcium from the processed MDS diet is more biologically available than the HDS diet with a similar dietary calcium level from calcium carbonate.

The 30-44% retention of fluoride in MDM from the bone material is very similar to other data for rats, and should pose no health hazard for children (with the possible exception of infants) or adults.

Iron bioavailability from MDS, as determined by percent absorption, terminal Hb (g/dl), and liver iron was similar to that from HDS with the exception of the autoclaved MDS. An alteration of the iron state to Fe_2O_3 due to the autoclaving is a possible explanation. A decreased iron bioavailability has also been observed in processed HDS diets (Tso, 1979).

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APPENDICES

APPENDIX A

Inorganic Phosphorus Procedure

Reagents

Prepare MS solution of 1% $MgCl_2$, 1% $(NH_4)_2MoO_4.2H_2O$, and 2.8% concentrated H_2SO_4.

Prepare elon solution by dissolving 1 g of elon (p-methyl-aminophenol sulfate) in 100 ml of 3% sodium bisulfite ($NaHSO_3$). (Prepare elon fresh monthly and store in refrigerator).

Procedure

Make standards of 10, 25, 50, 100, 150, 200 and 250 ppm P from a 1000 ppm P solution of dipotassium hydrogen phosphate (K_2HPO_4). Prepare blank and standards by mixing 50 µl of deionized water and the P standards each with 5 ml MS and .5 ml elon. Mix well. Prepare appropriate dilutions of samples using same amounts of MS and elon. Mix well. Allow tubes to stand 45 minutes and read at 700 mµ on a spectrophotometer. Calculate ppm P in sample using the prediction equation from the standards.

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APPENDIX B

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 distilled water in a 1 liter beaker. Add 47 ml glacial acetic acid, 58 g NaCl and 4 g CDTA (cyclohexylene dinitrilo tetraacetic acid or 1, 2-diaminocyclohexane N, N, N', N'-tetraacetic acid). Stir to dissolve. Cool the beaker in a water bath. Slowly add approximately 5 M NaOH until pH is between 5.0 and 5.5. Cool to room temperature and dilute to 1 liter with distilled water.

Procedure

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

Weigh 25-50 g of sample ashed 16 hr. at 550^{o}C. into a polyethylene cup. Dissolve with 5 ml of 0.25 M HCl. Neutralize with 10 ml of 0.125 M NaOH. Add 5 ml of 0.05 M NaOAC buffer, 5 ml distilled H_2O, and 25 ml TISAB. (If the pH has been adjusted by the addition of acid or base, add an equ-l volume of TISAB, carefully noting the total volume).

Using a magnetic stirrer, read samples and standards using a fluoride specific ion electrode with a digital pH/mv meter with the function switch set to REL MV. Wait for a stable reading (approximately 5 minutes) and record. Rinse electrodes with distilled water and blot dry between readings.

F content was calculated using the following formula:

$$\frac{(\text{Total g ash}) \left(\frac{\text{Total ml soln}}{\text{g ash in soln}}\right) (\mu\text{g F/ml})}{\text{g sample}} = \mu\text{F/g sample}$$

Reference: Orion Research, Inc. 1977. Instruction Manual. Form 1M 94, 96-09/7721. U.S.A.