IDENTIFYING A NON-INVASIVE MEASURE OF BONE STATUS IN DAIRY CATTLE

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ABSTRACT

The objectives of this research were to evaluate non-invasive measures of bone mineral content (BMC) and bone mineral density (BMD) as rapid, on-farm tools to assess phosphorus (P) status in dairy cows. In addition, the effects of parity and stage of lactation on measures of BMC of the fused 3rd and 4th metacarpal bone and of caudal vertebrae 14 and 15 were assessed. The caudal vertebrae and right front metacarpal (sample pairs) were excised from 107 Holstein cull cows following slaughter. Parity, age, and days in milk (DIM) of the donor animal were obtained for 43 pairs of samples. Samples were grouped by parity (1, 2, 3, and ≥ 4) and stage of lactation (Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250 DIM). Samples were analyzed for BMC and BMD with dual energy X-ray absorptiometry (DXA), BMC with radiographic photometry (RP), breaking strength with mechanical methods, and mineral content with chemical procedures. Estimates of BMC obtained with RP and DXA were poorly related to chemical measures of actual BMC and to measures of breaking strength. In caudal vertebrae 14 and 15, increasing stage of lactation decreased energy to peak load with the lowest values observed in late lactation. Stage of lactation had no effect on BMC measured chemically in the caudal vertebrae or metacarpal. Parity did not affect breaking strength of the metacarpal or caudal vertebrae or total ash or P content of any bone. Results indicated that imaging techniques are not useful measures of BMC in mature dairy cattle.

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INTRODUCTION

Nutrient contamination of ground and surface water is a leading concern facing farmers and animal agriculture. Overabundance of phosphorus (P) in surface water, primarily a result of runoff from land, causes accelerated algae growth. Degradation of this algae consumes dissolved oxygen, threatening fish and other aquatic life. When manure is applied to crops, nitrogen (N) and P are in imbalance relative to crop needs. When N is applied to land to meet crop needs, P over-accumulates in the soil. Because of these concerns, the focus of new environmental water quality regulations is now broadening from an exclusive spotlight on N to include P as well. Many states are now requiring P-based nutrient management plans. These regulations will require more land to dispose of manure. Phosphorus-based nutrient management plans have a significant impact on areas of confined animal agriculture.

Animal manure is applied to land for a number of reasons. Manure is beneficial for plant growth, may increase crop yields, improves soil structure, and increases soil fertility. If manure is not properly managed, its application can increase N and P concentrations in ground and surface water and cause contamination with bacteria.

Farmers need to reduce the P content of manure by more precisely meeting the animal's dietary requirements. By reducing dietary P, farmers can reduce feed costs, reduce the P content of manure, and reduce the risk of water contamination. An improved understanding of P digestion and utilization by animals is needed to reduce P excretion. Incremental increases in dietary P (g) in dairy cattle increases P excretion linearly (Morse et al., 1992; Knowlton et al., 2002).

Further reductions in dietary P may be achieved by accounting for the normal metabolism of bone in early lactation. Bone serves as a metabolic reservoir for Ca, P and other minerals and is a source of cells responsible for bone formation and resorption. All bone consists of mineral deposited in an organic matrix. The primary minerals found in bone and teeth enamel are Ca and P, imbedded in the organic matrix as the salt crystals, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Phosphorus mobilized from bone may meet a significant proportion of the cow's P requirements in early lactation, even though P mobilization through bone is not usually accounted for when establishing P requirements for lactation. The duration and extent of bone P stores in early lactation and the timing and extent of bone P is still not fully understood.

Increased understanding of factors affecting the P requirement of dairy cows (bone P resorption and feed P availability), and identification of non-invasive markers of P status of the animal are needed to increase adoption of these nutritional techniques. A field-level indicator of P status will allow fine-tuning of rations in the field through nutritional management on livestock farms and identify opportunities to reduce overfeeding of P on the farm.

Radiographic photometry (RP) is an inexpensive, non-invasive and accurate method of estimating bone mineral content (BMC) in horses (Meakim et al., 1981; Hoffman et al., 1999) and beef cattle (Williams et al., 1991). This method can be used to evaluate sequential changes in BMC in the same animal over a period of time, and may have potential as a non-invasive measure of P status in dairy cattle.

The objectives of this study were to evaluate RP assessment of BMC as a practical on-farm measure of P status in dairy cows by comparing this method to a more established imaging technique, mechanical measures of bone strength, and chemical measures of BMC. In addition, the effects of parity and stage of lactation on BMC in lactating cows were evaluated.

CHAPTER 1: REVIEW OF LITERATURE

Phosphorus

Functions in the body

Phosphorus (P) is an essential mineral that is involved in a wide variety of functions that are vital in cattle. More than 80% of P is found in bones and teeth, providing rigidity and ensuring bone formation and sound function of the musculoskeletal system. Phosphorus and calcium (Ca), are found in bone as the salt hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and Ca phosphate ($Ca_3(PO_4)_2$; Omnell, 1957).

Phosphorus is the second most abundant mineral found in the body, is required for milk secretion, is involved in a wide array of biochemical reactions, and is present in numerous structural components. Quantitatively, the most important function of P is in the formation of the organic bone matrix and mineralization. The approximately 20% of P that is not found in bone tissue serves important functions in fluids and soft tissues of the body. Phosphorus is a component of cell walls and cell contents as phosphoproteins and nucleic acids, maintaining the structure of all cell walls in the body. Nearly every form of energy exchange inside living cells involves the forming or breaking of high-energy bonds that link oxides to P and carbon or to carbon-nitrogen compounds. Phosphorus is a component of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) with essential functions in cell growth and differentiation. Phosphorus plays an important role in metabolic functions including energy utilization through ATP, gluconeogenesis, fatty acid transport, amino acid and protein synthesis, and the activity of the sodium/potassium (Na^+/K^+) pump. As phosphate, it helps to regulate osmotic pressure and acid-base balance. In the non-osseous tissue of a 300-kg steer, approximately 450 g of P are involved in a great variety of vital functions; probably more functions than any other mineral (Corbridge, 1985). Phosphorus is also required by rumen microorganisms for digestion of cellulose (Burroughs et al., 1951) and synthesis of microbial protein (Breves and Schroder, 1991).

Dietary requirements

The requirement for absorbed P for lactation is based on daily milk yield multiplied by the P content of milk. In the 2001 the National Research Council (NRC), this is assumed to be

0.90 g P/kg of milk. The maintenance requirement for P can be defined as the endogenous fecal loss when the supply of P is either just below or just meets the true requirements. The maintenance requirement for non-lactating pregnant and lactating cows was set at 1.0 g/kg of dietary dry matter (DM) consumed in the latest NRC (2001). This is based primarily on a study conducted by Spiekers (1993) in which two groups of cows were fed 37 or 21.5 g/d of P. These animals were fed very near or below true requirements respectively. Excretion of fecal P was 1.20 and 1.22 g/kg of DM intake per day for the high and low P intake groups. Dietary P was deliberately underfed to these animals to determine the absolute minimum P excretion and the effect of DMI on this excretion. By underfeeding dietary P, Spiekers found these animals could avoid excretion of any excess P. This particular study was not designed to determine pregnancy or milk yield requirements. Instead it was determined to define maintenance requirements as the minimal endogenous fecal excretion.

The requirement for absorbed P to support growth is the sum of the amount of absorbed P laid down in soft tissue plus the amount deposited in bone. Because bone matures early in the body, P requirements per kg of gain decline with maturity in growing dairy cattle. Requirements for P during pregnancy are low up until the last trimester. House (1993) slaughtered 18 multiparous Holstein cattle at various times from 190 to 270 d of gestation. Fetal mass and P content were measured over the sampling period. The accretion rate of P by the fetal mass increased from 1.9 g/d at 190 d of gestation to 5.4 g/d at 280 d of gestation.

Net absorption of P mainly occurs in the upper small intestine (Reinhardt et al., 1988) and only small amounts are absorbed in the rumen, omasum and abomasum. Absorption of P in ruminants takes place by way of active and passive diffusion. Efficiency of absorption of P depends on a number of factors including age of the animal, physiologic state, amount of DM consumed, P intake, ratio of Ca and P in the diet, intestinal pH, source of P and dietary concentrations of other minerals (Irving 1964; Peeler 1972; Agriculture and Food Research Council, 1991; Soares, 1995b). Several authors have found that P is absorbed in direct relation to the supply of potentially absorbable P in the lumen of the small intestine (Care et al., 1980), although Calla (1988) and Morse et al., (1992) both reported that the absorption coefficient declined as the dietary P concentration increased. The contradiction between these studies may be because some authors are comparing P deficient diets to P sufficient diets at the requirement, while others are comparing adequate P diets to excessive P diets. In the latter case, decreased

efficiency of P absorption is usually observed with the higher P diets. These studies all evaluated apparent P absorption, as fecal P contains endogenous P as well as undigested dietary P. True absorption may be determined by feeding animals less than their true dietary requirements so maximum absorption efficiency can be determined for the source of feed. Alternatively, infusion of ^{32}P may be used to differentiate between absorbed P of dietary origin and P of endogenous origin. The 1989 NRC publication used apparent absorption coefficients when calculating the amount of dietary P available for dietary requirements, but the current NRC uses the best available estimates of true absorption.

Phosphorus that is absorbed may be retained in meat, muscle and milk or secreted into the saliva to be reabsorbed, or excreted in the feces. Phosphorus retention is not usually affected by dietary P intake (Knowlton and Herbein, 2002), but P is retained in direct relation to the rate of Ca retention (Young et al., 1966; Braithwaite, 1979). In cows, between 30 and 90 g/d of P is secreted into saliva (Reinhardt et al., 1988; Scott, 1988). Almost all P in saliva is inorganic (Reinhardt et al., 1988) and the amount secreted is regulated by parathyroid hormone (Wasserman, 1981). Salivary P contributes to fecal excretion of P, the primary route of excretion.

A major portion of the P in dairy cattle diets comes from forages and cereal grains. Over half of the P in cereal grain is bound in the compound phytate, but leaves and stems contain very little phytate (Nelson et al., 1976). Phytate is degraded by the enzyme phytase, produced by rumen microorganisms. Phytase hydrolyzes organically bound P and makes it available for absorption. Ruminants are able to absorb and utilize the P released by phytate hydrolysis, but non-ruminants have very limited ability to digest phytate. While they are able to utilize phytate P, ruminant diets are still often supplemented with inorganic forms of P including dicalcium phosphate, monocalcium phosphate and deflourinated phosphate.

Phosphorus deficiency

Signs of deficiency can occur rapidly if dietary P is insufficient. Shupe (1988) concluded that12 g/d of supplemental P is adequate for proper growth and performance in beef cattle. Diets containing less than 6 g/d of P cause unthriftiness, rough hair coat, stiff gait, poor growth and infertility in cattle; however, these signs are often complicated by other deficiencies. Signs of severe P deficiencies include osteomalacia (lack of bone matrix mineralization in mature animal), decrease in bone mineral mass, brittle bones (Call et al., 1986; Blair-West et al., 1992)

and a craving for abnormal materials (pica) including wood, bones, flesh and soil. Phosphorus depleted cows show an avid appetite for old bones, preferring old weathered bones to fresh bones (Blair-West et al., 1992). Cows do not eat Na or Ca phosphate salts, or bones that are heated to greater than 250°C. Jubb (1985) reported that cattle are more susceptible to P deficiency than sheep or horses. Phosphorus deficiencies are seen in grazing cattle on low P soils or crops with a dietary P content less than 0.25% on a DM basis. Williams (1991) found that beef heifers receiving adequate dietary P (0.20% dietary P) consumed 11% more feed and tended to be more efficient in both total and average daily gain than heifers receiving low dietary P (0.12% dietary P). Shupe (1988) reported that beef cattle fed low dietary P diets (7.84 g/d to 8.85 g/d) for 14 m developed an abnormal stance, "humped back" appearance, loss of body condition, and a reluctance to stand. Osteoporosis, vertebral fractures and very thin bone cortices in these cattle resulted in lameness.

Normal serum P concentrations are maintained between 4.0 and 8.0 mg/dL, but when the mobile reserve of bone P is depleted, serum P concentrations may fall to less than 2 mg/dL (Forar et al., 1982). In P-deficient animals, bone P is mobilized to maintain normal serum inorganic P, although this mobilization is not rapid. Rapid changes in dietary P may be reflected in serum P concentrations, while prolonged dietary P deficiency is more likely to be reflected in bone status (Duncan, 1958). Phosphorus concentrations in milk are not affected by dietary P.

With P deficiency, plasma 1,25 dihydroxyvitamin D (1,25 $(OH)_2$ D) is elevated, increasing Ca absorption and bone resorption (Blair-West et al., 1992; Reinhardt et al., 1998; Goff, 1998). These changes cause decreased parathyroid hormone (PTH) concentrations, and decreased osteocalcin concentrations. At low P intakes, bone formation decreases in both sheep and cattle (Corlett and Care, 1988).

In cattle, once dietary P levels fall below 0.30% DM, microbial growth is inhibited and protein and energy supplied to the animal is reduced (Satter et al., 2002). Komisarczuk (1987) found rumen microbial activity can be maintained when rumen inorganic P levels are 75to100 mg/L. Ternouth (1990) reported rumen P levels are normally above the aforementioned level for optimal microbial activity even when animals are P deficient.

Milk yield is relatively unaffected by dietary P. Dietary P levels between 0.33 and 0.38% of DM were adequate for high producing cows > 11,900 kg of milk produced annually (Wu et

al., 2001). A level of 0.31% dietary P was marginally deficient for cows producing 9,000 kg/lactation (Wu, et al., 2001). Braintrip (1993), found no significant differences in DIM, milk yield, milk protein content or P concentrations in milk in cows fed diets with different concentrations of P. Brodison (1989) found that chemical composition of milk was the same for dairy cows fed low (0.24% DM) or high (0.42%) dietary P. Cows fed the low P diet had lower butterfat content than cows on the high P diet. Wu et al., (2001) found no differences in milk yield in cows fed diets supplemented with P (0.48% P) and un-supplemented with P (0.38% P). Satter et al., (2002) concluded that feeding diets containing 0.35% P meets the requirements of lactating cows, and provides a margin of safety. In their work, 0.30% P was border-line deficient, with reduced milk yield observed after two or three years.

Phosphorus toxicity can occur if animals consume a single large dose of P, but under normal conditions, P is absorbed according to the needs of the animal and the rest is excreted. Maximum tolerance level for P in ruminants is 1.0% of dietary DM (National Research Council, 1980). The 1980 NRC states animals consuming dietary P concentrations two to three times requirements may cause diarrhea and severe metabolic disorders associated with Ca absorption and metabolism.

Phosphorus excretion

The major route of P excretion in the dairy cow is through feces, and fecal excretion can be altered by P intake. In a total collection study (Morse et al., 1992), twelve lactating cows were fed a control diet (0.41% P) for four weeks, and then assigned to diets varying in dietary P (0.30% P; 0.41% P; and 0.56% P). At week four of the study, when all cows were on the control diet, cows excreted 88% of P consumed daily. Of this amount, 68% of P was excreted in feces, 30% was secreted in milk and 1% excreted in urine. The remainder was retained.

Fecal P excretion is affected by dietary P. Morse et al. (1992) reported that excretion in feces was decreased by 0.55 g/d for each gram of P intake decreased per day. In that study, cows consuming a diet of 0.30% P had 22.7% lower P excretion than cows fed 0.56% P.

Similarly, in dairy cows fed three different levels of dietary P (0.35% P, 0.51% P and 0.67% P), Knowlton and Herbein (2002) observed that total P excretion increased linearly with increased dietary P. Call (1978) reported that fecal P excretion was significantly higher in growing beef heifers fed 0.41% P compared with those fed 0.16% P. Lactating cows excreted

23% less P in feces when diets containing P were reduced from 0.49 to 0.40% P (Wu et al., 2000).

Only small amounts of P are secreted in the urine because kidneys do not serve as a major excretion route of P in ruminants (Harvey, 1989; Tamminga, 1992). Cattle do not usually excrete more than 0.5 g P/d in urine (Manston and Vagg, 1970), but this varies with diet and management. Ternouth (1990) reported that P the presence of measurable P in the urine is a sign of adequate P. Sato (1981) found diurnal effects on urinary P excretion in goats and sheep highest after feeding. Manston (1970) reported that permanently housed cattle excreted 10% more urinary P than grazing cattle, and observed that urinary P was increased when cattle were fed high concentrate diets, were restricted from exercise, or were prevented from grazing. Wu et al., (2001) reported that P concentration of urine in dairy cattle increases during the latter part of lactation because P requirements for production are lower at this time compared with early lactation.

Dietary P requirements for lactating cows range from 0.34 to 0.38% of dietary P, % DM (NRC, 2001), but rations of lactating cows often contain greater than 0.50%, approximately 20 to 30% in excess of 2001 NRC recommendations (Sink et al., 2000; Wu et al. 2001). Generally, overfeeding P has had neither detrimental nor beneficial effects on cow health and performance, but one study indicated that feeding excess dietary P may interfere with feed digestibility and metabolism at the tissue level (Minson, 1990). Carstairs (1981) concluded that cows fed 98% of dietary P requirements produced 1.8 kg/d more milk than cows fed 138% of dietary requirements.

Overfeeding P is often thought to improve reproductive performance. Eckles (1932) observed impaired reproductive performance in range cattle with dietary P concentrations at 0.20% P, well below requirements. Studies in England (Hignett and Hignett, 1951) and in the U.S. (Shupe et al., 1988) showed a decrease in first service conception rates when animals were fed low dietary P. Again, in these studies, dietary P was much lower than recommended current NRC levels, and microbial growth may have been impaired. To date there is no published research that shows that increasing dietary P above ~ 0.35% P will improve reproductive performance (Brodison et al., 1989; Braintrip et al., 1993; Wu et al., 2000).

Effect of overfeeding phosphorus on feed costs

By feeding less dietary P to cattle, producers stand to gain both ecologically and economically. Satter (2002) estimated that supplementation of P is costing dairy farmers and industry approximately $100 million per year. On a 1,000-cow dairy, Van Horn (1991) observed a reduction in manure P from 16 to 27% by reducing dietary P from 0.52 to 0.40%. This reduction in manure P lowered yearly feed costs to dairy farmers from $13,988 to $23,314.

According to several studies conducted throughout the U. S. (Wu et al., 2001), the average dietary P level for lactating dairy cows is 0.48%. Reducing dietary P from 0.48 to 0.38% would result in 23 g/cow/d less P fed, saving approximately $0.06/cow/d or $18/cow/yr in mineral supplement costs**.**

Environmental implications of manure P

Nutrient contamination of ground and surface water is a leading environmental concern facing farmers. Excess P in water leads to eutrophication and causes algae to grow quickly. The degradation of algae consumes dissolved oxygen in the water and decreases growth of aquatic life. The Chesapeake Bay watershed is one of the largest and most diverse in the country. The Chesapeake Bay provides use for recreation and tourism, and is an intensive site for agriculture. Animal agriculture is responsible for 25% of the P loading to the Chesapeake Bay watershed (Smith and Alexander, 2000).

Animal manure is a valuable resource to farmers, providing nutrients, improving soil structure, and increasing vegetative cover. If manure is applied in excess of crop needs, water contamination can occur. Land application of manure to meet N needs of crops results in over application of P and leads to an access accumulation in the soils, resulting in P run-off in surface water. Manure phosphate in the Shenandoah Valley in Virginia increased by 90% from 1978 to 1992 (Pease et al., 1990).

Increased public concern over and awareness in water quality, and the impact of animal production has led to the development of more stringent environmental regulations. Farmers are experiencing an increased level of regulatory pressure from the federal government. One key change in water quality regulations is the shift from a primary focus on N to an increased focus on P contamination on surface water. Nutrient management plans for all poultry farms in Virginia are now P-based. These regulations limit manure application to the P needs of the crop,

and require an increased amount of land to dispose of manure. Research conducted at Virginia Tech estimated that total farm income may be reduced by 11to 23% as a result of P-based management plans (Pease et al., 1990). Farmers must implement practices that reduce nutrient losses without decreasing profitability.

Bone Metabolism

Functions and properties of bone

Bone is a dynamic tissue influenced by physiological, nutritional, and physical factors, and it performs mechanical, biological , and chemical functions (Rath et al., 2000). Bone development, and chemical and physical properties are affected by age, nutrition, hormonal status, and disease (Williams et al., 1991). The skeletal system forms the sound external structure and appearance of most mammalian vertebrate species, and serves as a large storage area for minerals. The most abundant minerals in the mammalian skeletal structure are Ca and P. Obvious functions of bone include locomotion, structural support of the body , and protection of soft tissue including the brain, heart, spinal cord, and lungs. Bone also has less obvious functions, serving as a metabolic reservoir for Ca, P and other minerals, and houses cells responsible for bone formation and resorption.

All bone consists of mineral deposited in an organic matrix. The mineral crystal found in bone and teeth enamel is primarily hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). The length of a bone apatite crystal is approximately 50 to100 Å, and the size of these crystals will increase with age (Carter et al., 1999). In the molar teeth of 3-wk-old bull calves (Quint, et al., 1980), Mg and carbon dioxide (CO_2) content of bone was higher than the Ca and P content during pre and early stages of mineralization.

The fact that bone mineral is deposited in large numbers of small crystals gives bone a large surface area. The surface area of 1 g of bone mineral is estimated to be more than 100 m^2 (Omnell, 1957). This large surface area allows for a rapid interchange of ions between interstitial fluid and bone.

The inorganic matrix of bone provides compressional strength, and allows bone to keep its rigidity and hardness. Hydroxyapatite crystals bound to collagen fibers provide shear strength to bone, and provide approximately 60 to 65% of bone weight. Hydroxyapatite salts also make

bone resist the passage of x-rays (Omnell, 1957). Because the inorganic matrix is a major component of bone structure, bone mineral density (BMD) is considered to reflect the status of bone health (Rath et al., 2000), and low BMD is considered to be a risk factor for fractures.

Collagen

Collagen is the major constituent of the organic matrix. Collagens are insoluble extracellular glycoproteins found in all animals. Nearly 90% of the organic matrix is collagen; the chief collagen in bone is type I collagen. Intermolecular cross-linked collagens increase tensile strength, provide support to the mineralized matrix of bone, and help bone withstand physical stress (Knott et al., 1995; Eyre, 1996). Collagen provides bone with its flexibility, and holds the inorganic matrix in place (Riggs et al., 1993; Einhorn, 1996). Because collagen is the major organic constituent of bone, it is likely to affect the biochemical bone strength (Rath et al., 1999; 2000).

The most well known of the collagen cross-links are the pyridinium cross links, which are important in bone strength. Many bone strength and quality characteristics are related to the bone matrix where collagen molecules are arranged so they overlap and produce holes in collagen fiber. This process allows calcification and mineralization to occur (Turek, 1984). Together the inorganic and organic components of bone produce a material that is one-third the weight of cast iron but ten times as flexible, and has approximately equal tensile strength (Albright, 1987).

Types of bone

Bone is organized into two types that are intermixed within each bone: Cortical (compact, dense) and trabecular (cancellous, spongy) bone (Figure 1). Cortical bone is a hard layer of bone, forming the shaft of long bones (diaphysis), and outside surface of bone. The mid shaft of long bones is entirely cortical bone, while the distal metaphysis contains a ratio of 6:1 of cortical to trabecular bone (Adams, 1997). The primary structural unit of cortical bone is the osteon (haversian system). Cortical bone has a porosity ranging from 5 to 30%, and gives long bones their hardness, rigidity, and compressional strength (Carter and Spengler, 1978). Mechanical properties of cortical bone are greatly influenced by porosity, level of mineralization, and organization of the matrix.

Trabecular bone fills the epiphysis of long bones, and forms most of the structure of vertebral bones (Figure 1). The major difference between cortical and trabecular bone is the high

degree of porosity. Porosity is reflected by measurements of apparent density. This measurement is the mass of bone tissue divided by the bulk volume of tissue (Carter and Spengler, 1978).

The ultradistal (the furthest distance from the long axis of the body) site of bone contains 95% trabecular bone (Adams, 1997). Trabecular bone has porosity ranging from 30 to more than 90%, and has approximately twenty times more surface area per unit of volume than cortical bone (Adams, 1997). The makeup of trabecular bone gives it a spongy, mesh-like consistency designed for strength. The overall effect of the combination of cortical bone and trabecular bone is similar to steel rods within a concrete structure.

The structure of trabecular bone makes this bone less calcified compared with cortical bone, and it plays a larger role in metabolic functions, undergoing a continuous remodeling process (Seifert and Watkins, 1997). The large surface area of trabecular bone allows for a rapid rate of bone turnover (Albright, 1987). A study involving lactating ewes found that trabecular bone is more sensitive to resorption than the rest of the skeleton. Benzie (1955) found that compact bone resorbs only during mineral deficiency. Vertebral bones (made up primarily of trabecular bone) are the most sensitive to bone resorption, but are also the first to be repaired. In lactating ewes, trabecular bone lost 50% of its mineral, but mineral was restored shortly after the resorption process began (Benzie, et al., 1955). In contrast, Benzie (1959) found the diaphysis in the metacarpal lost 15% of total mineral but made no immediate recovery.

The medullary cavity (Figure 1) is the space surrounded by the cortex of the long bone shaft. At birth, the marrow is haematopoietic (red marrow), but as animals age, this marrow turns fatty and yellow. Bone marrow contains stem cells from which osteoblasts and osteoclasts originate. There is evidence that rate of bone turnover is related to marrow composition (Compston, 2002). Higher rates of turnover are found in trabecular bone in sites of haematopoietic marrow rather than in fatty marrow (Krempien, et al., 1978; Eventov, et al., 1991).

Classification of bone

Bones are classified as either long or short, flat or sesamoid bones. Long bones are greater in one dimension than any other, and are chiefly used for levers, which aid in support, locomotion, and prehension. The humerus, radius, ulna, metacarpals, and phalanges are examples of long bones. Long bones consist of a diaphysis (shaft) and the epiphysis (ends of the bone). The epiphysis contains a thin layer of cortical bone surrounding trabecular bone (Figure

1). The metaphysis lies between each epiphysis and diaphysis, and contains both cortical and trabecular bone. The inner surface of the bone is referred to as the endosteum; the outer surface the periosteum. A plate of cartilage, known as the epiphyseal growth plate, separates the metaphysis and epiphysis. The growth plate is the site of long bone growth and separates the diaphysis and epiphysis within the widest part (metaphysis) in an immature, growing bone. This is the only place where bone can grow and increase in length.

Short bones appear to be equal in all dimensions, and contain no marrow cavity, but consist mainly of spongy, trabecular bone, surrounded by a cortex of compact bone. They aid in absorbing concussions and are found in the complex joints of the carpus (knee) and tarsus (hock) where a lot of movement and shock absorption occur.

Flat bones are thin, expand in two directions, and provide protection to the vital organs. These bones have large amounts of trabecular bone (Rath et al., 2000) and may attach to muscle. Examples of flat bones include the skull, ribs, vertebrae, scapula, and mandible. In addition to protection, these bones aid in structural support and muscle attachment

Sesamoid bones are developed along tendons to help reduce friction (Cooper et al., 1998). This bone may change the angle a muscle pulls, allowing the muscle to have a greater mechanical advantage. The kneecap is the largest sesamoid bone in the human body (Frandson, 1986)**.**

Based on its structural organization, bone is either lamellar or woven. Lamellar (secondary) bone is a mature form of bone and can be made up of both cortical and trabecular bone. Lamellar bone is bound by cement lines. These cement lines are formed where osteoclastic activity stops and osteoblastic activity begins. These bones are very organized arrangements of collagen. In contrast, woven (primary) bone contains loosely packed and distributed collagen fibers, is lower in bone mineral density, and has higher water content (Turek, 1984; Gorski, 1998). Woven bone is found during embryonic development, fracture healing, and diseases (Recker, 1992). With increasing age, this bone is remodeled and replaced with cortical or trabecular bone.

A type of woven bone is medullary bone that develops on the endosteal side of long bones and has a high rate of remodeling. This type of bone was evident in poultry where bone remodeling provided Ca to meet the demands of eggshell formation (Dacke, et al., 1993). Although medullary bone lacks intrinsic strength, it can have a large influence on the mechanical

strength of cortical bone (Knott, et al., 1995). In comparison to cortical and trabecular bone, medullary bone is low in collagen but is high in mineral proteoglycans and carbohydrate content (Dacke, et al., 1993; Rath, et al., 1999). Medullary bone may be the only true type of metabolic bone in the animal kingdom (Loveridge, 1999).

As maturation of bone occurs, the area between the growth plates become calcified; the growth plate closes. This process ends longitudinal bone growth. Work in poultry (Orth, 1999) shows that proper long bone formation requires the growth plate to maintain a balance of bone degradation followed by formation.

Bone mineralization

Mineralization of bone involves the formation of hydroxyapatite crystals within an organic matrix (osteoid) in new, woven bone. In lamellar bone, mineral is deposited into tightly spaced collagen fibrils but in woven bone, matrix vesicles provide a site for hydroxyapatite crystal formation. When first synthesized, bone matrix requires a period of ten days before mineralization occurs (Loveridge, 1999). A layer of unmineralized bone matrix not calcified marks the site of active bone formation. Rate of mineralization is related to inhibitor molecules associated with calcified tissues (Compston, 2002). The ideal bone for assessing mineralization may vary with age, sex, and other biological and factors (Crenshaw, et al., 1981.).

In adult mammals, approximately 25% of the bone-wet weight is water, 45% is ash, and 35% is organic matter on a wet basis (Carter and Spengler, 1978). Calcium contributes 37% of the ash content and P, 18.5%. On a dry weight basis mineral content is 65-70% and inorganic matter is 30-35%.

Bone tissue contains a number of different cell types, including osteoblasts, osteocytes, and osteoclasts. These are responsible for matrix synthesis, bone mineralization, and resorption. These cells are the key to determining the bone chemistry, geometry, and strength (Rath,, 2000). Bone serves as a mineral reservoir and is continuously being turned over by the processes of bone resorption and bone formation.

Osteoblasts

Osteoblasts are cells responsible for bone formation. They secrete collagen, essential for bone matrix synthesis. Collagen strands form osteiods (new bone), which are the spiral fibrils of bone matrix. Osteoblasts regulate the mineralization process of hydroxyapatite crystals. They

cause Ca salts and phosphates to precipitate from the blood and bond with the newly formed osteoids to mineralize bone tissue.

Osteoblasts originate from *mesenchymal* cells and divide readily, however only a portion of these cells actually form true bone. The rest are held in reserve as layers of the periosteum or endosteum within the marrow cavity. The reserve cells function whenever more bone is needed in repairs of fractures, responses to stress or bone growth. When osteoblasts finish filling in bone cavities, the cells resemble a pancake-like structure lining the surface of the bone.

Resting osteoblast cells are known as bone lining cells or surface osteoclasts, and help regulate the passage of Ca in and out of the bone. Bone lining cells cover bone surfaces undergoing neither bone formation nor resorption. These cells are flatter than osteoblasts in appearance and are inactive (Wasserman, et al., 1993). Bone lining cells are precursors to osteoblasts, and may play a function in bone formation but can only be added on the surface because of the unyielding intercellular surface. Osteoblasts secrete the enzyme phosphatase needed to deposit Ca salts in osteoid tissue to form new bone. Alkaline phosphatase is a characteristic of the cartilage growth plate that becomes mineralized. Ostoblasts contain receptor sites for the Ca regulating hormones, parathyroid hormone (PTH) and 1,25 dihydroxyvitamin D (1,25 $(OH)_2$ D). Thus, osteoblastic cells control the maintenance of bone formation and bone turnover.

Osteocalcin (OC) is a non-collagenous protein found in bone. Depending on species and age (Fedarko, 2002), OC can comprise between 10-20% of the non-collagenous proteins in bone. Osteocalcin concentrations are normally low in early stages of bone development, increasing with age. Plasma OC concentrations were measured in fetal lambs killed between 80 and 145 days of gestation (Collignon, et al., 1996). Plasma concentrations of OC in the lambs increased from day 80 to day 132 of gestation. Naito (1990) observed that plasma OC concentrations decreased in dairy cows 3 days before calving, decreased rapidly one day before calving and reached the lowest point one day after calving. The authors concluded that decreased of OC around calving signify a decrease in bone mineralization.

Osteonectin is a Ca^{+2} binding glycoprotein found in connective tissue (Fedarko, 2002). Relatively high concentrations of osteonectin are present in bone, but additional research is needed to more fully understand the role osteonectin plays in bone formation.

Osteoclasts

Osteoclasts are multinucleated, large motile cells capable of resorbing both bone mineral and matrix (Wasserman, et al., 1993). When attached to bone, they are characterized by finger-like projections, extending into the bone's surface and releasing mineral from the bone. As bone is resorbed, the pH is lowered in the space between the osteoclast and bone surface through the secretion of hydrogen (H^+) ions. The precursors of osteoclasts are stem cells that originate in the bone marrow and spleen. Macrophages**,** phagocytic cells in the bone marrow, may be another precursor to osteoclasts.

Osteocytes

Osteocytes are cells inside the bone derived from osteoblasts, and have the capability of becoming either bone forming or bone resorbing cells. Osteocytes maintain bone and play a role in controlling extra-cellular concentrations of Ca and P.

Skeletal homeostasis

The balance between bone formation and resorption maintains skeletal homeostasis. Diet can influence bone resorption and formation. Bone loss occurs when resorption exceeds bone formation, when diets do not provide enough mineral to be deposited back into skeletal reserves. A classic example of bone imbalance due in part to diet is osteoporosis in humans. This chronic condition results in fractures and loss of bone mass. Shupe (1988) reported signs of osteoporosis in beef cattle fed low levels of P (6 to 12 g/d) for two years. Another disorder of improper bone homeostasis is osteomalacia. Osteomalacia is the failure of normal osteoclastic bone resorption. This disease results in dense bones and an over abundance of bone mass, but with poor mineralization of bone.

Hormonal influences on bone metabolism

Hormones affecting bone metabolism and homeostasis include parathyroid hormone (PTH), secreted by the parathyroid glands, and dihydroxyvitamin D_2, secreted by the kidneys. These act to stimulate bone resorption, but calcitonin, secreted by the thyroid gland, inhibits resorption (Horst et al., 1994). In horses, Day (1955) reported that growth hormone, a protein hormone produced by the anterior pituitary gland, may be a regulator of long bone growth by stimulating insulin-like growth factor-I, associating growth hormone with ostoblastic activity.

Changes in bone with age

Growth and maturation of bone is normally accompanied by an increase in bone density (Field, et al., 1974) with an early growth in bone length followed by a later growth in circumference, characterized by bone thickening and ossification (Mc Meekan, 1940). This was observed in swine, where in the early stages of bone development, growth in bone length predominated over growth in thickness and density (Mitchell, et al., 2001). Pigs weighing from 3 to 138 kg showed positive allometric growth, with bone mineral deposited faster than bone area is increased (Mitchell, et al., 2001). Increasing bone density with age results in a gradual dehydration of bone, and an increase in fat and mineral content (Field, et al., 1974).

As species age (Carter and Spengler, 1978) there is increased bone porosity at the endosteal surface of bone that gradually shifts to the periosteal surface. As cortical bone matures, there is a shift in distribution of bone strength. In younger people (Carter and Spengler, 1978), the strongest and stiffest cortical bone is at the bone cortex. As bone shifts and remodels with age, the periosteal surface of bone becomes the strongest and stiffest section. This gradual shift in bone strength toward the periosteal surface helps to maintain the structural strength of long bone (Carter and Spengler, 1978).

As animals mature, bone development causes more mineral to be deposited in weight bearing bones (Williams, et al., 1990). The metacarpal, ulna, and radius are examples of weight-bearing bones used for daily locomotion and activity. Optimal BMC produces the lightest bone contained in the smallest area to give adequate support for locomotion (Lawrence, 1986). Below this optimal level, bone is less organized in the matrix. Bone mineral content above optimal mineralization does not change the organization of the mineral in the matrix, but a greater amount of total bone will be deposited.

Growing animals are born with poorly mineralized bones. Currey (1959) reported that ash content of cortical bone (obtained via biopsy) increases with age in men and women. Field (1974) found ash content increases with age in dairy, swine, chickens, and turkeys (Table 4). Ash content increased with age in the metacarpal bone of growing swine (Combs, et al., 1991; Table 4). Management also affects BMC, as caged poultry of growing chicks had higher ash content of the tibia bone than floor poultry of the tibia bone (Wilson, et al., 2000; Table 4).

Changes in bone with stage of lactation

Bone resorption and formation occur at the same time, and net changes in bone mineral

content occur throughout stages of lactation. During early lactation, cows mobilize both Ca and P from their bone reserves to meet their needs, but replenish bone supplies in later lactation (Beighle, 1999). These authors concluded that bone P peaked between 180 and 230 days of gestation, followed by a decline to parturition. Cortical bone thickness decreased from beginning to end of gestation. Cortical bone P concentrations were higher in the dry period compared to the first 30 days of lactation (Beighle, 1999). Braithwaite (1976) reported increases in bone resorption at the beginning of lactation to accommodate increased Ca needs.

Phosphorus mobilized from bone may meet a significant portion of the cow's need for P during early lactation. Ternouth (1990) estimated that as much as 30% of bone P could be removed from bone. Based on this estimate from growing beef cattle, Satter (2002) calculated that dairy cows weighing 600 kg could mobilize 600 to 1000 g of P from bone. Knowlton and Herbein (2002) found that dairy cows may mobilize as much as 25-40g/d of P from bone in the first weeks of lactation, and that cows mobilize P to the same extent regardless of dietary P supply. This mobilization of P may meet 30-50% of the cow's total dietary need for P. These reserves need to be replenished, but Judkins (1985) and Shupe (1988) reported when lactation stress had been removed from beef cattle, bone P could be replenished during the dry period without any supplementation.

Calcium homeostasis and absorption

Calcium is required for physiologic functions including muscle contractions, blood clotting, and nerve transmission. Calcium is most noted for its function in bone formation, as nearly 98-99% of all Ca in the body is found in the skeleton and teeth. The other 1-2% of Ca is found in the extra cellular fluids within the body**.** According to the 2001 NRC, the concentration of intracellular Ca is 1/10,000 the extra-cellular concentration. Calcium acts as a second messenger on the interior of the cell and regulates the actions of many hormones, including PTH and 1,25-$(OH)_2$D.

Normal blood Ca concentrations fall in the range of 9-10 mg/dl in adult animals. Calcium homeostasis is important to minimize disorders that may occur in dairy cattle, particularly around parturition. Calcium exits the extra-cellular system via bone resorption, sweat, urine excretion, and milk secretion. Calcium can be mobilized from soft tissue as well as from bone (Matsui, et al., 1992).

Hormonal regulation maintains a balance of Ca leaving and entering the extra-cellular system. The loss of Ca through different routes leads to a decreased plasma Ca and stimulates the release of parathyroid hormone and vitamin D. These stimulate Ca absorption from the upper small intestine and Ca resorption from bone to raise blood Ca. When too much Ca leaves the system, hypocalcaemia occurs, more commonly known as milk fever. Common signs are a loss of nerve and muscle function and recumbancy. Hypercalcemia can occur when too much Ca enters the extra-cellular system, leading to soft tissue deposition of Ca.

Passive absorption of Ca is regulated through Ca concentration in the digesta, while active absorption is regulated by 1,25-dihydroxyvitamin D. Braithwaite (1979) reported that in sheep Ca absorption is constant when Ca intake is between 40 and 100 mg/d per kg body weight. Above 100 mg/d per kg body weight, Ca absorption increased in direct relation to intake.

The efficiency of Ca absorption depends on factors including age and physiological state. Young animals absorb Ca more efficiently as compared to older animals (Reinhardt, et al., 1988). The decline in Ca absorption with age is due to a decrease in the number of vitamin D receptors in the small intestine (Horst, et al., 1994). Several authors have found a relationship with hypocalcemia and age, either due to the cow's inability to mobilize bone (Marquardt, et al., 1977; Van de Braak and A. T. H. Kloester, 1987; Anderson, 1991), reduced DM intake (Marquardt, et al., 1977), or inefficient intestinal absorption (Hansard, et al., 1954; Anderson, 1991). Anderson (1991) found that in humans, the number of both bone forming and bone resorbing cells decrease with age. Reinhardt (1988) found older cows have lower bone Ca content than younger cows and concludes that hypocalcemia should be considered normal in older cows.

Cows producing large quantities of milk at parturition have high Ca requirements because of the Ca secreted into colostrum. Increased Ca demands at parturition are due to the increased demands of Ca on production (Kronfeld, 1971; Horst, 1986). These authors reported a cow producing 10 L of colostrum loses 23 g of Ca in a single milking. This amount is nearly nine times as much Ca present in the plasma pool. Cows at parturition have a "lag time" to achieve Ca homeostasis (Littledike, 1976; Goff, et al., 1986). These authors reported cows cannot react fast enough to high Ca demands. Milk fever most likely occurs 2-3 days post partum. Cows given high Ca diets in during lactation and pregnancy are less able to mobilize Ca from bone and are less able to increase intestinal absorption in response to need (Braithwaite, 1979).

Magnesium homeostasis

Like Ca, magnesium (Mg) is vital for normal nerve and muscle function and bone formation. Normal blood concentrations for Mg range between 1.8 and 2.4 mg/dl. In young animals Mg absorption occurs in the small intestine. In an adult ruminant however, Mg absorption occurs primarily in the rumen and to a lesser extent in the reticulum (Pfeffer, et al., 1970; Martens and Y. Rayssiguier, 1980; Martens and G. Gabel, 1986). Magnesium absorption is via a sodium (Na) linked active-transport system (Martens and G. Gabel, 1986) and is largely dependent on the solubility of Mg in the rumen. Magnesium solubility and availability declines as rumen pH rises above 6.5 (Miller, et al., 1972). Miller (1972) found Mg to be more available in concentrates than in forages because of the lower ruminal pH content common in high grain diets.

Adding Na to a ration otherwise deficient can increase transport of Mg across the rumen wall, but adding excessive Na will increase Mg excretion. High dietary potassium (K) can also interfere with Mg absorption (Newton, et al., 1972). Efficiency of Mg absorption can vary depending on feedstuffs, but absorption efficiency of Mg is normally between 20-30% (Forbes, 1916; Rook, 1958; Rook, 1962; National Research Council, 1980; Agriculture and Food Research Council, 1991; Henry, 1995;).

Potassium homeostasis

Potassium is the third most abundant mineral in the skeletal system (Underwood and Suttle, 1999) and the dietary requirement for K is the highest of the mineral cations. Little K is stored in the body, so supplementation in the diet is essential. Potassium's major roles in the body are acid base balance, water balance, osmotic pressure regulation, nerve transmission, and muscle contraction. Most K in blood is located within red blood cells, and the K concentration in saliva is less than 10 meq/L (Hemken, 1983; Aitken, 1976). Normal K concentrations in rumen fluid are 40 – 100 meq/L (Hemken, 1983). The main absorption site for K in ruminants is the rumen while some K absorption will occur in the large intestine (Underwood and Suttle, 1999). Hemken (1983) found a true absorption coefficient of 95% for most feeds. Thompson (1972) reported the need for K for milk production is greater for Ca or P because of the higher concentration in milk (1.5 g/l).

Non-invasive measures of bone mineral content

Radiographic photometry

Radiographic photometry (RP) is an inexpensive, non-invasive, and accurate method of estimating BMC in horses (Meakim, et al., 1981) and beef cattle (Williams, et al., 1991). This method can be used to evaluate sequential changes in BMC in the same animal over a period of time. Radiographic photometry has been used in horses (Meakim, et al., 1981; Porr, et al., 1998; Hoffman, et al., 1999) and beef (Williams, et al., 1991) to evaluate the effects of nutrition and conditioning on BMC in the metacarpal of growing animals.

Radiographic photometry utilizes a portable x-ray unit, x-ray film, scanning equipment, and imaging software to estimate BMC. When an x-ray is projected onto one side of an object, the intensity of the beam on the opposite side of the object is related to the thickness, density, and chemical composition of the object. X-rays are absorbed by aluminum (Al) to the same degree as bone mineral (Ekman, et al., 1970), allowing use of an aluminum step-wedge as a reference standard. Omnell (1957) found Al to be best suited for use as a standard because it most closely matches the atomic number of the substance (hydroxyapatite) being analyzed. Aluminum has an atomic number of 13 while hydroxyapatite has an atomic number of 16.5. The Al step wedge is exposed to radiographic cassettes simultaneously with the bone of interest (Figure 2; Meakim, et al., 1981).

An image analysis system transfers images of the radiographic film to an imaging system where a calibration curve is developed from the standard Al step wedge (Hoffman, et al., 1999). Logarithmic regressions are used to relate the thickness of the steps of the Al step wedge and percent transmittances. This relationship is used to determine BMC from the readings obtained from the radiograph (Figure 3). Bone mineral content estimated via RP is expressed in radiographic bone aluminum equivalents (RBAE) in millimeters of aluminum (mmAl; Meakim, et al., 1981). Bone mineral content as estimated by RP will be abbreviated BMC_{RP} in the remainder of this paper.

Absorption of x-rays by bone is affected by exposure time, voltage, screens, and film type. Use of the Al step wedge in each x-ray as a standard corrects for variation in these factors for most consistent results (Ekman, et al., 1970). Work by Lachman (1955) reported that without the use of a step wedge exposed simultaneously, sensitivity is decreased, and the smallest change in Ca loss that could be detected with RP is 30%.

The absorption of x-rays by bone is largely dependent on its mineral content. Bone contains approximately 85% hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). BMC_{RP} values from photometric scans of radiographs taken from the third metacarpal in equine are highly correlated to BMC (Meakim, et al., 1981; Hoffman, et al., 1999). Similar work in beef cattle (Williams, et al., 1991) showed RP to be useful in predicting BMC.

Using RP to estimate BMC_{RP} in horses, Hoffman (1999) found the greatest BMC to be on the medial side of the metacarpal. Stashek et al., (1987) reported that horses carry the largest portion of their weight on the medial side of their metacarpal during conditioning and training exercises. Significant variation in peak BMC_{RP} on the medial (peak medial RBAE) and lateral (peak lateral RBAE) side of the metacarpal has been observed in various studies with horses (Stashak, 1987; Hoffman, et al., 1999), likely because horses walk on their front legs differently depending on the season, weather conditions, and their daily maintenance, training and exercise programs. Hoffman (1999) observed that season affects BMC_{RP} of horse's front legs . In the winter months, horses may change their walking patterns on snow and ice, affecting BMC in their weight-bearing bones.

BMC_{RP} in the metacarpal increases as animals age to maturity, reflecting increased bone mineralization. Meakim (1981; 1999) found that BMC_{RBAE} is positively related to body weight, girth measurements, and age. Weanling foals had a lower BMC_{RP} for both the medial and lateral side of the metacarpal as compared to mature equine (20.7, 23.4 vs. 22.5, 23.7 mm Al; Table 1).

Dual energy X-ray absorptiometry

Dual energy x-ray absorptiometry (DXA) is a non-invasive method of bone densitometry that provides precise, quick, and accurate measures of bone mass, including BMC, BMD, and bone area (BA) with very low levels of radiation (Mazess, et al., 1990; Combs, et al., 1991; Lukaski, 1993; Adams, 1997; Sievanen, 2000; Koo, et al., 2001). DXA is considered the "gold standard" method to access the potential for bone fractures in humans (Mazess, et al., 1990; Ellis, 2000; Sievanen, 2000), and is the accepted standard for assessing osteoporosis intervention in humans. This method assesses regional and whole body BMD and BMC (Figure 4), and fat and fat-free soft tissue masses in vivo with very low precision error (<1%; Mazess, et al., 1990; Lukaski, 1993). Multiple measurements for skeletal BMC and BMD are accurate enough that a change of 15 g in skeletal Ca can be detected (Mazess, et al., 1990). This change corresponds to

a net Ca balance of approximately 100 mg/d over a 6-month period. In a typical total body projection, 40% of the body is bone and soft tissue while 60% contains soft tissue alone. Calcium hydroxyapatite is a constant fraction (37%) of the mineral component (Mazess, et al., 1990). DXA has been used extensively in human bone research, and some research has been conducted with DXA in swine (Mitchell, et al., 2001) and chickens (Mitchell, et al., 1997). Only limited work has been done using DXA in dairy cattle (Bascom, 2002). DXA has proven to be a good predictor of bone status and fractures in humans (Mazess, et al., 1990; Lukaski, 1993; Adams, 1997; Nelson and Koo, 1999; Sievanen, 2000; Koo, et al., 2001) but has limited use in animals because of its high cost and weight limit (<136 kg).

The first DXA unit became available in 1987. In a DXA unit **,** x-ray beams from two peak energies are produced to optimize separation of mineralized and soft tissue components of the analyzed area (Mazess, et al., 1990; Lukaski, 1993; Ellis, 2000). Attenuation of these x-rays through bone, lean tissue, and fat is different, and reflects the differences in densities and chemical composition. Measurements of relative intensities of the attenuating beams allow estimates of the bone mass and overlaying soft tissue to be calculated. Two separate sets of equations are used to describe a two-compartment model of body composition. Use of DXA yields estimated of three body composition values (BMC_{DXA}, lean tissue mass, and fat mass).

Bone mineral density as measured by DXA (BMD_{DXA}) is a measure of the increase in the BMC per unit of bone area (BA) where BA is the total area in the scanned image for all pixels containing bone. Bone mineral density does not reflect some of the chemical changes that may occur in bone (Mitchell et al., 2001). Bone mineral density is defined as the ratio of BMC in grams to BA, in sites including the spine, femur, and forearm (Lunt, et al., 1997). Bone mineral density is an area density, expressed as BMC/BA in g/cm^2, and is used to describe the bone mass per unit of projected bone area. Areal BMD is not true (apparent) volumetric bone density (g/cm^3). True bone density can be obtained using computed tomography. BMD_{DXA} is a non-linear function of apparent BMD (Sievanen, 2000).

BMD_{DXA} is considered to reflect the status of bone health, indicates the porosity in the bone, and is a reliable criterion for assessing mineral status of bone. One of the primary reasons for estimating BMD_{DXA} is to reduce the variation associated with BMC_{DXA} in varying ages in humans, and to increase ability to detect abnormal changes by correcting for differences in BA

(Ellis, 2000). BMD_{DXA} is a less accurate measure than BMC_{DXA}; however BMD_{DXA} compensates for the effect of patient size on bone density, and is considered a better indicator of bone fracture risk (Adams, 1997).

DXA has been used in small animals and is the most common method of measuring bone mass in human infants and small children (Nelson and Koo, 1999; Koo, 2000). DXA measurements of total BMC are highly correlated with total body ash content in pigs (Mitchell, et al., 1996; Table 2). Yang (1998) used DXA to measure BMC, BMD, and BA of swine vertebrae. Mitchell (2001) used DXA to measure changes in total body and regional BMC and BMD in pigs weighing between 3 and 138 kg in body weight. Van der Peere (1994) looked at parameters of bone structure using DXA and found a strong relationship between BMD_{DXA} and wall thickness of the diaphysis; BMC_{DXA} and cross sectional area of long bone; and BMC_{DXA} and effective area of trabecular bone. In growing children, Nelson and Koo (1999) found weight to be a better predictor of bone mass than age.

Serum and urinary markers of bone metabolism

Other potential indicators used in other species to measure rapid changes in bone turnover include various serum and urinary markers of bone resorption and formation. Bone is continuously turned over by the coupled processes of osteoclast-mediated bone resorption, and osteoblast-mediated bone formation. During resorption, osteoclasts dissolve bone mineral and they are involved in enzymatic digestion of collagen and other bone matrix proteins. During bone formation, osteoblasts function to influence bone mineralization thru synthesis of the organic matrix of bone. The end products and by-products of these processes have potential as non-invasive (or minimally invasive) measures of bone status.

Markers of bone resorption

Markers of bone resorption include urinary hydroxyproline (HYP), plasma tartrate-resistant acid phosphatase (TRAP), and urinary pyridinoline/deoxypyridinoline (Lester, 1995). Plasma and urinary HYP are the most common measures of bone resorption (Braithwaite, 1976), largely because the HYP assay is easy and inexpensive. However this marker is relatively insensitive and is non-specific, as it reflects breakdown of total collagen from all tissues (Lester, 1995; Liesegang, et al., 2000). When released into circulation, HYP breaks down to peptide bound HYP and free amino acids. These amino acids are degraded in the liver and only 10% of

the peptide bound form of HYP is found in urine. Its usefulness is also limited around calving, when HYP from uterine collagen is released (Naito, et al., 1990), masking changes in bone metabolism.

More sensitive markers of bone resorption may be hydroxylysylpiridinoline and deoxypyridinoline (DPD; Lester, 1995). The collagen cross links pyridinoline (Pyd) and DPD are formed during maturation of collagen fibrils. During bone resorption, these cross-links are released into circulation and excreted in free and peptide-bound forms in the urine. While Pyd can be found in the collagen of bone, cartilage, and many soft tissues, DPD is located primarily in type I bone collagen (Liesegang, et al., 2000). A strong correlation has been demonstrated between bone turnover and urinary excretion of these markers (Liesegang, et al., 1998). These markers are measured with HPLC or enzyme immunoassay.

Carboxyterminal telopeptide of type I collagen (ICPT) is another marker of bone resorption. Serum concentrations of ICPT reach a peak in dairy cattle 5 days after calving, while DPD concentrations increase in urine and reach a peak 9 days post partum (Liesegang, et al., 1998). Deoxypyridinoline appears to be a better marker of bone resorption than ICPT, as Sassi (2001) found that ICPT is not sensitive to physiological bone turnover. A protein antigen is destroyed during bone resorption by cathepsin K, a protein enzyme found in most cells. This process makes ICPT insensitive to physiological changes in bone turnover.

Markers of bone formation

Markers of bone formation include alakaline phosphatase (AP), procollagen extension peptides, and osteocalcin (OC, a.k.a. bone gla protein). Serum alkaline phosphatase activity assesses bone metabolism in periods of high turnover (Philipov, 1992). Total AP includes AP from bone, liver, and kidney, however, limiting the usefulness of the metabolite because of its origin in tissues other than bone. The development and refinement of assays for bone specific alkaline phosphatase may improve it's utility. Bone specific AP is an isoenzyme of AP found in bone, and is localized in the cell membrane of osteoblasts.

Serum collagen III amino-terminal propeptide (PIIINP) is another indicator of bone formation, released during synthesis of collagen III (Liesegang, et al., 1998). In ruminants, PIIINP is a relatively insensitive marker of changes in bone metabolism that occur post-calving and parturient paresis (Liesegang, et al., 1998).

Plasma OC may be the most promising measure of bone formation because it is a non-collagenous protein found only in mature bone (Lester, 1995). Osteocalcin is synthesized by osteoblasts and incorporated into bone matrix. A fraction of the newly synthesized OC is released into circulation and binds Ca to hydroxyapatite. It is expressed exclusively in bone (Naito, et al., 1990; Lester, 1995; Liesegang, et al., 2000). Osteocalcin is metabolized primarily by the kidneys and, to a lesser extent, the liver. Its production is dependent on 1,25 dihydroxycholicalciferal, and vitamins C, D, and K. Increases in both plasma OC and BSAP concentrations during the first two weeks after birth in lambs confirmed an increase in bone formation (Collignon, et al., 1996). In cows, plasma concentrations of OC decreased 3 days before calving, decreased rapidly one day before calving and reached a low point one day after calving (Naito, et al., 1990), indicating decreased rate of bone mineralization and formation in the pre-parturient period, due to increased levels of 1,25 (OH) 2 D and PTH after calving. Liesegang (1998) showed that cows had decreased plasma concentrations of OC 14 days before parturition, and return to prepartum concentrations one month after calving.

Invasive measures of bone mineral content

Bone breaking strength

BMC is highly correlated to breaking strength (BS), breaking load, and elasticity of bone, all of which may influence the resistance to injury. Breaking strength is assessed mechanically, yielding several specific measures. Breaking strength measurements include peak load, energy to peak load, and stress. Higher peak load and energy to peak load values are associated with larger bones, and more mineralized bones. In contrast, stress values are higher for smaller bones (i.e., vertebrae as compared to the metacarpal), as the fibrous bonds in bones hold together more strongly in smaller bones and take more stress to break.

Stress

Stress is defined as the force per unit area required to break bone, expressed in newtons/mm^2 (N/mm^2). Stress is the internal resistance of bone fibers to an externally applied load. This measurement allows the comparison between bones of different shapes and sizes. A more common term for stress is strength, and the terms are used interchangeably. Stress takes into account the force applied to the geometric shape of the area (Crenshaw, et al., 1981.). Bone's ultimate strength is related to its physical (shape, size, and mass), architectural (collagen

fiber arrangement), and material (matrix) properties. Bone's ability to withstand stress is related to the ultimate load at which bone will break (Nigg and Grimstone, 1994). Stress values increased with age (Table 3) in both growing pigs and poultry (Combs, et al., 1991; Rath, et al., 1999).

Cortical bone is both a viscoelastic and anisotropic material. Bone is a viscoelsatic (rate of deformation is dependent on properties of tested material) material because its elastic properties and strength of bone are dependent on both the rate and duration of applied load to bone. Bone is an anisotropic (having unlike properties in different directions) material since its elastic properties and strength are dependent on the orientation of bone structure in relation to the direction of loading. Cortical bone is stronger and stiffer in longitudinal direction than in transverse direction (Carter and Spengler, 1978).

The slope of the stress to strain curve (force-deformation curve; Figure 5) is known as Young's modulus of elasticity and reflects the intrinsic stiffness and material properties of bone. Modulus of elasticity is the resistance of a solid to a change in its length. A high modulus of elasticity refers to a bone that is more rigid, is stiff and hard to bend, where a low modulus refers to a bone that is less flexible. The elasticity of bone and its resistance to fracture is related to the degree of mineralization (Loveridge, 1999). The author concluded that increased stiffness and brittleness of bone follows the replacement of water by mineral content.

Strain denotes the percentage of deformity, and is higher in less mineralized bones. Load increases at a linear rate as increasing load is applied until a yield is reached . A constant increase of load on a bone results in elastic deformation, a point where bone is no longer resilient. The load required to reach this point is called the yield point (Turner and Burr, 1993; Figure 5) At yield point, bone enters a non-elastic (plastic) region where structural changes occur and permanent deformation results.

Energy at peak load

Energy at peak load, expressed in N-mm, is the amount of energy needed to break the bone, and is the total area under the force/deformation curve Energy lost at this point in the loading process is not recovered when the load is removed. At this point, bone reaches an ultimate load where bone fracture occurs (Figure 5). The tibia is the primary load bearing bone used for walking. Exercise results in a denser bone and requires more energy to break.

Energy at peak load was higher in the tibia than in the radius of caged poultry (Wilson, et al., 2000) and higher than the tibia of caged poultry than in fresh bone samples from growing hens (Wilson and Mason, 1992; Table 3). Energy at peak load values reported for the metacarpal of dairy cows (Wu, et al., 2001) was higher than reported for the tibia and radius bones of poultry (Wilson, et al., 1984; Table 3). Because of its size and shape, the metacarpal of a cow logically requires more energy to break than the smaller weight bearing bones in poultry.

Peak load

Peak load is the force, expressed in Newtons (N) at which any further increase results in failure (break) of the bone (Figure 5). It is a structural property affected by both bone size and quality (Lawrence, 1986).

Bone mineral content

Bone mineral content measured chemically and expressed as g/2 cm bone section ($BMC_{CHEMICAL}$, a.k.a. bone ash), is the true standard measure of bone mineralization, but as a terminal measurement, is not practical in research with humans or high value livestock. Higher $BMC_{CHEMICAL}$ were reported for the equine metacarpal (Porr, 1998) than was reported for growing beef animals (Williams, 1990; Table 4). Porr (1998) observed higher $BMC_{CHEMICAL}$ in the metacarpal of mature horses (age = 4 to 7 years) than were reported in the metacarpal of beef (Williams et al., 1990). Values reported in the equine study were $BMC_{CHEMICAL}$ at the diaphysis (bone from the long shaft), and did not include the medullary cavity, which tends to be less mineralized. The diaphysis is comprised primarily of densely packed layers of mineralized collagen and is a major component of cortical, compact bone. In contrast, the $BMC_{CHEMICAL}$ values reported in the study of Williams (1990) were based on the whole bone.

In mature animals, $BMC_{CHEMICAL}$ of bone was higher in the metacarpal of horses (Porr, 1998) and beef (Williams, 1990) than in the ribs of horses (Porr, 1998), beef (Little, 1972; (Williams, 1990; Beighle, 1993), ribs of dairy (Wu et al., 2001), tibia of poultry (Wilson, 2000), metacarpal of swine (Combs, 1991) and vertebrae of beef (Williams, 1990; Table 4). These latter bones contain more cancellous, spongy bone, which are not as mineralized as trabecular bone. $BMC_{CHEMICAL}$ content in all animals (Field et al., 1974) was higher in the femur compared to cervical and lumbar vertebrae, synsacrum and rib bones. (Table 4). Higher $BMC_{CHEMICAL}$ is observed in the femur because this is a weight bearing bone. Animals receiving diets with higher

P content tended to have higher $BMC_{CHEMICAL}$ than those fed diets deficient in P (Williams, et al., 1990; Table 4).

Effects of age on bone mineral density and bone mineral content

Measured a variety of ways, BMC increases with age, until maturity. Bone mineral content in the front leg of growing pigs and humans (measured by DXA) increases with age (Table 2). BMD_{DXA} in the vertebrae also increases with age, but at a decreasing rate (Mitchell et al., 2001). Mitchell et al. (2001) found that younger pigs less than 30 kg had high growth coefficients for BMD_{DXA} in the spine. In pigs greater than 30 kg, BMD_{DXA} increased in the back legs with increasing age but decreased with age in the front legs. Regional changes in BMD_{DXA} with age are related to changes in body weight and redistribution of body proportions (Lukaski, 1993; Mitchell et al., 2001). Gershon-Cohen (1955) found increasing bone density with increasing age in a study determining the degree of osteoporosis in hands of aged humans. Increases in BMD_{DXA} with age result from a gradual dehydration and an increase in fat and bone mineral content (Field et al., 1974). Younger animals will normally deposit a larger portion of mineral in the non-weight bearing bones (head, trunk, ribs, spine, and pelvis) and then will increase BMC in the major load bearing bones of the body (front and back legs) with age.

Increases in BMD_{DXA} are a function of increases in BMC_{DXA} both for total body and within regions (Mitchell et al., 2001). Lower BMC_{DXA} and BMD_{DXA} were reported for the spine in growing pigs and humans and the caudal vertebrae in mature dairy cows than were reported for the metacarpal and distal radius in humans (Sievanen, 2000; Table 2). The bones of the spine are more porous and play less of a role in load bearing activities; these bones don't have high amounts of mineral. Gershon-Cohen (1955) found that BMD_{DXA} of the human metatarsal increased with age. Higher BMD_{DXA} and BMC_{DXA} in men compared to women may suggest a difference in both bone size (the diameter of the diaphyisis) as well as hormonal differences (Sievanen, 2000).

Field (1974) found that increasing age increases total ash content ($BMC_{CHEMICAL}$) for all species, with the highest reported values in dairy animals between 48 and 96 mo (Table 4). Highest $BMC_{CHEMICAL}$ of cortical bone were found in horses between 4 and 7 yr (Porr, et al., 1998). Ash content in cortical bone was higher in humans (2-48 yr) than in ribs of beef fed a lower P content (Williams, 1990), ribs of dairy (Wu et al., 2001), all rib bones of beef and dairy

animals except the 9th dorsal rib (Beighle, 1993), tibia bone of caged and floor poultry (Wilson, 2000), metacarpal of swine aged 27 to 138 d (Combs, 1991), dairy heifers two to 24 months old (Field, 1974), swine six to 26 mo (Field, 1974), turkeys five to 13 mo (Field, 1974), the lumbar and cervical vertebrae ribs and femur of dairy and swine (Field, 1974), and the synsacrum, cervical vertebrae, ribs and femur of turkey (Field, 1974; Table 4). Field et al. (1974) reported that $BMC_{CHEMICAL}$ increased with age in all species (Table 4). Past maturity, however, bone becomes more porous and $BMC_{CHEMICAL}$ tends to decline as demonstrated in biopsies of cortical bones of men and women (Currey, 1959). In horses, $BMC_{CHEMICAL}$ is positively related to age and weight (Hoffman, et al., 1999).

Wilson (1984) found that both shear force and shear strength of the radius of otter legs increased with age, while Combs (1991) found that bone length and stress increased at a decreasing rate in pigs from weaning to market (Table 3). Strength of bones, measured by peak load, varies with age within species. In male and female otters at one year of age, the ulna, tibia and fibula had higher peak load values than the radius (Table 3). In five-year-old otters, however, peak load was higher in the radius than the ulna, tibia and fibula (Wilson et al., 1984). These changes may be because as animals age, different bones in the body are used for weight bearing activities.

Effect of lactation, diet and exercise on bone mineral content

Cows undergo a net loss of Ca and P from bone in early lactation, but this is reversed in late lactation. Ternouth (1990) suggested that as much as 30% of the bone mineral can be resorbed in beef cattle. Braithwaite (1983) found that ewes mobilize bone mineral stores in both late pregnancy and early lactation, regardless of dietary P supply. Reserves were replaced in mid to late lactation. In dairy cows, Beighle (1999) found that net bone resorption (measured via rib cortical bone biopsy) was occurring at both parturition and at the end of lactation. In the same study (Beighle, 1999), $BMC_{CHEMICAL}$ was highest in animals at 180 to 230 days of gestation (mid lactation) , and lower in animals in late lactation. Beyond 230 days, P content of bone was at its lowest value due to advancing pregnancy.

Diet and exercise are major factors affecting bone density and breaking strength. Deconditioning in horses leads to decreased $BMC_{CHEMICAL}$ by 0.45%, or 1.1g/cm^2 (Por et al.,

1998). Supplementing dietary Ca at twice the recommended levels did not prevent loss of mineral in response to deconditioning.

Williams (1989) found that chemical and physical properties of bone are sensitive to dietary P. Beef heifers fed a high P diet (0.20 % P) had increased bone density and bone mineralization as compared to cattle fed 0.12 % P (Table 4). Little (1972) found higher $BMC_{CHEMICAL}$ in yearling beef cattle fed a control (normal P) diet compared to cattle fed a diet containing 0.08 % P. In contrast, Erickson et al., (1999) fed different concentrations of dietary P (0.14, 0.19, 0.24, 0.29, and 0.34%) and Ca (0.35 and 0.70%) to crossbred steers and found no effects of dietary P on bone ash or breaking strength. Work completed by Wu (2000) found no effects on bone strength characteristics when dairy cows were fed diets with three different P contents (0.31, 0.39, and 0.47%). Ash and P content measured on wet weight, dry weight and wet bone volume were not different in bones from cows fed the 0.39 and 0.47% P diets, but values were lower in bones from cows fed the 0.31% P diet. In studies in which $BMC_{CHEMICAL}$ was not affected by diet, or was minimally affected, one must conclude that the low P diets may have been only borderline deficient (Wu, 2000) or may have met the animal's P needs (Erickson et al., 1999).

Dietary Mg content can affect BMC as well. In bone rib samples, van Mosel (1991) found that an inadequate dietary supply of Mg (0.22% Mg) resulted in higher Ca content in bone ash in low parity cows, but a higher percentage of bone ash in higher parity cows. Magnesium content of cortical rib bone (Beighle, 1999) was higher at parturition compared to other periods of lactation.

Calcium content of bone, and ratio of calcium to phosphorus

On average, bone ash contains 36% Ca and 17% P, giving approximate Ca to P ratio of 2:1 (Williams et al., 1991; Table 5 and 6), although some variation is observed with bone type and diet. The ratio of Ca to P was higher in the coocygeal vertebrae and rib bones compared to the metacarpal (Williams et al., 1991). In the same study, beef fed high P diets (0.20 % P) had a higher Ca to P ratio in the metacarpal, coccygeal vertebrae and ribs than cattle fed the lower P diet (0.12 % P). There was no seasonal effect on ratio of Ca to P in these beef cattle (Table 6). Horses had higher Ca to P ratio in the rib bone compared to the metacarpal (Cooper et al., 1998).

If Ca and P are not found in adequate amounts in the matrix, bone will not become fully mineralized (Shupe et al., 1988).

Calcium content of bone (Table 6) increased with age in all species (Field et al., 1974). In dairy cows, bone Ca content was higher in rib bones than in the lumbar or cervical vertebrae and femur bone. In swine, however, Ca content was higher in the femur bone than in vertebrae or rib bones. Sheep had a higher Ca content in the cervical vertebrae than in the lumbar vertebrae, rib, or femur (Field et al., 1974). Turkeys had a higher Ca content in the cervical vertebrae than in the lumbar vertebrae, rib or femur bones (Field et al., 1974). Chickens had a higher Ca content in the femur than in the lumbar and cervical vertebrae and rib bone (Field et al., 1974).

Summary

Overabundance of phosphorus (P) in surface water, primarily a result of runoff from land, causes accelerated algae growth. Degradation of this algae consumes dissolved oxygen, threatening fish and other aquatic life. Manure applied to crops, leaves N and P at an imbalance relative to crop needs. Nitrogen applied to land to meet crop needs results in an over accumulation of P in the soil. Because of these concerns, the focus of new environmental water quality regulations is now broadening from an exclusive spotlight on N to include P as well. Many states are now requiring P-based nutrient management plans. By reducing the P content of manure, farmers can more precisely define and meet the animal's dietary requirements. An improved understanding of P digestion and utilization by animals is needed to reduce P excretion.

Further reductions in dietary P may be achieved by accounting for the normal metabolism of bone in early lactation. Phosphorus mobilized from bone may meet a significant proportion of the cow's P requirements in early lactation, even though P mobilization through bone is not usually accounted for when establishing P requirements for lactation. The duration and extent of bone P stores in early lactation and the timing and extent of bone P is still not fully understood.

Increased understanding of factors affecting the P requirement of dairy cows (bone P resorption and feed P availability), and identification of non-invasive markers of P status of the animal are needed to increase adoption of these nutritional techniques. A field-level indicator of P status will allow fine-tuning of rations in the field through nutritional management on livestock farms and identify opportunities to reduce overfeeding of P on the farm.

Radiographic photometry is an inexpensive, non-invasive and accurate method of estimating BMC in horses (Meakim et al., 1981; Hoffman et al., 1999) and beef cattle (Williams et al., 1991). This method can be used to evaluate sequential changes in BMC in the same animal over a period of time, and may have potential as a non-invasive measure of P status in dairy cattle.

The objectives of this study were to evaluate RP assessment of BMC as a practical on-farm measure of P status in dairy cows by comparing this method to a more established imaging technique, mechanical measures of bone strength, and chemical measures of BMC. In addition, the effects of parity and stage of lactation on BMC in lactating cows were evaluated.

Table 1. Radiographic photometric measures of bone mineral content (BMC_{RP}) in various species.

Species	Bone type	Age	n	Peak medial RBAE, mmAl[1]	SE	Peak lateral RBAE, mmAl[2]	SE	Medullary low RBAE, mmAl[3]	Circular area index, mm^2	SE	Citation[4]
Equine	Metacarpal	Weanling	1	20.74	3.23	23.47	1.96	18.92			1
Equine Thoroughbreds	Metacarpal	Growing foals	56	20.00		22.40		18.40			1
Equine quarter horse	Metacarpal	Growing foals	88	20.80		22.00		18.90			1
Equine	Metacarpal	120.4 days	22	17.9		19.3		17.1			1
Equine	Metacarpal	177.1 days	20	18.5		20.1		17.3			1
Equine	Metacarpal	233.7 days	22	19.9		21.0		18.4			1

[1]Peak medial RBAE = Most mineralized part of the bone on the medial side, expressed in mm Al.

[2]Peak lateral RBAE = Most mineralized part of the bone on the lateral side, expressed in mm Al.

[3]Medullary Low = Least mineralized part of the bone, expressed in mm Al.

[4]Citations: 1 = Meakim et al., 1981.

Table 1. (cont.). Radiographic photometric measures of bone mineral content (BMC_{RP}) in various species.

Species	Bone type	Age	n	Peak medial RBAE, mmAl[1]	SE	Peak lateral RBAE, mmAl[2]	SE	Medullary low RBAE, mmAl[3]	Circular area index, mm^2	SE	Citation[4]
Equine	Metacarpal	297.7 days	20	21.6		23.3		20.8			1
Equine	Metacarpal	355.7 days	20	20.6		23.5		20.2			1
Equine	Metacarpal	412.8 days	21	21.6		24.3		21.3			1
Equine	Metacarpal	469.7 days	19	22.5		23.7		21.5			1
Beef (0.12% P DM)	Metacarpal	7 months to 2.5 yrs	14						932.00	24.40	2
Beef (0.20% P DM)	Metacarpal	7 months to 2.5 yrs	14						1,048.00	24.40	2

[1]Peak medial RBAE = Most mineralized part of the bone on the medial side, expressed in mm Al.

[2]Peak lateral RBAE = Most mineralized part of the bone on the lateral side, expressed in mm Al.

[3]Medullary Low = Least mineralized part of the bone, expressed in mm Al.

[4]Citations: 1 = Meakim et al., 1981; 2 = Williams et al., 1990.

Table 2. Dual energy x-ray absorptiometry estimates of bone mineral content (BMC_{DXA}) in various species.

Species	Age	n	Bone Type	BMC, g	SE	BMD, g/cm^2	SE	Citation[1]
Swine, 12 kg	Growing pigs	180	Front leg	57	3.1	0.6	0.0076	1
Swine, 33 kg	Growing pigs	127	Front leg	173	3.4	0.86	0.0063	1
Swine, 62 kg	Growing pigs	324	Front leg	364	3.5	1	0.0048	1
Swine, 92 kg	Growing pigs	239	Front leg	520	4.6	1.14	0.0051	1
Swine, 120 kg	Growing pigs	183	Front leg	629	6.5	1.21	0.0066	1
Swine, 12 kg	Growing pigs	180	Spine			0.60	0.0076	1
Swine, 33 kg	Growing pigs	127	Spine			0.84	0.0063	1
Swine, 62 kg	Growing pigs	324	Spine			1.06	0.0048	1
Swine, 92 kg	Growing pigs	239	Spine			1.11	0.0051	1
Swine, 120 kg	Growing pigs	183	Spine			1.12	0.0066	1
Swine	4-6 months	12	Thoracic vertebrae 1-4	4.73	0.27	0.46	0.20	2
Swine	4-6 months	12	Thoracic vertebrae 5-8	4.67	0.23	0.48	0.20	2
Swine	4-6 months	12	Thoracic vertebrae 9-12	5.73	0.27	0.50	0.14	2
Swine	4-6 months	15	Lumbar vertebrae 1-5	6.98	0.19	0.54	0.13	2

[1]Citations: 1 = Mitchell et al., 2001; 2 = Yang et al., 1998.

Table 2. (cont.). Dual energy x-ray absorptiometry estimates of bone mineral content (BMC_{DXA}) in various species.

Species	Age	n	Bone Type	BMC, g	SE	BMD, g/cm^2	SE	Citation[1]
Human	18-77 yrs	34	Distal radius			0.46	0.08	3
Men	Young, growing adults	6	Legs	1249	10.61	1.36	0.004	4
Women	Young, growing adults	6	Legs	862	6.94	1.15	0.004	4
Men	Young, growing adults	6	Spine	307	4.08	1.09	0.02	4
Women	Young, growing adults	6	Spine	267	4.08	1.02	0.01	4

[1]Citations: 3 = Sievanen, 2000; 4 = Mazess et al., 1990.

Table 3. Breaking strength of bone in various species.

Species	Age	n	Bone Type	Diameter, mm	SE	Thickness, mm	SE	Area,mm2	SE	Stress, N/mm^2	SE	Peak load, N	SE	Energy Peak Load, N-mm	SE	Citation[1]
Poultry, male	7 wks	7	Tibia	8.50	0.44					17.24	2.16					1
Poultry, female	7 wks	7	Tibia	7.49	0.34					19.31	3.92					1
Poultry, male	72 wks	7	Tibia	11.88	0.46					46.94	7.64					1
Poultry, female	72 wks	7	Tibia	9.46	0.18					73.99	8.13					1
Poultry (Caged, 25 mg/kg nickel)	Growing	40	Tibia	8.88	0.23	1.47	0.05	39.1	2.0	18.0	0.09	1,253	64	2309	224	2
Poultry Caged, control	Growing	40	Tibia	9.13	0.25	1.34	0.07	36.8	2.2	16.3	1.5	1,030	95	1662	211	2

[1]Citations: 1 = Rath et al., 1999; 2 = Wilson et al., 2000.

Table 3. (cont.) Breaking strength of bone in various species.

Species	Age	n	Bone Type	Diameter, mm	SE	Thickness, mm	SE	Area, mm^2	SE	Stress, N/mm^2	SE	Peak Load, N	SE	Energy Peak Load, N-mm	SE	Citation
Poultry Caged, 25 mg/kg nickel	Growing	40	Radius	3.25	0.07	0.64	0.03	6.19	0.35	24.5	1.7	260	24	243	44	2
Poultry Caged, 25 mg/kg nickel	Growing	40	Radius	3.24	0.06	0.67	0.04	6.38	0.39	19.4	1.3	210	20	120	31	2
Poultry, fresh samples	Growing hens	30	Tibia							20.9	1.5			378	0.03	2
Dairy, 0.39 %P	Multiparous	37	12^{th} rib			5.1	0.1			28.1	2.2			6,050	420	3
Dairy, 0.47 %P	Multiparous	37	12^{th} rib			5.2	0.1			27.5	2.2			6,500	420	3

[1]Citations: 2 = Wilson et al., 2000; 3 = Wu et al., 2001.

Table 3. (cont.) Breaking strength of bone in various species.

Species	Age	N	Bone type	Stress, N/mm^2	SE	Peak Load, N	SE	Citation[1]
Otter, male and female	1 yr	41	Radius	48.3	6.30	915	153	4
Otter, male and female	5 yr	41	Radius	92.1	21.80	2,082	343	4
Otter, male and female	1 yr	41	Ulna	89.4	4.80	1,545	122	4
Otter, male and female	5 yr	41	Ulna	97.8	5.60	2,041	223	4
Otter, male and female	1 yr	41	Tibia	38.2	3.30	1,021	114	4
Otter, male and female	5 yr	41	Tibia	33.9	7.60	1,432	384	4
Otter, male and female	1 yr	41	Fibula	135.1	10.00	958	95	4
Otter, male and female	5 yr	41	Fibula	132.7	6.60	1,058	139	4
Swine (Daily Ca and P intake 100%NRC)	27 days	45	Metacarpal	7.2	0.46			5
Swine (Daily Ca and P intake 100%NRC)	55 days	45	Metacarpal	8.17	0.45			5
Swine (Daily Ca and P intake 100%NRC)	83 days	45	Metacarpal	10.91	0.46			5
Swine (Daily Ca and P intake 100%NRC)	112 days	45	Metacarpal	13.42	0.45			5
Swine (Daily Ca and P intake 100%NRC)	138 days	45	Metacarpal	14.06	0.47			5

[1]Citations: 4 = Wilson et al., 1984; 5 = Combs et al., 1991.

Table 4. Total bone mineral content measured chemically ($BMC_{CHEMICAL}$) in various species.

Species	Age	n	Bone Type	BMC, g/2 cm	SE	Ash, % DM	SE	Citation[1]
Equine	1-20 yrs	20	Metacarpal			69.5	0.4	1
Equine	1-20 yrs	20	12^{th} rib			63	0.4	1
Equine	4-7 yrs	11	Metacarpal, diaphyseal cortical	20.47	0.4			2
Equine	4-7 yrs	11	Metacarpal, diaphyseal medial	18.75	0.72			2
Beef, 0.12 P% DM	7 months to 2.5 yrs	14	Metacarpal	11.20	0.33	67.2	0.2	3
Beef, 0.20 P% DM	7 months to 2.5 yrs	14	Metacarpal	12.60	0.33	68	0.2	3
Beef, 0.12 P% DM Nov 1983	7 months to 2.5 yrs	14	12^{th} rib			55.7	0.97	3
Beef, 0.20 P% DM May 1983	7 months to 2.5 yrs	14	12^{th} rib			61.9	0.97	3
Beef, 0.12 P% DM Nov 1984	7 months to 2.5 yrs	14	12^{th} rib			63.1	0.97	3
Beef, 0.20 P% DM May 1984	7 months to 2.5 yrs	14	12^{th} rib			63.8	0.97	3
Beef, 0.12 P% DM	7 months to 2.5 yrs	14	Coccygeal vertebra V			62.8	0.39	3
Beef, 0.20 P% DM	7 months to 2.5 yrs	14	Coccygeal vertebra V			63.8	0.39	3
Beef, 0.12 P% DM	7 months to 2.5 yrs	14	Coccygeal vertebra VI			63.4	0.28	3
Beef, 0.20 P% DM	7 months to 2.5 yrs	14	Coccygeal vertebra VI			63.8	0.28	3
Beef, 0.12 P% DM	7 months to 2.5 yrs	14	Coccygeal vertebra VII			62.8	0.37	3
Beef, 0.20 P% DM	7 months to 2.5 yrs	14	Coccygeal vertebraVII			63.8	0.37	3

[1]Citations: 1 = Cooper et al., 1998; 2 = Porr et al., 1998; 3 = Williams et al., 1990.

Table 4. (cont.) Total bone mineral content measured chemically ($BMC_{CHEMICAL}$) in various species.

Species	Age	n	Bone Type	Ash, % DM	SE	Citation
Beef, control	Yearlings	5	Rib	66.8	2.7	4
Beef, 0.08% P DM	Yearlings	5	Rib	61.8	1.5	4
Dairy and Beef	6 months to 3 yrs	45	9^{th} rib, Right.side	59.46	1.14	5
Dairy and Beef	6 months to 3 yrs	45	12^{th} rib, Right.side	58.41	1.14	5
Dairy and Beef	6 months to 3 yrs	45	9^{th} rib, left.side	59.3	1.14	5
Dairy and Beef	6 months to 3 yrs	45	12^{th} rib, left.side	58.33	1.14	5
Dairy and Beef	6 months to 3 yrs	45	9^{th} rib, dorsal	60.35	1.14	5
Dairy and Beef	6 months to 3 yrs	45	9^{th} rib, ventral	57.66	1.14	5
Dairy and Beef	6 months to 3 yrs	45	9^{th} rib, ventral	57.66	1.14	5
Poultry-cage	Growing chicks	40	Tibia	40.1	0.8	6
Poultry-floor	Growing chicks	40	Tibia	36.40	0.07	6

[1]Citations: 3 = Williams et al., 1990; 4 = Little, 1972; 5 = Beighle et al., 1993; 6 = Wilson et al., 2000.

Table 4. (cont.) Total bone mineral content measured chemically ($BMC_{CHEMICAL}$) in various species

Species	Age	n	Bone Type	Ash, % DM	SE	Citation
Female	2 yrs	4	Cortical bone biopsy	59.98	0.52	7
Male	2.5 yrs	4	Cortical bone biopsy	60.57	1.05	7
Male	3 yrs	4	Cortical bone biopsy	62.12	0.17	7
Male	3.5 yrs	8	Cortical bone biopsy	61.04	0.33	7
Female	4 yrs	10	Cortical bone biopsy	61.55	0.14	7
Male	6 yrs	5	Cortical bone biopsy	63.08	0.42	7
Female	8 yrs	11	Cortical bone biopsy	63.20	0.29	7
Male	13 yrs	7	Cortical bone biopsy	63.02	0.25	7
Male	14 yrs	6	Cortical bone biopsy	62.61	0.20	7
Male	16 yrs	9	Cortical bone biopsy	61.35	0.41	7
Male	17 yrs	10	Cortical bone biopsy	65.02	0.37	7
Male	26 yrs	12	Cortical bone biopsy	63.70	0.38	7
Male	28 yrs	6	Cortical bone biopsy	65.18	0.43	7
Male	32 yrs	8	Cortical bone biopsy	64.79	0.24	7
Male	39 yrs	6	Cortical bone biopsy	64.52	0.36	7
Male	44 yrs	7	Cortical bone biopsy	65.98	0.16	7
Female	46 yrs	6	Cortical bone biopsy	64.60	0.20	7
Male	48 yrs	6	Cortical bone biopsy	64.58	0.38	7

[1]Citations: 7 = Currey et al., 1959.

Table 4. (cont.) Total bone mineral content measured chemically ($BMC_{CHEMICAL}$) in various species.

Species	Age	n	Bone Type	Ash, % DM	SE	Citation[1]
Swine (Daily Ca and P intake 100%NRC)	27 days	45	Metacarpal	51.0	0.70	8
Swine (Daily Ca and P intake 100%NRC)	55 days	45	Metacarpal	55.6	0.70	8
Swine (Daily Ca and P intake 100%NRC)	83 days	45	Metacarpal	57.4	0.70	8
Swine (Daily Ca and P intake 100%NRC)	112 days	45	Metacarpal	57.7	0.70	8
Swine (Daily Ca and P intake 100%NRC)	138 days	45	Metacarpal	57.8	0.70	8
Dairy	2-3 Months	4		49.80	0.88	9
Dairy	12-24 Months	4		56.27	0.72	9
Dairy	48-96 Months	4		63.93	0.88	9
Dairy		4	Lumbar vertebrae	55.18	0.96	9
Dairy		4	Cervical vertebrae	53.36	0.96	9
Dairy		4	Rib	58.94	0.96	9
Dairy		4	Femur	59.20	0.96	9

[1]Citations: 8 = Combs et al., 1991; 9 = Field et al., 1974.

Table 4. (cont.) Total bone mineral content measured chemically ($BMC_{CHEMICAL}$) in various species.

Species	Age	n	Bone Type	Ash, % DM	SE	Citation[1]
Swine	6-8 months	4		52.53	0.59	9
Swine	24-48 months	4		57.25	0.59	9
Swine		4	Lumbar	53.93	0.84	9
Swine		4	Cervical	52.92	0.84	9
Swine		4	Rib	54.90	0.84	9
Swine		4	Femur	57.84	0.84	9

[1]Citations: 9 = Field et al., 1974.

Table 4. (cont.) Total bone mineral content measured chemically ($BMC_{CHEMICAL}$) in various species.

Species	Age	n	Bone Type	Ash, % DM	SE	Citation
Turkeys	5-6 months	4		54.47	0.64	9
Turkeys	12-13 months	4		55.36	0.64	9
Turkeys		4	Synsacrum	54.31	0.91	9
Turkeys		4	Cervical	55.99	0.91	9
Turkeys		4	Ribs	50.24	0.91	9
Turkeys		4	Femur	59.11	0.91	9
Dairy, 0.31 %P	Multiparous	37	12^{th} rib	53.9	0.8	10
Dairy, 0.39 %P	Multiparous	37	12^{th} rib	56.2	0.8	10
Dairy, 0.41 %P	Multiparous	37	12^{th} rib	55.6	0.8	10

[1]Citations: 9 = Field et al., 1974; 10 = Wu and Satter, 2001.

Table 5. Calcium and phosphorus content of bone in various species.

Species	Age	n	Bone type	Ca, % Ash	SE	P, % Ash	SE	Ca:P	Citation
Equine	1-20 yrs		Metacarpal	42.4	1.09	14.7	0.12	2.88	1
Equine	1-20 yrs		12th rib	42	1.09	13.1	0.12	3.21	1
Beef, 0.12 P% DM	7-8 months	14	Metacarpal	37.2	0.23	16.3	0.14	2.28	2
Beef, 0.20 P% DM	7-8 months	14	Metacarpal	37.1	0.23	16.4	0.14	2.26	2
Beef, 0.12 P% DM	7-8 months	14	Coccygeal vertebra V	38.1	0.3	14.7	0.3	2.59	2
Beef, 0.20 P% DM	7-8 months	14	Coccygeal vertebra V	37.1	0.3	15.3	0.3	2.42	2
Beef, 0.12 P% DM	7-8 months	14	Coccygeal vertebra VI	38.2	0.44	14.9	0.25	2.56	2
Beef, 0.20 P% DM	7-8 months	14	Coccygeal vertebra VI	37.2	0.44	15.4	0.25	2.42	2
Beef, 0.12 P% DM	7-8 months	14	Coccygeal vertebra VII	38.1	0.45	15.3	0.23	2.49	2
Beef, 0.20 P% DM	7-8 months	14	Coccygeal vertebra VII	36.8	0.45	15.5	0.23	2.37	2
Beef, 0.12 P% DM	7-8 months	14	12th rib	42.9	0.93	16.5	0.33	2.6	2
Beef, 0.20 P% DM May 1983	7-8 months	14	12th rib	42.4	0.93	16.6	0.33	2.55	2
Beef, 0.12 P% DM Nov 1984	7-8 months	14	12th rib	42.5	0.93	16.8	0.33	2.53	2
Beef, 0.20 P% DM May 1984	7-8 months	14	12th rib	42.3	0.93	17.5	0.33	2.42	2

[1]Citations: 1 = Cooper et al., 1998; 2 = Williams et al., 1990.

Table 5. (cont.). Calcium and phosphorus content of bone.

Species	Age	n	Bone type	SE	SE	P, % Ash	SE	P, % DM	SE	Citation
Beef, pasture Dec. 1969	7-8 months	15	Rib					12.4	0.20	3
Beef, pasture March 1970	7-8 months	15	Rib					11.3	0.20	3
Beef, pasture Sept. 1970	Yearlings	15	Rib					11	0.20	3
Beef, pasture Dec. 1970	Yearlings	15	Rib					13	0.20	3
Beef, before 0.08 %P	Yearlings	5	Rib	24.5	0.5			11.5	0.50	4
Beef, after 0.08 %P	Yearlings	5	Rib	23.8	1.70			11.1	0.40	4
Dairy, 0.31 %P	Multiparous	37	12th rib			17.7	0.3	9.5	0.20	5
Dairy, 0.39 %P	Multiparous	37	12th rib			17.3	0.3	9.7	0.20	5
Dairy, 0.41 P %DM	Multiparous	37	12th rib			17.9	0.3	9.9	0.20	5

[1]Citations: 3 = Cohen, 1973; 4 = Little, 1972; 5 = Wu et al., 2001.

Table 6. Bone calcium content in various species[1].

Species	Age	n	Bone type	Ca, % Ash	SE
Dairy	2-3 months	4		36.93	0.37
Dairy	12-24 months	4		36.92	0.30
Dairy	48-96 months	4		37.34	0.37
Dairy		4	Lumbar	37.00	0.40
Dairy		4	Cervical vertebrae	36.45	0.40
Dairy		4	Rib	37.52	0.40
Dairy		4	Femur	37.28	0.40
Swine	6-8 months	4		36.72	0.33
Swine	24-48 months	4		38.63	0.33
Swine		4	Lumbar	37.41	0.46
Swine		4	Cervical vertebrae	37.61	0.46
Swine		4	Rib	37.25	0.46
Swine		4	Femur	38.44	0.46
Sheep	5-6 months	4		37.62	0.34
Sheep	10-12 months	4		37.56	0.34
Sheep	48-96 months	4		38.44	0.34
Sheep		4	Lumbar	37.80	0.40
Sheep		4	Cervical vertebrae	38.17	0.40
Sheep		4	Rib	37.55	0.40
Sheep		4	Femur	37.97	0.40

[1]adapted from Field et al., 1974.

Table 6. (cont.). Bone calcium content in different species[1].

Species	Age	n	Bone type	Ca, % Ash	SE
Chickens	2-3 months	4		37.25	0.66
Chickens	12-13 months	4		38.44	0.66
Chickens		4	Synsacrum	37.22	0.93
Chickens		4	Cervical vertebrae	37.91	0.93
Chickens		4	Rib	37.16	0.93
Chickens		4	Femur	39.08	0.93
Turkeys	5-6 months	4		37.52	0.30
Turkeys	12-13 months	4		38.57	0.30
Turkeys		4	Synsacrum	38.01	0.43
Turkeys		4	Cervical vertebrae	38.20	0.43
Turkeys		4	Rib	37.90	0.43
Turkeys		4	Femur	38.08	0.43

[1]adapted from Field et al., 1974.

CHAPTER 2: MATERIALS AND METHODS

Sample collection

Caudal vertebrae and right front metacarpals (sample pairs) from Holstein cows (n = 107) were excised and collected at slaughter (Taylor Packing, Wyalusing, PA) and stored frozen. Information on parity, age, and DIM was obtained for donor animals for 43 sample pairs. Within this subset, sample pairs were grouped by DIM, age, and parity.

Dual energy X-ray absorptiometry

Samples were thawed and BMC and BMD were measured on each metacarpal and caudal vertebra 14 and 15 using DXA (QDR 4500 A: Hologic, Inc., Bedford, MA, USA) with the standard lumbar spine (L1-L4) and forearm protocols (Beiseigel and Nickols-Richardson, 2002). Caudal vertebrae and metacarpals were placed prone on the table, and anterior-posterior scans were performed. Bone mineral density, g/cm^2 and BMC, g were estimated for caudal vertebrae 14 and 15 and the upper 1/3 and mid section of the metacarpal.

Radiographic photometry

Dorsopalmer and anterior-posterior radiographs were taken of the right metacarpal and of caudal vertebrae in dairy cows using a high frequency portable x-ray unit (MinXRay HF-80, MinXRay, Northbrook, IL.). Radiographs of the metacarpal were taken (80 KV,0.06 s, 66-cm focal distance) on Kodak TMGRA radiographic film in a Kodak Lenex regular screen (24 cm x 30 cm). Radiographs of caudal vertebrae were taken (75 KV, 0.04 s, 66-cm focal distance) on Kodak TMGRA radiographic film in a Kodak Lenex regular screen (18 cm x 43 cm). An 11-step aluminum step wedge was taped to each screen and exposed simultaneously in each radiograph as a reference standard. Film was developed at the Virginia-Maryland Regional College of Veterinary Medicine using an automatic processor (Kodak RP X-Omat processor, Model M-6).

Bone mineral content was estimated using an Epson Expression 1680 scanner that transferred images of the films to imaging software (Image-Pro Plus for windows, version 4.5, Media Cybernetics, Silver Springs, MD). For each film, a calibration curve was developed using the 11-step standard aluminum step wedge. In the metacarpal, a location 10 cm from the nutrient foramen was evaluated. Radiographic bone aluminum equivalents (RBAE) were recorded as

outlined in Table 8. For caudal vertebrae, measurements were taken as for the metacarpal and a measurement of bone length was obtained as well.

Table 7. **Definition of BMC_{RP} terminology**.

Term	Definition
Medial	BMC of the very outermost part of the bone on the medial side, expressed in RBAE (mm Al)
Lateral	BMC of the very most outside part of the bone on the lateral side expressed in mm Al
X1	Bone width measurement on outside of bone, mm
X2	Bone width measurement on outside of bone, mm
Area	Area of bone, mm^2
Peak medial	BMC at the most mineralized part of the bone on the medial side expressed in mm Al
Peak lateral	BMC at the most mineralized part of the bone on the lateral side expressed in mm Al
Medullary low	BMC at the least mineralized part of the bone expressed in mm Al
Inside X1	Bone width measurement on inside of bone, mm
Inside X2	Bone width measurement on inside of bone, mm
Inside medial	BMC of the most inside part of the bone on the medial side expressed in mm Al
Inside lateral Y2	BMC of the most inside part of the bone on the lateral side expressed in mm Al
Inside area	Area on the inside of bone, mm^2
Bone width	X2 – X1, mm
Medullary width	Inside X2 – inside X1, mm
Cortical width	Bone width – medullary width, mm

Bone breaking strength

Caudal vertebrae and metacarpals were thawed and manually de-fleshed with a surgical scalpel. All soft tissue including fibrous periosteum was removed; care was taken not to scratch the surface of the bones. Samples were wrapped in moist gauze to prevent desiccation.

Caudal vertebrae bone dimensions were measured at the center of the diaphysis. Exterior bone diameter was determined by rotating the bone, measuring the largest and smallest bone diameters, then averaging the values. An average wall thickness was derived from two measurements after bone fracture. All dimensions were measured with digital calipers with a precision of +/- 0.01 mm.

Mechanical tests on caudal vertebrae were conducted on a MTS universal testing machine at a loading rate of 5 mm/min. Shear tests were performed on the 14^{th} and 15^{th} caudal vertebrae using a double block shear apparatus following ASAE standard testing procedures

(Wilson, 1984; ASAE Standards 1998). The shear force was exerted over a 12.7 mm section at the center of the diaphysis. Results from the breaking strength included peak force (N), stress (N/mm^2) and energy at peak load (N-mm). The software Test Works for Windows was used in conjunction with the MTS machine for data acquisition and analysis.

For the metacarpal samples, bone dimensions and shear force were measured at a distance 10 cm from the nutrient foramen. Exterior bone diameter was determined by rotating the bone, measuring the largest and smallest diameters, then averaging the values. Shear tests were performed using a double block apparatus on an Instron model 4206, 30,000-lb capacity screw driven universal testing machine at a loading rate of 5 mm/min using ASAE standard testing procedures (ASAE, 1998). Data acquisition was via a PC communicating with National Instruments Lab View software and a GPIB interface. Results from the breaking strength tests on metacarpal samples included peak force (N), stress (N/mm^2) and energy at peak load (N-mm).

Following fracture, a band saw was used to cut the metacarpal bone into 1 cm cross sections on either side of the 10 cm marking with a precision of +/- 0.01 mm. Average wall thickness was derived from four measurements of the cross sections with digital calipers. Caudal vertebrae and metacarpal cross sections were identified, placed in plastic bags and stored frozen.

Bone ash

After testing was completed, caudal vertebrae and metacarpal sections were analyzed for ash content (a.k.a. $BMC_{CHEMICAL}$). Samples were dried to a constant weight at 100°C and then ashed in a muffle furnace at 600°C for 12 hr. (AOAC standards, 1990). Bone ash was expressed as grams per 2-cm section and defined as $BMC_{CHEMICAL}$.

Statistical analysis

All samples for which all analyses were completed (n = 78 pair of caudal vertebrae and metacarpals) were used to calculate the relationship between various measures of BMC. The sample set was incomplete because 29 metacarpal samples were collected in a way that prevented identification of the chosen reference point for measurement (10 cm from the nutrient foramen). The nutrient foramen is an important landmark for several of the analyses.

Regression analysis was conducted using the simple regression model in PROC REG in

SAS (version 8.0).

$$Y_i = \beta_0 + \beta_1 X_{ij} + e_{ij}$$

Where

Y_i = Response for the dependent variable

X_{ij} = Independent variable

β_0 = Intercept estimate

β_1 = Slope estimate

e_{ij} = Residual error, assumed to be normally distributed

The dependent variables included all measures of $BMC_{CHEMICAL}$, BMC_{DXA}, BMD_{DXA} and breaking strength. The independent variables were all measures of BMC_{RP}, expressed in mm Al, BMC_{DXA}**,** and $BMC_{CHEMICAL.}$ In addition, the relationship between measures of breaking strength measures of $BMC_{CHEMICAL}$ were assessed.

Independent variable	**Dependent variable**
BMC_{RP} (i.e. Peak medial RBAE, Peak lateral RBAE, medullary low RBAE, etc)	$BMC_{CHEMICAL}$ (i.e. % ash, Ca as a % ash, Ca as a % DM, etc)
	BMC_{DXA} (i.e.BMCCV14, BMDCV14, etc)
BMC_{DXA} (i.e.BMCCV14, BMDCV14, etc)	$BMC_{CHEMICAL}$ (i.e. % ash, Ca as a % ash, Ca as a % DM, etc), Breaking strength (i.e. peak load, stress, energy to peak load)
$BMC_{CHEMICAL}$ (i.e. % ash, Ca as a % ash, Ca as a % DM, etc)	Breaking strength (i.e. peak load, stress, energy to peak load)

All sample pairs for which all analyses were completed (n = 78 pair of caudal vertebrae and metacarpals) were used to evaluate the effect of the site of measurement on various measures of BMC. Data was analyzed using PROC Mixed in SAS (version 8.0) with the following model:

$$Y_{ij} = u + \alpha_i + b_j + e_{ij}$$

Where

Y_{ij} = Response of dependent variable

u = Mean

α_i = Fixed effect of site (metacarpal, caudal vertebrae 14 and 15)

b_j = Random effect of cow (n=78)

e_{ij} = Residual error (Site by cow interaction)

Using pre-planned contrasts, values for the metacarpal were compared to values for the caudal vertebrae and values for caudal vertebrae 14 were compared to values for caudal vertebrae 15.

All samples for which parity and stage of lactation were available (n = 43 sample pairs of caudal vertebrae and metacarpals) were used to evaluate the effects of parity and stage of lactation on measures of BMC. Sample sets were grouped by stage of lactation, where Stage 1 = < 90 DIM, Stage 2 = > 90and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250 DIM.

The effect of stage of lactation was analyzed using Proc GLM of SAS (version 8.0) with the following model:

$$Y_i = u + \alpha_i + e_{ij}$$

Where

Y_i = Response (All measures of BMC)

u = Mean

α_i = Fixed effect of stage of lactation, based on DIM and

e_{ij} = Residual error, assumed to be normally distributed.

Sample sets were grouped by parity (1, 2, 3, 4+). The effect of parity was analyzed using Proc GLM of SAS (version 8.0) with the following model:

$$Y_i = u + \alpha_i + e_{ij}$$

Where

Y_i= Response (All measures of BMC)

u = Mean

α_i = Fixed effect of parity of animal (i = 1-4 lactations)

e_{ij} = Residual error, assumed to be normally distributed.

Linear and quadratic effects of stage of lactation and parity were assessed using pre-planned contrasts. In this study, metacarpal and caudal vertebrae samples were grouped by stage of lactation and parity in order to get results that were biologically meaningful, and to obtain a relatively consistent number of cows in stratification of stage of lactation and parity. It should be noted that the sample set was not ideally suited to an evaluation of the effects of stage of lactation and parity. Repeated measures within the same animal over time would be desirable for this analysis, but it is not possible to obtain all measures (invasive and non-invasive) repeatedly in live animals. . A regression of all data on DIM (linear, quadratic and cubic), was also evaluated. Results were not significant, likely due to unequal distribution of data, and are not presented.

For all statistical analyses, effects were declared significant at $P < 0.05$ and trends at $P < 0.10$. Data are presented as least square means.

CHAPTER 3: RESULTS AND DISCUSSION

Measures of bone mineral content

Radiographic photometry

Interspecies comparisons

In the metacarpal of dairy cows, peak lateral RBAE was somewhat numerically lower than reported for horses (Meakim et al., 1981; Table 1and Table 8). This difference may be due to differences in how horses walk on their front legs or to differences in exercise activity between the species. In both species, the metacarpal is a weight-bearing bone, used for locomotion and support, daily maintenance activities (i.e., milking and heat detection) and exercise. Hoffman (1999) found that the greatest BMC_{RP} is on the medial side of the bone in horse. Stashek, (1987) reported that horses carry the largest portion of their weight on the medial side of their metacarpal during conditioning and training exercises.

Differences between values observed for horses and dairy cattle may be because most dairy cattle do not go through rigorous training programs and are not exposed to snow and ice conditions as are horses. Diet and seasonal changes alter the way horses walk on their front legs (Hoffman, et al., 1999) because in the winter months, horses change the weight-bearing load of the metacarpal on snow and ice. Horses may walk and exert a different force of impact on their front legs depending on the season, weather conditions and their daily maintenance, training and exercise programs. Porr (1998) reported diet and exercise in horses are two major factors affecting BMC. During deconditioning, BMC decreased by 0.45% or 1.1 g/cm^2 per week.

Circular area index

The circular area index (CAI, mm^2) of the metacarpal observed in this study (Table 8) was similar to values observed in beef cattle (Williams et al., 1991), but was less variable. Cortical area index is calculated from bone and medullary diameters (CAI, mm^2 = bone diameter $(B)^2$ – medullary diameter $(b)^2$). If bovine cortical bone is uniformly mineralized, a linear relationship should exist between the CAI of the metacarpal and its BMC (Lawrence et al., 1986). In this study, there was no relationship between CAI and BMC (Figure 6). This may be explained by the lack of variation in BMC observed in the sample set used in this study (see discussion in section titled “Relationships between measures of BMC in dairy cows”).

Effect of site of measurement

Lower RBAE is observed in caudal vertebrae than in metacarpal because of differences in bone composition (Figure 1). Caudal vertebrae are comprised of more trabecular bone than cortical bone. Trabecular bone is less calcified and more porous than cortical bone (Seifert and Watkins, 1997) and has up to twenty times more surface area per unit of volume than cortical bone (Buckwalter et al., 1996). Trabecular bone plays a larger role in metabolic functions. There was a significant difference between the metacarpal and caudal vertebrae 14 and 15 on all radiographic photometry measurements (Table 9). A significant difference between the metacarpal and caudal vertebrae 14 and 15 was observed for cortical width, bone width, peak medial and peak lateral (Table 9). There are no other studies that provide RBAE data for caudal vertebrae.

The medullary cavity was consistently the low point in BMC in this study and in studies with other species (Table 1 and Table 8). The medullary cavity had lower values because the mineral content of the marrow is low (Meema et al., 1964).

Dual energy x-ray absorptiometry

Interspecies comparison

Bone mineral density as estimated by DXA was higher in the metacarpal of dairy cattle than reported for the front leg of pigs (Mitchell et al., 2001), legs and spine of men and women (Mazess et al., 1990) and distal radius of humans of varying ages (Sievanen, 2000; Table 2 and Table 10).

In caudal vertebrae 14 and 15 of dairy cattle, BMD_{DXA} was lower than reported for front legs of swine (Mitchell et al., 2001) and legs and spine of men and women (Mazess et al., 1990; Table 2). Bone mineral density was higher in caudal vertebrae 14 than caudal vertebrae 15 in dairy cattle, although BMD_{DXA} of caudal vertebrae 15 was similar to that reported for the distal radius of humans (Sievanen, 2000).

Effect of site of measurement

A significant difference between the metacarpal and caudal vertebrae 14 and 15 was observed for BMC_{DXA} and BMD_{DXA}, but there were no significant difference between caudal vertebrae 14 and 15 (Table 9). Because of its physical size and function, the metacarpal is higher in BMD compared to other bones within the body. We have no data on body weight of the donor

animals, but mature dairy cattle could range from 450 to 680 kg. The weight and force exerted on the metacarpal by dairy cows to support daily maintenance activities results in more mineral deposited into the load-bearing bones than in non-load-bearing bones.

Gender, diet and exercise all influence BMC_{DXA} (Mitchell et al., 2001). All of our donor animals were female, but we have no information on their previous diet or exercise. Mazess (1990) reported higher BMC_{DXA} in the legs and spine of men compared to women, which may suggest a difference in hormonal regulation of the metacarpal diaphyisis. Various hormones released in the body affect bone resorption. For instance, increased levels of PTH increase bone resorption (Harris et al., 1993). As resorption takes place, mineral is being pulled from the bone, reducing BMC.

Bone breaking strength

Interspecies comparisons - Stress

Stress is defined as the force per unit area, expressed in newton/mm^2 (N/mm^2). Stress is the internal resistance of bone fibers to an externally applied load and can be related to the physical and material properties of bone. Stress allows for comparison of bones of different shapes and sizes and accounts the force applied to the geometric area (Crenshaw et al., 1981.)

In the metacarpal of dairy cattle (Table 11), stress was lower than reported for the tibia of poultry (Rath et al., 1999), poultry on a nickel containing diet (Wilson et al., 2000), radius, ulna, tibia and fibula of otter (Wilson et al., 1984) and rib of dairy cattle (Wu et al., 2001). Stress of the metacarpal in dairy cattle was higher than reported for the metacarpal of swine (Combs, et al., 1991), tibia of caged poultry (Wilson, et al., 2000) and radius of poultry (Wilson et al., 2000; Table 3 and Table 11).

In caudal vertebrae 14 and 15 in dairy cattle (Table 11), stress was higher than reported for the metacarpal of swine (Combs et al., 1991), tibia of 7-wk-old poultry (Rath et al., 1999); tibia and radius of poultry (Wilson et al., 2000), tibia of hens (Wilson and Mason, 1992) and metacarpal of dairy cattle (Wu et al., 2001). Stress of caudal vertebrae 14 and 15 of dairy cattle was lower than the tibia of 72-wk-old poultry (Rath et al., 1999) and radius, tibia, ulna and fibula of otter (Wilson et al., 1984; Table 3 and Table 11).

Interspecies comparisons - Energy at peak load

Energy at peak load, expressed in N-mm, is the amount of energy to break the bone and is the total area under the force/deformation curve to the point of fracture. In the metacarpal of dairy cows, energy to peak load was higher than reported for the radius and tibia of poultry (Wilson et al., 2000), tibia of hens (Wilson and Mason, 1992) and ribs of dairy cows (Wu et al., 2001; Table 3 and Table 11). The metacarpal, because of its size and shape, requires a greater amount of energy to break compared to the vertebrae, rib, tibia, ulna and radius of growing animals (Field et al., 1974).

In caudal vertebrae 14 and 15 of dairy cattle, energy to peak load was higher than in the tibia and radius of poultry (Wilson and Mason, 1992; Wilson et al., 2000), but lower than in the rib of dairy cattle reported by Wu (2001; Table 3 and Table 11).

Interspecies comparisons - Peak load

Peak load is the force, expressed in newtons (N), at which any further increase in force results in failure (break) of the bone. Peak load is a structural test of the bone and is affected by both size and quality of the bone (Lawrence, 1986). In the metacarpal and caudal vertebrae 14 and 15 of dairy cows, peak load was higher than the radius, ulna, tibia and fibula of otters reported by Wilson (1984) and tibia and radius of poultry reported by Wilson (2000; Table 3 and Table 11).

Load increases linearly until it reaches yield, then reaches peak followed by a decrease in load (Wigderowitz, et al., 2000). An increase in breaking strength and breaking load up to a point of maximum mineralization reflects the increased mineralization in the bone matrix and the total amount of bone present. Above a point of optimal mineralization, the organization in the matrix is not changed but a greater amount of bone is deposited (Williams et al., 1991). The higher peak load for caudal vertebrae and metacarpal samples indicate that there was more mineral deposited in the bone matrix of the metacarpal and caudal vertebrae of dairy cattle in this study than in other studies.

During daily activities of an animal, different bones are exposed to a variety of forces (walking, standing to be mounted while in estrous, standing and lying daily). Williams (1991) concluded that the ability of bone to withstand constant loading without breaking plays an important role in fatigue and stress fractures. Bone size and cortical thickness are two factors

contributing to bone breaking strength.

The shear test used in this study is considered a more desirable test of bone strength than the bending (flexure) test. The shear test is independent of length, is less sensitive to the orientation of the bone, shows less variability and is easier to calculate compared to the bending test (Crenshaw et al., 1981.; Wilson et al., 1984). Mechanical breaking strength tests have been performed in many different species using the three point bending test (Combs et al., 1991; Williams et al., 1991; Rath et al., 1999).

Effect of site of measurement

There was a significant difference on breaking strength measurements between the metacarpal and the caudal vertebrae (Table 9). In dairy cattle, the stress required to break the metacarpal was less compared with the caudal vertebrae (Table 9). As discussed earlier, strong fibrous bonds increase the stress required to break smaller bones compared with larger bones, but peak load and energy to peak load are generally lower for smaller bones. Because of its overall size, shape and mass, the metacarpal can withstand a greater load before breaking than smaller bones of the caudal vertebrae, tibia, radius, ulna and fibula. Because of their smaller bone structure, more stress is needed to break the tibia and radius in poultry (Rath et al., 1999; Wilson et al., 2000) than the metacarpal and caudal vertebrae of dairy cows.

A significant difference in breaking strength measurements (diameter, peak load and energy to peak load) was also observed between caudal vertebrae 14 and 15. A trend for a significant difference between the two caudal vertebrae was observed for stress ($P < 0.07$). Less stress was required to break caudal vertebrae 14, the larger bone with greater diameter, greater peak load, and greater energy to peak load. The size and shape of caudal vertebrae decrease rapidly in the caudal direction until the last few caudal vertebrae are small rods of bone (Frandson, 1986).

Total bone mineral content (bone ash or $BMC_{CHEMICAL}$)

Interspecies comparison

In the metacarpal of dairy cattle $BMC_{CHEMICAL}$ was lower than for values for horses reported by Porr (1998; Table 4 and Table 12) and Hoffman (1999), but was slightly higher than for beef cattle reported by Williams (1990). Bone mineral content in horses (Porr et al., 1998) may have been higher than observed in this study because of sample location (Table 4). Samples

for $BMC_{CHEMICAL}$ in these horses were taken at the cortical and medial bone diaphysis, comprised primarily of densely packed layers of mineralized bone. Bone mineral content data in the horse study did not include the medullary cavity, which tends to be less mineralized.

Hoffman (1999) reported that diet and season impacted BMC in horses. Bone mineral content was higher during spring and summer than winter . Bone mineral content was higher in horses fed a corn grain and molasses supplement compared with horses fed a corn oil and fiber supplement (Hoffman et al., 1999). Dietary P may also impact BMC. In beef cattle, higher dietary P increased BMC (Table 4; Williams, et al., 1990).

In the metacarpal of dairy cattle, ash content was similar to the ash content of rib biopsies of dairy and beef animals reported by Beighle (1993); rib biopsies of dairy cattle fed diets of 0.39% P and 0.41% P reported by Wu et al., (2001), rib bones and coccygeal vertebrae of beef reported by Williams (1990), rib biopsies of beef fed 0.08% P as reported by Little (1972), metacarpals of swine 83 days to 138 days old reported by Combs (1991), and rib bones of dairy; femur of dairy, swine and turkeys (Field et al., 1974; Table 4). Ash content of the metacarpal in dairy cattle was higher than in swine 27 to 55 days old (Combs, et al., 1991), higher than the tibia bone of caged and floor poultry reported by Wilson (2000), dairy calves 2 to 3 months old and dairy heifers 12 to 24 months old (Field et al., 1974) rib of swine, swine 6 to 8 months old, turkeys 5 to 6 months old (Field et al., 1974) and turkeys 12 to 13 months old (Field et al., 1974), lumbar and cervical vertebrae of dairy, (Field et al., 1974) swine (Field et al., 1974) and turkey (Field et al., 1974) and synsacrum of turkey (Field et al., 1974), and rib biopsies of dairy cattle fed 0.31% P (Wu et al., 2001; Table 4 and Table 12). Ash content of the metacarpal of dairy cattle in this study was lower than dairy cattle 48 to 96 months old reported by Field (1974), metacarpal and rib biopsies of horses reported by Cooper (1998), metacarpal of beef (Williams, et al., 1990), and rib biopsies of beef fed a control diet reported by Little (1972; Table 4 and Table 12).

Ash content of caudal vertebrae 14 and 15 of dairy cattle was lower than in rib biopsies and metacarpal of horses (Cooper, et al., 1998), ribs and coccygeal vertebrae of beef (Williams et al., 1990), rib biopsies of beef (Little, 1972), rib biopsies of dairy and beef (Beighle et al., 1993), tibia of poultry (Wilson et al., 2000), metacarpal of growing swine (Combs et al., 1991), various ages and bones of dairy, swine and turkeys, (Field et al., 1974), and rib biopsies of dairy cattle

(Wu et al., 2001; Table 4 and Table 12).

Caudal vertebrae in this study had lower ash content than the coccygeal vertebrae of beef cattle reported by Williams (1990; Table 4 and Table 12). Generally, the term "coccygeal vertebrae" is used to refer to a subset of the caudal vertebrae. The number of caudal vertebrae in dairy and beef cattle may vary from 18 to 20 bones (Frandson, 1986). Caudal vertebrae 14 and 15 are located toward the caudal end of the tail, are visibly smaller and less mineralized as compared to coccygeal vertebrae 5, 6, and 7. Coccygeal vertebrae are located at the top of the tail head, are visibly larger in size, and are heavier and more mineralized.

Effect of site of measurement

In caudal vertebrae 14 and 15, ash content of bone was lower than in the metacarpal of dairy cattle (Table 9). One possible explanation for lower BMC in the caudal vertebrae compared with the metacarpal is the composition of bone. Caudal vertebrae are classified as flat bones. They are comprised primarily of trabecular bone and contain less mineral than metacarpals. Trabecular bone is resorbed faster than cortical bone. The surface area to volume ratio of trabecular bone is higher than cortical bone and makes this bone more sensitive to bone turnover (Currey, 1959).

Within this study, $BMC_{CHEMICAL}$ of caudal vertebrae 14 was similar to caudal vertebrae 15. There are no other studies with comparable data on $BMC_{CHEMICAL}$ for different caudal vertebrae within different species. Compared with other bones in the body, caudal vertebrae tend to be more sensitive to changes in mineralization and to undergo a higher rate of bone resorption (Benzie, et al., 1955).

Calcium and phosphorus content of bone

Interspecies comparison

In the metacarpal of dairy cattle, Ca as a percent of the total bone ash was higher in the metacarpal and coccygeal vertebrae than for beef reported by Williams (1990; Table 5 and Table 13). Calcium as a percent of DM in the metacarpal of dairy cattle was similar to that reported for rib biopsies in beef cattle (Little, 1972), but was lower than reported in the metacarpal and rib bone biopsies of horses (Cooper et al., 1998) and rib bones in beef cattle (Williams et al., 1990). Calcium content of bones in the metacarpal in this study was higher than values reported for dairy, swine, sheep, chickens and turkeys of different ages and different bone types as reported

by Field (1974).

In the metacarpal of dairy cattle, P as percent of total bone ash was higher than in biopsies of rib bones and metacarpal of horses (Cooper et al., 1998; Table 5 and Table 13), metacarpal, coccygeal vertebrae and ribs bones of beef (Williams et al., 1990) and rib bones biopsies of dairy (Wu et al., 2001). Phosphorus content as a percent of DM for the metacarpal in dairy cows was higher than rib biopsies of dairy cattle (Wu et al., 2001), but lower than rib biopsies of beef (Little, 1972; Cohen, 1973a). Differences between these studies may have been due to differences in diet, age, and bone type.

In caudal vertebrae 14 and 15 of dairy cattle, Ca expressed as a percentage of ash was higher than in the metacarpal and coccygeal vertebrae of beef cattle (Williams et al., 1990; Table 6 and Table 13) and was higher than for dairy, swine, sheep, turkey, and chickens of varying ages and bone types (Field et al., 1974; Table 7 and Table 13). In caudal vertebrae 14, Ca as a percentage of ash was similar to the femur in chickens as reported by Field (1974; Table 7 and Table 13). Calcium as percentage of DM in caudal vertebrae 14 and 15 in dairy cattle was lower than reported for rib bone biopsies of beef cattle (Little, 1972; Table 6 and Table 13).

In caudal vertebrae 14 and 15 of dairy cattle, P as a percentage of total ash was similar to rib bone biopsies of dairy cattle (Wu et al., 2001), higher than biopsies of metacarpal and rib bones of horses (Cooper et al., 1998), metacarpal and coccygeal vertebrae of beef (Williams et al., 1990; Table 5 and Table 13). Phosphorus expressed as a percentage of total ash of dairy cattle in this study was higher than beef fed diets of 0.12 and 0.20% P in May and November of 1983 and November of 1984, but was lower than beef fed 0.20% P DM in May of 1984 (Williams, et al., 1990). Phosphorus content as a percent of DM was lower for rib biopsies of beef (Little, 1972; Cohen, 1973 a.) and rib bones biopsies of dairy (Wu et al., 2001).

In the metacarpal and caudal vertebrae 14 and 15, the Ca:P ratio was lower than that reported for horses (Cooper et al., 1998) and beef (Williams et al., 1990; Table 6 and Table 13). On average, bone ash typically contains 36% Ca and 17% P, giving an approximate Ca to P ratio of 2:1. For this study an average value of 2.21:1 was observed (Table 13). One possible explanation for a higher Ca:P ratio in our study is the origin of bone samples and timing of sampling. Because donor animals in this study were obtained from a slaughterhouse, they may have had health problems. Animals sent to slaughter right after calving may have been depleted

of Ca or P due to metabolic disorders (i.e., milk fever). This depletion leads to an imbalance of minerals in the body. Donor animals for our samples were slaughtered and then researched, while just the opposite was true for the animals used in the beef study.

Time of sampling may also have an effect on mineral content and Ca:P ratio. Samples taken of horses by Cooper (1998) were biopsy samples of the metacarpal and ribs of live animals. Beighle (1994) found biopsy samples from live animals have significantly more mineral (Ca, P and Mg) content than those obtained from slaughter cattle. An osteolytic membrane system allows for rapid Ca and P resorption (Guyton, 1991). A long film process connects with osteocytes throughout the bone structure to surface osteocytes. This system provides a membrane that separates bone from extra-cellular fluid. This osteolytic membrane pumps Ca ions from bone fluid to extra-cellular fluid This results in lower bone Ca concentration compared to extra-cellular fluid. Bone Ca concentrations then fall to lower concentrations than original after slaughter. As a result, bone salts are resorbed from bone. The osteolytic membrane may have caused a rapid rate of Ca and P out of the bone of donor animals in this study at the time of slaughter. Beighle (1994) concluded biopsy samples and samples taken from slaughtered and dead animals should not be compared.

Effect of site of measurement

A significant difference was observed between the metacarpal and caudal vertebrae 14 and 15 for Ca as a percent of ash and Ca as a percent DM (Table 9). Ca as a percentage of ash was higher in caudal vertebrae 14 than in caudal vertebrae 15, though no difference was observed between these bones for Ca as a percentage of DM.

Phosphorus content of bone, as a percent of ash and as a percentage of DM, was higher in the metacarpal than in the caudal vertebrae (Table 9). Phosphorus as a percentage of ash was lower in caudal vertebrae 14 than in the smaller caudal vertebrae 15, although no significant difference was observed for P as a percentage of DM.

Relationships between measures of BMC in dairy cattle

$BMC_{CHEMICAL}$ and BMC_{RP}

A weaker relationship was observed between $BMC_{CHEMICAL}$ and BMC_{RP} (Table 14, Table 15, Table 16, and Figure 7), and between measures of breaking strength and BMC_{RP} in the

metacarpal and caudal vertebrae 14 and 15 for dairy cows (Table 17 and Table 18) than was reported in the metacarpal of horses (Meakim et al., 1981; Lawrence 1986; Hoffman et al., 1999; Hoffman et al., 1999) and beef (Williams et al., 1990). BMC_{RP} appears not to be sensitive enough to detect small changes that occur in $BMC_{CHEMICAL}$ in mature cows. In this study there was one observation per cow. Changes were from one cow to the next. Lachman (1955) reported that a 30% loss of Ca was needed to visually see changes in BMC_{RP} in an x-ray if a step-wedge is not simultaneously exposed. In the current study, a step wedge was simultaneously exposed with the bone samples.

Work using RP to predict BMC (Meakim et al., 1981; Lawrence 1986; Hoffman et al., 1999) was based primarily on growing animals. Bone mineral content of the sample set in the current study was less variable than often observed (Meakim et al., 1981; Hoffman et al., 1999), likely due to the maturity of the donor animals (age range of 3 to10 years). Dairy cattle in this study may have reached a maximum $BMC_{CHEMICAL}$ and did not have the variation in $BMC_{CHEMICAL}$ seen in growing animals. Radiographic photometry may not be sensitive enough to detect small differences in $BMC_{CHEMICAL}$.

$BMC_{CHEMICAL}$ and BMC_{DXA}

In the metacarpal and caudal vertebrae 14 and 15 in dairy cattle, a weaker relationship was observed between $BMC_{CHEMICAL}$ and BMC_{DXA} (Table 19, Table 20, Figure 13) than was reported for sheep (Pouilles et al., 2000) and pigs (Mitchell et al., 1998). Similar to these results, though, Bascom (2002) found a poor relationship between $BMC_{CHEMICAL}$ and BMC_{DXA} in Jersey calves.

The relationship between $BMC_{CHEMICAL}$ and BMC_{DXA} may be influenced by state of hydration of the bones and soft tissue thickness (Mazess et al., 1990). Skin and tissue thickness were not measured in this study. It is possible that skin and soft tissue in dairy cattle in this study may be thicker than in the animals used in other studies, explaining the weak relationship between $BMC_{CHEMICAL}$ and BMC_{DXA}. Mitchell (1998) concluded that variation in $BMC_{CHEMICAL}$ and BMC_{DXA} could also be due to animals in a certain weight range, make of the DXA instrument, and software version. Similarly, a weak relationship was reported for BMC_{DXA} and breaking strength in the metacarpal and caudal vertebrae 14 and 15 in dairy cattle (Table 21 and Figure 10).

$BMC_{CHEMICAL}$ and breaking strength

In the metacarpal and caudal vertebrae 14 and 15 of dairy cows, a weaker relationship was observed between breaking strength and $BMC_{CHEMICAL}$ for all measures of breaking strength (Table 22, Table 23, Figure 11) than was reported for the metacarpal bone in beef cattle (Williams et al., 1991), and metacarpal bone in horses (El Shorafa et al., 1979). Breaking strength analysis in beef cattle and horses were performed using a three point bending test while breaking strength tests in the present study were performed using the shear test.

Optimal bone mineralization occurs when bone strength is maximized. Below this level, the mineral matrix is less organized. As the degree of calcification (mineralization) increases, both bone stress and force increase (Williams et al., 1991). Vose (1959) suggested a relationship should exist between stress and bone ash although Wilson (1984) and Kornegay (1981) have shown experimentally this does not exist.

Williams (1991) reported that as ash content reaches 63 to 68 %, bone matrix organization produces maximum strength. Increasing mineralization density increases bone's ability to absorb impact energy (Loveridge, 1999). Microfractures can travel more easily through bone that is highly mineralized (Loveridge, 1999), and cortical bone above 60 % ash content has increased risk of fracture. Below this point, hydroxyapatite crystals may disrupt the collagen molecules when a load is applied. A higher Young's modulus of elasticity implies that the greater ratio of bone mineral to protein in the bone matrix makes a bone stiffer under normal stress. Mineralization beyond an optimum value may decrease the resiliency of a bone and reduce its ability to resist dynamic loading.

Effect of stage of lactation on measures of BMC in dairy cattle

Stage of lactation and $BMC_{CHEMICAL}$

No significant effect of stage of lactation was observed on any measure of $BMC_{CHEMICAL}$ in the metacarpal or caudal vertebrae 14 and 15 in dairy cows (Tables 24, Table 25, and Table 26). There was a tendency for a quadratic effect of stage of lactation on Mg as a percentage of total ash ($P < 0.07$) in caudal vertebrae 15 with highest Mg quantities in stages 1 and 4.

Braithwaite (1983) found sheep mobilized bone the most in early lactation and late pregnancy, but replenished bone stores in mid lactation as Ca demands for lactation were met.

Similar changes in bone mineral were not observed in this study, possibly because of the differences in the experimental unit. Rather than obtaining repeated measures in live animals over time, this study measured $BMC_{CHEMICAL}$ from a set of samples obtained at slaughter. This sample set was not ideally suited for measuring changes in mineral content with time.

Stage of lactation and BMC_{RP}

The poor relationships observed between $BMC_{CHEMICAL}$ and BMC_{RP} make interpreting the effects of stage of lactation on BMC_{RP} problematic at best, as the biological meaning of BMC_{RP} measures is limited. Figure 3 and Table 7 provide a diagram and definitions of x-ray terminology used to interpret RP data from the metacarpal and caudal vertebrae 14 and 15 in dairy cattle. There was a significant effect of stage of lactation on BMC_{RP} of the metacarpal in dairy cows (X1, X2, peak lateral, inside X1, inside X2), and tendency for an effect of stage of lactation on cortical width ($P < 0.08$; Table 24). A linear effect of stage of lactation was observed in the metacarpal for X1, X2, inside X1 and inside X2 with highest RBAE measurements observed in stage 3 (151 to 250 DIM), however highest peak lateral RBAE was observed for stage 4 (>250 DIM). Stage of lactation tended to have a linear effect on peak medial ($P < 0.08$) with highest RBAE in stage 3. Stage of lactation tended to have a linear effect on medullary low ($P < 0.08$), and area ($P < 0.07$) with highest RBAE recorded in late lactation (>250 DIM).

There was no significant effect of stage of lactation on BMC_{RP} of caudal vertebrae 14 in dairy cows, but there tended to be an effect of stage of lactation on medullary low ($P < 0.06$; Table 28). A quadratic effect was seen for X2, area, inside medial, bone width and cortical width. Highest RBAE measurements were reported during stage 2 and stage 3. A tendency for a quadratic effect of stage of lactation was observed for medullary low ($P < 0.06$) and inside X2 ($P < 0.09$) where RBAE measurements were highest in stages 2 and 3.

A significant effect of stage of lactation on RBAE of caudal vertebrae 15 was observed for both peak medial and peak lateral RBAE (Table 29). A trend for an effect of stage of lactation on RBAE of caudal vertebrae 15 was observed for inside medial and lateral. A tendency for a linear effect of stage of lactation was observed for peak medial ($P < 0.09$) with the highest RBAE reported in stage 3. A quadratic effect of stage of lactation was seen for X2, peak lateral and inside lateral. Highest measurements were in stage 2 and stage 3. A tendency for a quadratic

effect if stage of lactation was seen for area ($P < 0.07$) and inside X2 ($P < 0.07$). Highest RBAE measurements were reported in stages 2 and 3.

Overall, in dairy cattle, stages 2 and 3 of lactation were the periods of highest BMC_{RP}. During these stages, cows should be in positive Ca and P balance. Instead of drawing on bone reserves as in early lactation (stage 1), animals are able to replace bone reserves in mid lactation. In all three bones of dairy cattle in this study, X1 and X2 (width) measurements were the highest during stages 2 and 3. Lateral and peak medial RBAE measurements were greatest during stages 2 and 3, confirming that BMC is optimized during this time. Bone is replenished by mineral at this time, resulting in greater BMC and larger bone dimensions.

Stage of lactation and BMC_{DXA}

There was no effect of stage of lactation on BMC_{DXA} in the metacarpal in dairy cows (Table 30). Stage of lactation tended to affect caudal vertebrae 14 ($P < 0.09$) BMC_{DXA} (Figure 12); however there were no linear or quadratic effects. In caudal vertebrae 15, there was a significant effect ($P < 0.05$) of stage of lactation on BMC_{DXA}. No linear or quadratic effects were observed for stage of lactation and BMC_{DXA} in caudal vertebrae 15.

There was no effect of stage of lactation on BMD_{DXA} of the metacarpal, caudal vertebrae 14 and 15 (Table 30), however there was a trend for effect of stage of lactation on BMD_{DXA} in caudal vertebrae 15 ($P < 0.09$). No linear or quadratic effect of stage of lactation on BMD_{DXA} of the metacarpal or caudal vertebrae 14 and 15 was observed in dairy cattle. There are no other studies that report the effects of stage of lactation on BMC_{DXA} and BMD_{DXA}.

The caudal vertebra is more sensitive to changes in BMC in different stages of lactation than is the metacarpal, suggesting that this site may allow detection of short-term changes in BMC. This difference between caudal vertebrae and the metacarpal is due to bone composition. Caudal vertebrae are made largely of spongy, cancellous bone, and have a higher turnover rate than cortical bone found in the metacarpal. Because of bone composition, the metacarpal may be used to detect long term changes in BMC. Mazess (1990) found that using DXA to make serial measurements in a single tail, a change in 15 g of Ca could be detected. This could result in a Ca balance change of around 100 mg/d over a 6-mo. period.

Stage of lactation and breaking strength

No significant effects of stage of lactation were observed on breaking strength

measurements of the metacarpal in dairy cows (Table 31). In caudal vertebrae 14 a significant effect on the stage of lactation was observed on breaking strength (peak load and energy to peak load) measurements in dairy cattle (Table 32). Stage of lactation tended to affect thickness of caudal vertebrae 14 ($P < 0.08$), but this effect was neither linear nor quadratic (higher in stage 1 and 3 than stages 2 and 4). A linear effect of stage of lactation for energy at peak load, and a trend for a linear effect on peak load ($P < 0.10$) were observed, with the highest measurement observed in stage 3. A significant effect of stage of lactation on energy to peak load of caudal vertebrae 15 in dairy cattle was observed (Table 33). A linear effect was observed for stage of lactation on energy to peak load ($P < 0.04$) of caudal vertebrae 15 in dairy cows (Table 33).

The effect of stage of lactation on breaking strength in the caudal vertebrae is consistent with changes expected in bone resorption. Highest values were generally observed in stage 3 (151-250 DIM), while net resorption occurred after parturition (stage 1) and just before calving (stage 4) when demands from the fetus was the greatest. During lactation, dairy cattle may not need to draw on bone reserves from the metacarpal. Glade (1993) found breaking strength in the metacarpal of horses increased 12 wk after initiation of lactation. In the horses that were fed a sufficient Ca diet, bone mineral was fully restored 24 wk post partum.

As observed for the BMC_{DXA} data, stage of lactation had more effect on breaking strength in the caudal vertebrae than in the metacarpal. Caudal vertebrae may be more sensitive to changes in BMC throughout lactation. Benzie (1959) found that in sheep, resorption was greater in bones with cancellous tissue like caudal vertebrae as compared to cortical bone. Only when severe resorption took place were significant losses of bone mineral observed in the shaft of long bones.

Of real interest is the complete lack of effect of stage of lactation on $BMC_{CHEMICAL}$, despite several observed effects of stage of lactation in BMC_{RP}, BMC_{DXA}, and breaking strength in the caudal vertebrae. Factors other than actual BMC must be behind these observations in other measures.

Effect of parity on measures of BMC in dairy cattle

The terms parity and age are often used interchangeably in regards to dairy cattle. Similar effects for age and parity on various measurements of BMC in the metacarpal and caudal

vertebrae 14 and 15 in dairy cattle in this study were observed, however more differences were observed for parity than for age. Only the effects of parity will be discussed; effects of age can be found in Appendix Tables 1-10.

Parity and $BMC_{CHEMICAL}$

Relatively few effects of parity were observed on measures of $BMC_{CHEMICAL}$, with no effects observed for total ash or P content in any bone (Table 34, Table 35, and Table 36). A trend for an effect of parity on Mg as a percentage of DM ($P < 0.08$) of the metacarpal in dairy cattle was observed (Table 34). Increasing parity decreased Mg as a percent of ash ($P < 0.05$) and Mg as a percent of DM ($P < 0.05$) with the highest values in the first parity.

There was a significant effect of parity on Ca as a percent of DM and Mg as a percent of DM in caudal vertebrae 14 in dairy cattle (Table 35). A linear effect of parity was observed for Mg as a percent of DM with highest concentrations observed in parity 1 and 2. A quadratic effect of parity was observed for Ca as a percent of DM, with a peak observed in parity 2 and the lowest quantity seen in parity 4.

In caudal vertebrae 15 there was a significant effect of parity on mineral content for Ca as a percent of DM and Mg as a percent of DM with highest concentrations observed in parity 2 (Table 36). There was a linear effect of parity observed for Mg as a percent of DM with peak concentrations appearing in parity 2 and declining values in parity 3 and 4. There were no quadratic effects of parity on mineral analysis of caudal vertebrae 15 in dairy cows.

Calcium and Mg concentrations in bone peaked in second lactation cows and reached a low in the fourth lactation. As cows age, they are less able to respond to intestinal and bone Ca absorption to meet lactation demands (Reinhardt et al., 1988). Older animals have fewer osteoclastic cells which delays the ability of bones to respond to plasma Ca. This could explain why older cows are more susceptible to milk fever than younger lactating animals.

Parity and BMC_{RP}

As mentioned previously, the poor relationships observed between $BMC_{CHEMICAL}$ and BMC_{RP} make interpreting the effects of parity on BMC_{RP} problematic. A significant effect of parity was observed on BMC_{RP} of the metacarpal in dairy cows for X1 with the highest RBAE taking place in parity 3 (Table 37). Similarly, parity tended to affect inside X1 ($P < 0.09$), inside

X2 ($P < 0.06$) and X2 ($P < 0.09$), with highest values observed in parity 3. No linear or quadratic effects of parity on BMC_{RP} of the metacarpal were observed.

A significant effect of parity on BMC_{RP} of caudal vertebrae 14 in dairy cows was observed for inside X1 and cortical width (Table 38). A tendency for an effect of parity was observed for medullary low ($P < 0.09$) and X2 ($P < 0.07$). Increasing parity increased X2, inside X2, bone width and cortical width with highest measurements recorded in parity 3. The highest RBAE for medullary low was recorded in parity 4. A tendency for a linear effect of parity was observed for area ($P < 0.07$), inside X1 ($P < 0.06$) with highest RBAE measurements observed in parity 3. Parity tended to have a linear effect on medullary low ($P < 0.08$), with highest values observed in parity 4.

A trend for an effect of parity on RBAE of caudal vertebrae 15 was observed for X2 ($P < 0.07$) and inside X1 ($P < 0.09$; Table 39). Increasing parity increased RBAE measurements for X2, inside X1 and inside X2 with highest values observed in parity 3. A tendency for a linear effect of parity was reported for X1 ($P < 0.08$) and medullary low ($P < 0.09$) in parity 3. Peak medial($P < 0.09$) and peak lateral($P < 0.07$) tended to increase linearly with parity, with highest measurements reported in parity 4.

As measured by radiographic photometry, BMC in dairy cows appeared to reach a peak in a cow's third parity (at approximately 5 yr old). In horses, BMC_{RP} in the metacarpal increased as animals age (Meakim et al., 1981; Hoffman et al., 1999). Hoffman (1999) found that BMC_{RP} was positively related to body weight and girth measurements. Growing foals had a lower peak medial RBAE as compared to the mature equine (17.9 when compared to 22.5 mm Al) and a lower peak lateral RBAE (19.3 vs. 23.7). These authors concluded that as animals age, bone formation and mineralization increased.

Parity and BMC_{DXA}

There was a significant effect of parity on total BMC_{DXA} in the metacarpal of dairy cows (Table 40). Parity tended to affect BMC-upper ($P < 0.07$) and BMC-mid ($P < 0.06$). Highest BMC_{DXA} of the metacarpal was observed in the second and fourth lactation. There were no significant effects of parity on BMC_{DXA} or BMD_{DXA} in caudal vertebrae 14 and 15 in dairy cattle, and no effects of parity on BMD_{DXA} in the metacarpal (Table 40).

A biological explanation for the changes observed in BMC_{DXA} with parity (peaking in 2nd and fourth parity, rather than a linear or quadratic effect) is not apparent. Bone mineral content in the front leg of growing pigs and adults increased with age although BMD_{DXA} in the vertebrae increases with age at a decreasing rate (Mitchell et al., 2001). Mitchell (2001) found that younger pigs (< 30kg) had high growth coefficients for BMD_{DXA} in the spine. In pigs > 30 kg, BMD_{DXA} increased in the back legs but decreased in the front legs and trunk regions (Mitchell et al., 2001). Regional changes in BMD_{DXA} are related to body weight with changes occurring from birth to maturity. These changes result in a redistribution of body proportions (Mitchell et al., 2001; Lukaski, 1993). Gershon-Cohen (1955) found increasing levels of bone density as estimated by DXA with increasing age in metatarsals of elderly people.

Increases in BMD with age result from a gradual dehydration and an increase in fat and bone mineral content of the body (Field et al., 1974). Younger animals will normally deposit a larger portion of mineral in the non-weight-bearing bones (head, trunk, ribs, spine and pelvis). With age, BMC increases in the major body and load bearing bones (front and back legs).

Reinhardt (1988) found that bone mineral deposition in bone is higher in younger animals than older animals. These authors reported that the amount of mineral deposited reaches a peak at one year old and then the rate of deposition is then dramatically reduced. Bone mineral content in ruminants will level off at approximately 9 yrs of age (Reinhardt et al., 1988). Bone accretion and remodeling decrease with age (Parfitt, 1984) and as animals age, they undergo less active bone resorption.

Parity and breaking strength

There were no effects of parity on breaking strength measurements in the metacarpal or caudal vertebrae 14 and 15 in dairy cattle (Table 41, Table 42, Table 43). Strength characteristics in this study were independent of lactation in animals three to 6 yr old, perhaps because animals had achieved bone maturity. Bone maturity is defined as the completion of structural development and mineralization, and is associated with optimal bone strength. Generally, bone mass increases with growth to maturity, and declines thereafter (Frost, 1997; Seeman, 1999). Animals are born with poorly mineralized bones. In younger animals BMC tends to be lower than in older animals as they are still depositing mineral into the bone matrix. Wilson (1984) found that stress values increased with age in the radius and ulna bones of the otter, and peak

load increased with age in all otter bone types. Combs (1991) observed increased stress values in pigs with increasing age and Lawrence (1986) reported that breaking strength of the metacarpal in horses reached a maximum between 1.5 and 4 yr of age.

McCalden (1993) found in humans aged 20 to 102 yr, mechanical breaking strength properties decrease with age. Mechanical breaking strength properties decreased by five, nine and 12 % per decade with increasing age. The author concluded that bone porosity increases with age, but mineral content was not affected. Changes in porosity were responsible for 76 % of strength reduction.

CHAPTER 4: FINAL CONCLUSIONS

A weak relationship was observed between BMC_{RP}, $BMC_{DXA,}$ and BS, and measures of $BMC_{CHEMICAL}$ in the metacarpal and caudal vertebrae 14 and 15 in dairy cattle. Similarly, BMC as estimated by DXA was not a good predictor of either $BMC_{CHEMICAL}$ or BS. The much weaker relationships between estimates of BMC from two imaging techniques and actual measures of BMC and BS observed in this study compared with other studies may be due to differences in the experimental units (growing animals vs. mature), or to sampling frequency (repeated measures vs. one time, terminal measurements).

Overall, there was less variability in observed BMC in the mature animals in this study (age range three to ten years) compared with growing animals in other studies. Also, there was no opportunity for repeated measures within the same animal in the current study. Radiographic photometry may not be sensitive enough to detect small differences in BMC in mature animals. Bone strength measurements and $BMC_{CHEMICAL}$ are real, biological phenomena, while RP and DXA are tools to estimate bone status. Although $BMC_{CHEMICAL}$ is an invasive measure, this is the most meaningful measure of bone mineral status. If BMC_{RP} and BMC_{DXA} have no relationship with $BMC_{CHEMICAL}$ or BS in mature animals, the biological meaning of the imaging measures is limited, as is their use in research with mature animals. Effects of stage of lactation and parity on BMC_{RP} and BMC_{DXA} are reported, and some are of statistical significance. Interpretation is problematic, however, as these are such poor predictors of $BMC_{CHEMICAL}$ and BS.

A weak relationship was also observed between breaking strength and $BMC_{CHEMICAL}$. This weak relationship may be because of maximized bone strength and mineralization in the sample set. As animals age and mature, they reach optimal bone mineralization, and animals in this study had reached a mature age.

Significant effects of stage of lactation were observed on breaking strength (energy to peak load and peak load) in caudal vertebrae 14 and 15 in dairy cattle, although no effects of stage of lactation were observed on breaking strength measures in the metacarpal. Highest values for breaking strength in the caudal vertebrae were generally observed in stage 3 (151-250 DIM). Caudal vertebrae may be more sensitive than the metacarpal to changes in BMC throughout lactation.

There were no effects of stage of lactation on bone ash or bone P content, and relatively minor effects on bone Ca and Mg (highest values were observed in parity one and two). As animals mature, bone development causes more mineral to be deposited in weight bearing bones such as the metacarpal, but advancing age past maturity generally results in a decline in BMC. Neither was observed in the current study, likely because of the relative uniformity of the donor animals.

A lower BMC and lower Ca:P ratio of dairy cows in this study compared with other studies may be due to the origin of bone samples and timing of sampling. Animals sent to slaughter after calving may be Ca or P depleted due to metabolic disorders (i.e., milk fever). This depletion leads to an imbalance of minerals in the body. Time of sampling may also have an effect on mineral content and Ca:P ratio. Biopsy samples from live animals have significantly more mineral (Ca, P and Mg) content than slaughter cattle, due to the effects of the osteolytic membrane system on Ca and P resorption at the time of slaughter.

Others have found that RP and DXA offer opportunity to detect short-term changes in BMC and BMD in young, growing animals of different species. If $BMC_{CHEMICAL}$ measures are the gold standard or true measure of BMC in animals, however, the current work suggests that non-invasive techniques are not useful measures of BMC in mature dairy cattle.

Table 8. Radiographic photometry in dairy cattle.

Bone Type	n	Peak medial RBAE, mm Al	SD	Peak lateral RBAE, mm Al	SD	Medullary Low RBAE, mm Al	SD	CAI, mm_1^2	SD
Metacarpal	78	22.19	2.30	21.73	2.12	18.12	2.21	1,399.24	142.87
Caudal vertebrae 15	78	4.86	0.71	4.89	0.71	3.69	0.53	51.18	26.07
Caudal vertebrae 14	78	5.30	0.88	5.32	0.79	3.72	0.57	46.50	24.75

[1] Definition: Circular area index (CAI, mm2) = B2 – b2 where B = bone diameter; b = medullary diameter

Table 9. Effect of site of measurement (metacarpal and caudal vertebrae 14 and 15) on measures of BMC in dairy cattle.

Measurement	Caudal vertebrae 14	Caudal vertebrae 15	Metacarpal	SEM[3]	*P* < Metacarpal vs. CV	*P* < CV 14 vs. CV 15
$BMC_{CHEMICAL}$						
Ca, % ash	39.79	39.12	40.97	0.10	<.0001	<.0001
Ca, % DM	11.31	10.95	24.65	0.29	<.0001	0.33
P, % ash	17.12	17.87	18.01	0.13	0.0015	0.0003
P, % DM	4.87	4.99	10.85	0.14	<.0001	0.41
Mg, % ash	0.65	0.64	0.58	0.005	<.0001	0.0006
Mg, % DM	0.19	0.21	0.35	0.005	<.0001	<.0001
% Ash	29.29	28.03	60.15	0.69	<.0001	0.15
BMC_{DXA}						
[1]BMC_{DXA}, g	1.59	0.92	56.25	1.14	<.0001	0.65
[2]BMD_{DXA}, g/cm^2	0.49	0.46	1.64	0.03	<.0001	0.43
Breaking strength						
Diameter, mm	6.92	5.98	35.05	0.17	<.0001	<.0001
Thickness, mm	1.97	1.82	10.07	0.09	<.0001	0.21
Peak load, N	2,014.19	1,670.53	25,191.00	60.69	<.0001	0.0003
Stress, N/mm^2	33.41	35.83	17.30	0.98	<.0001	0.07
Energy to peak load, N-mm	3,883.71	2,865.22	34,360.00	157.11	<.0001	<.0001

[1]BMC_{DXA} = BMC as measured by DXA, g.

[2]BMD_{DXA}= BMD as measured by DXA, g/cm^2.

[3]n = 78

Table 9 (cont.) Effect of site of measurement (metacarpal and caudal vertebrae 14 and 15) on measures of BMC in dairy cattle.

Measurement	Caudal Vertebrae 14	Caudal Vertebrae 15	Metacarpal	SEM	*P* < Metacarpal vs. CV	*P* < CV 14 vs. CV 15
BMC_{RP}						
[3]Medial, mm Al	3.67	3.33	12.21	0.36	<.0001	0.52
[4]Lateral, mm Al	2.95	2.96	11.76	0.38	<.0001	0.98
[5]X1, mm	4.67	4.73	11.80	0.27	<.0001	0.86
[6]X2, mm	12.83	12.05	53.84	0.32	<.0001	0.04
[7]Area, mm2	420.68	350.24	9,142.52	84.03	<.0001	0.53
[8]Peak medial, mm Al	5.30	4.84	22.12	0.17	<.0001	0.04
[9]Peak lateral, mm Al	5.29	4.86	21.70	0.16	<.0001	0.03
[10]Medullary low, mm Al	3.91	3.68	18.12	0.16	<.0001	0.26
[11]Inside medial, mm Al	4.60	4.25	20.03	0.19	<.0001	0.18
[12]Inside lateral, mm Al	5.05	4.59	19.81	0.25	<.0001	0.17
[13]Inside X1, mm	6.86	6.90	23.64	0.29	<.0001	0.93
[14]Inside X2, mm	10.85	10.11	42.81	0.32	<.0001	0.07
[15]Inside area, mm2	205.55	154.50	4,378.60	45.81	<.0001	0.44
[16]Bone width, mm	8.10	7.35	41.99	0.21	<.0001	0.002
[17]Cortical width, mm	4.13	4.20	22.88	0.20	<.0001	0.79
[18]Medullary width, mm	4.04	3.23	19.17	0.21	<.0001	0.001

[3]Medial = BMC_{RP} expressed in RBAE (mm Al) on the very most outside part of the bone on the medial side.

[4]Lateral = BMC_{RP} expressed in mm Al on the very most outside part of the bone on the lateral side.

[5]X1= Bone width measurement on outside of bone, mm.

[6]X2 = Bone width measurement on outside of bone, mm.

[7]Area = Area of bone, mm^2.

[8]Peak medial= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the medial side.

[9]Peak lateral= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the lateral side.

[10]Medullary low = BMC_{RP} expressed in mm Al at the least mineralized part of the bone.

[11]Inside X1 = Bone width measurement on inside of bone, mm.

[12]Inside X2 = Bone width measurement on inside of bone, mm.

[13]Inside medial = BMC_{RP} expressed in mm Al on the most inside part of the bone on the medial side.

[14]Inside lateral Y2 = BMC_{RP} expressed in mm Al on the most inside part of the bone on the lateral side.

[15]Inside area = Area on the inside of bone mm^2.

[16]Bone width = X2 – X1, mm.

[17]Medullary width = Inside X2 – inside X1, mm.

[18]Cortical width = Bone width – medullary width, mm.

Table 10. BMC_{DXA} and BMD_{DXA} in dairy cattle.

Bone Type	n	BMC, g	SD	BMD, g/cm^2	SD
Metacarpal	78	55.71	1.68	1.63	0.03
Caudal vertebrae 14	78	1.53	0.06	0.49	0.006
Caudal vertebrae 15	78	1.01	0.06	0.46	0.006

Table 11. Breaking strength of bone in dairy cattle.

Bone Type	n	Diameter, mm		Thickness, mm		Area, mm^2		Stress, N/mm^2		Peak Load, N		Energy Pkld, N-mm	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Metacarpal	78	35.09	1.32	10.07	1.06	9197.48	1163.44	17.23	4.86	27,767	2225.16	28,937	13,863.1
Caudal vertebrae 14	78	7.44	1.41	2.11	0.44	411.48	125.83	33.65	9.54	2,265	591.61	4,429	1845.47
Caudal vertebrae 15	78	6.41	1.59	1.83	0.26	306.00	47.59	35.71	3.71	1,824	671.08	3,273	1677.70

Table 12 Total bone mineral content in dairy cattle.

Bone Type	n	BMC, g	SD	Ash %DM	SD
Metacarpal	78	14.52	1.94	60.21	4.68
Caudal vertebrae 14	78	1.01	0.62	29.43	6.80
Caudal vertebrae 15	78	0.68	0.53	28.00	5.39

Table 13. Calcium and phosphorus content of bone in dairy cattle.

Bone Type	n	Ca, % Ash	SD	Ca, %DM	SD	P, %Ash	SD	P, %DM	SD	Ca: P
Metacarpal	78	40.97	0.97	24.65	1.85	18.00	0.44	10.83	0.79	2.28
Caudal vertebrae 14	78	39.05	0.79	11.95	4.59	17.18	0.44	4.84	1.06	2.27
Caudal vertebrae 15	78	39.14	0.79	10.94	2.03	17.89	1.85	5.02	1.15	2.19

Table 14. Relationship between % ash ($BMC_{CHEMICAL}$) and BMC_{RP} in the metacarpal in dairy cattle[1].

	medial	lateral	X1	X2	Area	Peak medial	Peak lateral	Medullary low	Inside medial	Inside lateral	Inside x1	Inside x2	Inside area	Bone width	Medullary Width	Cortical Width
Y intercept	60.59	59.60	58.80	59.16	62.37	57.09	60.51	58.43	63.10	61.41	56.99	63.27	65.85	72.26	68.45	53.43
Regression coefficient	-0.03	0.05	0.12	0.02	0.0002	0.14	-0.01	0.10	-0.14	-0.06	0.14	-0.07	-0.001	-0.29	-0.43	0.30
Std. Error	0.10	0.09	0.15	0.13	0.0004	0.24	0.26	0.24	0.20	0.15	0.14	0.13	0.0007	0.25	0.19	0.22
Prob > t	0.75	0.58	0.42	0.89	0.59	0.55	0.96	0.69	0.46	0.69	0.33	0.58	0.08	0.26	0.02	0.18
R^2	0.001	0.004	0.009	0.0003	0.004	0.005	0.00	0.002	0.007	0.002	0.01	0.004	0.04	0.02	0.06	0.02

[1]n = 78

Table 15. Relationship between measures of BMC_{RP} and all measures of $BMC_{CHEMICAL}$ in the caudal vertebrae of dairy cattle[1]

		Ca, % DM	Ca, % Ash	P, % DM	P, % Ash	Mg, % DM	Mg, % Ash	Ash, %DM
					r^2			
Caudal vertebrae 14	Medial	0.04	0.0003	0.03	0.00	0.02	0.02	0.03
	Lateral	0.05	0.005	0.06	0.05	0.06	0.005	0.06
	Peak medial	0.16	0.01	0.14	0.02	0.13	0.01	0.15
	Peak lateral	0.21	0.02	0.18	0.009	0.17	0.006	0.20
	Medullary low	0.04	0.04	0.03	0.03	0.02	0.02	0.03
	Inside medial	0.07	0.06	0.05	0.01	0.05	0.01	0.06
	Inside lateral	0.17	0.02	0.15	0.003	0.14	0.004	0.16
	Bone length	0.006	0.02	0.01	0.04	0.007	0.004	0.007
	Bone width	0.004	0.02	0.008	0.05	0.008	0.007	0.005
	Medullary width	0.002	0.006	0.002	0.0001	0.007	0.0005	0.003
	Cortical width	0.001	0.01	0.006	0.11	0.002	0.0002	0.002
Caudal vertebrae 15	Medial	0.11	0.001	0.11	0.07	0.02	0.04	0.09
	Lateral	0.01	0.006	0.02	0.003	0.002	0.0008	0.02
	Peak medial	0.03	0.03	0.003	0.05	0.02	0.02	0.03
	Peak lateral	0.02	0.03	0.002	0.05	0.04	0.03	0.03
	Medullary low	0.02	0.02	0.002	0.05	0.03	0.007	0.03
	Inside medial	0.009	0.002	0.00	0.03	0.02	0.002	0.01
	Inside lateral	0.03	0.04	0.002	0.07	0.03	0.05	0.04

Bone length	0.003	0.007	0.0007	0.00	0.005	0.0004	0.002
Bone width	0.003	0.001	0.00	0.01	0.002	0.008	0.0005
Medullary width	0.0004	0.005	0.0005	0.002	0.02	0.0007	0.0001
Cortical width	0.001	0.0002	0.0006	0.03	0.02	0.008	0.001

[1]n = 78

Table 16. Relationship between measures of BMC_{RP} and all measures of $BMC_{CHEMICAL}$ in the metacarpal of dairy cattle[1].

	Ca, % DM	Ca, % Ash	P, % DM	P, % Ash	Mg, % DM	Mg, % Ash	Ash, %DM
				r^2			
Medial	0.0007	0.003	0.001	0.05	0.001	0.02	0.001
Lateral	0.002	0.008	0.0001	0.05	0.0004	0.02	0.004
Peak medial	0.002	0.007	0.0004	0.02	0.01	0.07	0.005
Peak lateral	0.002	0.01	0.001	0.005	0.005	0.01	0.00
Medullary low	0.002	0.001	0.00	0.02	0.01	0.05	0.002
Inside medial	0.01	0.003	0.01	0.01	0.06	0.09	0.007
Inside lateral	0.004	0.002	0.005	0.005	0.02	0.02	0.002
Bone width	0.02	0.0001	0.02	0.0002	0.001	0.02	0.02
Medullary width	0.03	0.07	0.04	0.05	0.01	0.04	0.06
Cortical width	0.02	0.009	0.02	0.002	0.005	0.01	0.02

[1]n = 78

Table 17. Relationship between measures of BMC_{RP} and BS in the caudal vertebrae of dairy cattle[1].

		Diameter	Thickness	Peak Load	Stress	Energy Pkld
				r^2		
Caudal vertebrae 14	Medial	0.03	0.003	0.003	0.01	0.01
	Lateral	0.05	0.0002	0.20	0.005	0.03
	Peak medial	0.10	0.10	0.12	0.0004	0.11
	Peak lateral	0.13	0.10	0.12	0.005	0.14
	Medullary low	0.07	0.05	0.10	0.003	0.11
	Inside medial	0.06	0.12	0.11	0.0002	0.10
	Inside lateral	0.10	0.09	0.12	0.002	0.10
	Bone length	0.06	0.005	0.05	0.001	0.05
	Bone width	0.19	0.05	0.12	0.004	0.16
	Medullary width	0.27	0.02	0.07	0.08	0.14
	Cortical width	0.005	0.03	0.05	0.04	0.03
Caudal vertebrae 15	Medial	0.0009	0.01	0.01	0.002	0.002
	Lateral	0.0001	0.004	0.002	0.04	0.0004
	Peak medial	0.02	0.26	0.16	0.02	0.14
	Peak lateral	0.02	0.25	0.15	0.03	0.14
	Medullary low	0.02	0.25	0.16	0.003	0.12
	Inside medial	0.01	0.20	0.09	0.01	0.08
	Inside lateral	0.008	0.21	0.15	0.01	0.13
	Bone length	0.01	0.002	0.008	0.009	0.02
	Bone width	0.01	0.08	0.04	0.02	0.03
	Medullary width	0.00	0.004	0.0002	0.06	0.01
	Cortical width	0.02	0.15	0.06	0.00	0.01

[1]n = 78

Table 18. Relationship between measures of BMC_{RP} and BS in the metacarpal of dairy cattle[1].

	Diameter	Thickness	Peak Load	Stress	Energy Pkld
			r^2		
Medial	0.003	0.005	0.05	0.05	0.05
Lateral	0.02	0.002	0.05	0.040	0.07
Peak medial	0.003	0.004	0.0001	0.0006	0.006
Peak lateral	0.02	0.008	0.005	0.009	0.02
Medullary low	0.008	0.00	0.0004	0.003	0.008
Inside medial	0.005	0.004	0.001	0.001	0.002
Inside lateral	0.07	0.001	0.01	0.03	0.008
Bone width	0.30	0.003	0.06	0.01	0.04
Medullary width	0.13	0.15	0.009	0.008	0.0008
Cortical width	0.005	0.23	0.0005	0.04	0.02

[1]n = 78

Table 19. Relationship between BMC_{DXA} and $BMC_{CHEMICAL}$ in caudal vertebrae 14 in dairy cattle[1].

	Ca, %Ash	Ca, %DM	P, %Ash	P, %DM	Mg, %Ash	Mg, %DM	%Ash
Y intercept	4.30	1.94	1.74	1.72	2.75	2.11	1.67
Regression Coefficient	0.07	0.02	0.01	0.02	2.19	1.83	0.003
Standard error	0.08	0.04	0.16	0.09	2.31	2.34	0.01
Prob > t	0.37	0.63	0.93	0.79	0.35	0.44	0.82
R^2	0.01	0.003	0.001	0.001	0.01	0.008	0.007

[1]n = 78

Table 20. Relationship between all measures of BMC_{DXA} and $BMC_{CHEMICAL}$ in dairy cattle[1].

		Ca, % DM	Ca, % Ash	P, % DM	P, % Ash	Mg, % DM	Mg, % Ash	Ash, %DM
					r^2			
Metacarpal	BMC_{UPPER}	0.02	0.002	0.004	0.02	0.003	0.02	0.02
	BMC_{MID}	0.02	0.004	0.005	0.01	0.003	0.005	0.02
	BMC_{TOTAL}	0.02	0.02	0.005	0.01	0.005	0.009	0.02
	BMC_{UPPER}	0.001	0.02	0.0002	0.009	0.006	0.008	0.004
	BMC_{MID}	0.008	0.008	0.002	0.003	0.008	0.0007	0.007
	BMD_{TOTAL}	0.008	0.01	0.002	0.004	0.008	0.002	0.006
Caudal Vertebrae	BMC_{CV14}	0.02	0.08	0.03	0.003	0.004	0.001	0.03
	BMD_{CV14}	0.001	0.12	0.007	0.001	0.005	0.003	0.005
	BMC_{CV15}	0.03	0.06	0.03	0.0007	0.008	0.005	0.03
	BMD_{CV15}	0.004	0.06	0.009	0.0003	0.001	0.0001	0.008
	BMC_{CVTOT}	0.02	0.06	0.03	0.006	0.005	0.002	0.03
	BMD_{CVTOT}	0.001	0.10	0.006	0.001	0.004	0.0001	0.004

[1]n = 78

Table 21. Relationship between measures of BMC_{DXA} and BS in dairy cattle[1].

		Diameter	Thickness	Peak Load	Stress	Energy Pkld
				r^2		
Metacarpal	BMC_{UPPER}	0.008	0.002	0.007	0.001	0.01
	BMC_{MID}	0.0001	0.05	0.007	0.002	0.007
	BMC_{TOTAL}	0.0008	0.04	0.0003	0.00	0.00
	BMC_{UPPER}	0.00	0.003	0.01	0.01	0.007
	BMC_{MID}	0.002	0.03	0.00	0.004	0.002
	BMD_{TOTAL}	0.002	0.12	0.003	0.0002	0.0005
Caudal Vertebrae	BMC_{CV14}	0.04	0.05	0.02	0.02	0.02
	BMD_{CV14}	0.05	0.05	0.001	0.03	0.02
	BMC_{CV15}	0.04	0.05	0.04	0.004	0.03
	BMD_{CV15}	0.05	0.06	0.06	0.002	0.04
	BMC_{CVTOT}	0.04	0.04	0.02	0.01	0.02
	BMC_{CVTOT}	0.04	0.04	0.02	0.02	0.02

[1]n = 78

Table 22. Relationship between stress and $BMC_{CHEMICAL}$ in the metacarpal in dairy cattle[1].

	Ca, %Ash	Ca, %DM	P, %Ash	P, %DM	Mg, %Ash	Mg, %DM	%Ash
Y intercept	6.59	6.13	-1.21	5.42	-2.71	2.62	7.98
Regression Coefficient	0.26	0.45	1.03	1.09	34.44	41.99	0.15
Standard error	0.67	0.34	1.36	0.76	20.55	20.44	0.13
Prob > t	0.70	0.19	0.45	0.15	0.10	0.04	0.25
R^2	0.002	0.02	0.007	0.27	0.04	0.53	0.018

[1]n = 78

Table 23. Relationship between all measures of BS and $BMC_{CHEMICAL}$ in dairy cattle[1].

		Ca, % DM	Ca, % Ash	P, % DM	P, % Ash	Mg, % DM	Mg, % Ash	Ash, %DM
		r^2						
Metacarpal	Diameter, mm	0.006	0.01	0.002	0.04	0.0002	0.02	0.01
	Thickness, mm	0.002	0.008	0.002	0.01	0.006	0.06	0.006
	Peak Load, N	0.03	0.0001	0.04	0.0004	0.05	0.01	0.03
	Stress, N/mm^2	0.02	0.002	0.03	0.007	0.05	0.04	0.02
	Energy Pkld, N-mm	0.0009	0.0002	0.001	0.00	0.009	0.01	0.001
Caudal Vertebrae 14	Diameter, mm	0.003	0.003	0.002	0.05	0.0006	0.01	0.004
	Thickness, mm	0.004	0.001	0.002	0.02	0.002	0.001	0.0003
	Peak Load, N	0.001	0.02	0.00	0.03	0.0009	0.03	0.0004
	Stress, N/mm^2	0.002	0.06	0.004	0.009	0.008	0.009	0.005
	Energy Pkld, N-mm	0.0002	0.005	0.001	0.04	0.004	0.02	0.0003
Caudal Vertebrae 15	Diameter, mm	0.001	0.07	0.0002	0.02	0.0001	0.03	0.004
	Thickness, mm	0.00	0.02	0.008	0.01	0.0002	0.009	0.0001
	Peak Load, N	0.06	0.03	0.13	0.08	0.0006	0.03	0.06
	Stress, N/mm^2	0.07	0.006	0.07	0.03	0.005	0.03	0.06
	Energy Pkld, N-mm	0.03	0.03	0.06	0.04	0.002	0.03	0.03

[1]n = 78

Table 24. Effect of stage of lactation on $BMC_{CHEMICAL}$ of the metacarpal in dairy cattle.

Stage[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Ca, % Ash	41.03	40.74	41.48	40.87	0.28	0.16	0.78	0.55
Ca, %DM	24.80	24.65	23.95	24.60	1.00	0.92	0.69	0.69
P, % Ash	17.82	17.98	18.26	17.86	0.19	0.29	0.54	0.14
P, %DM	10.78	10.88	10.54	10.75	0.44	0.95	0.78	0.91
Mg, % Ash	0.57	0.56	0.57	0.59	0.01	0.45	0.26	0.40
Mg, %DM	0.35	0.34	0.33	0.35	0.02	0.63	0.90	0.36
% Ash	60.45	60.52	57.74	60.20	2.45	0.79	0.66	0.63

[1]Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]n = 43

Table 25. Effect of stage of lactation on $BMC_{CHEMICAL}$ of caudal vertebrae 14 in dairy cattle.

Stage[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Ca, % Ash	11.92	11.34	11.37	12.26	1.43	0.93	0.85	0.60
Ca, %DM	39.87	39.73	39.96	40.20	0.21	0.23	0.12	0.36
P, % Ash	4.36	4.81	5.10	4.75	0.83	0.87	0.94	0.72
P, %DM	17.15	17.40	16.87	17.12	0.22	0.40	0.94	0.72
Mg, % Ash	0.17	0.19	0.19	0.17	0.028	0.79	0.80	0.48
Mg, %DM	0.67	0.64	0.64	0.66	0.014	0.39	0.68	0.09
% Ash	29.9	28.6	28.0	30.6	0.037	0.93	0.92	0.58

[1]Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]n = 43

Table 26. Effect of stage of lactation on $BMC_{CHEMICAL}$ of caudal vertebrae 15 in dairy cattle.

Stage[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Ca, % Ash	11.92	11.35	11.37	12.26	1.43	0.93	0.13	0.89
Ca, %DM	39.87	39.73	39.96	40.20	0.21	0.23	0.30	0.87
P, % Ash	4.81	4.99	4.23	4.99	0.82	0.87	0.83	0.45
P, %DM	17.15	17.40	16.87	17.12	0.22	0.40	0.25	0.75
Mg, % Ash	0.19	0.18	0.16	0.19	0.028	0.79	0.78	0.07
Mg, %DM	0.67	0.64	0.64	0.66	0.014	0.39	0.24	0.30
% Ash	30.1	28.7	28.9	26.9	1.44	0.93	0.26	0.88

[1]Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]n = 43

Table 27. Effect of stage of lactation on BMC_{RP} in the metacarpal in dairy cattle.

Stage[1]	1	2	3	4	SEM[18]	*P* <	Linear	Quadratic
Medial[2]	8.90	12.24	14.58	12.48	2.28	0.30	0.10	0.20
Lateral[3]	14.40	13.85	8.19	12.23	2.16	0.17	0.13	0.29
X1[4]	10.14	9.63	15.43	11.86	1.28	0.01	0.02	0.23
X2[5]	52.12	51.27	57.16	54.55	1.48	0.02	0.01	0.55
Area[6]	8736.50	8518.45	9243.29	9495.90	451.62	0.20	0.07	0.59
Peak medial[7]	21.91	20.81	22.91	22.95	0.79	0.12	0.08	0.46
Peak lateral[8]	20.75	20.33	21.44	22.61	0.66	0.02	0.01	0.23
Medullary Low[9]	17.50	16.77	17.99	18.86	0.83	0.17	0.08	0.33
Inside X1[10]	21.26	21.75	26.97	24.30	1.33	0.01	0.01	0.23
Inside X2[11]	41.00	39.57	46.71	43.04	1.52	0.009	0.02	0.46
Inside medial[12]	18.97	18.82	21.13	20.43	1.10	0.30	0.10	0.80
Inside lateral[13]	18.92	19.01	19.98	20.37	1.04	0.52	0.16	0.88
Inside area[14]	4383.87	3800.72	4496.46	4507.75	263.84	0.16	0.27	0.26
Bone width[15]	41.98	41.64	41.73	42.70	0.80	0.61	0.45	0.41
Medullary width[16]	19.73	17.82	19.74	18.74	1.03	0.47	0.78	0.65
Cortical width[17]	22.25	23.82	21.99	23.96	0.79	0.08	0.26	0.80

[1] Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]Medial = BMC_{RP} expressed in RBAE (mm Al) on the very most outside part of the bone on the medial side.

[3]Lateral = BMC_{RP} expressed in mm Al on the very most outside part of the bone on the lateral side.

[4]X1= Bone width measurement on outside of bone, mm.

[5]X2 = Bone width measurement on outside of bone, mm.

[6]Area = Area of bone, mm^2.

[7]Peak medial= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the medial side.

[8]Peak lateral= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the lateral side.

[9]Medullary low = BMC_{RP} expressed in mm Al at the least mineralized part of the bone.

[10]Inside X1 = Bone width measurement on inside of bone, mm.

[11]Inside X2 = Bone width measurement on inside of bone, mm.

[12]Inside medial = BMC_{RP} expressed in mm Al on the most inside part of the bone on the medial side.

[13]Inside lateral Y2 = BMC_{RP} expressed in mm Al on the most inside part of the bone on the lateral side.

[14]Inside area = Area on the inside of bone, mm^2.

[15]Bone width = X2 – X1, mm.

[16]Medullary width = Inside X2 – inside X, mm.

[17]Cortical width = Bone width – medullary width, mm.

[18]n = 43

Table 28. Effect of stage of lactation on BMC_{RP} of caudal vertebrae 14 in dairy cattle.

Stage[1]	1	2	3	4	SEM[19]	*P* <	Linear	Quadratic
Medial[2]	3.40	3.49	3.98	3.63	0.34	0.54	0.31	0.49
Lateral[3]	3.04	2.84	3.39	2.85	0.22	0.11	0.98	0.41
X1[4]	4.78	4.33	5.46	4.44	0.71	0.50	0.95	0.67
X2[5]	12.19	14.19	14.98	12.92	0.97	0.14	0.37	0.03
Area[6]	372.12	466.24	530.72	417.72	51.66	0.12	0.26	0.03
Peak medial[7]	5.06	5.48	5.73	5.27	0.40	0.60	0.52	0.24
Peak lateral[8]	5.17	5.40	5.70	5.30	0.29	0.50	0.47	0.24
Medullary Low[9]	3.67	3.96	4.47	3.89	0.24	0.06	0.16	0.06
Length[10]	40.18	43.84	44.33	43.78	2.75	0.60	0.23	0.41
Inside X1[11]	6.88	7.28	8.15	6.55	0.70	0.21	0.95	0.13
Inside X2[12]	10.33	11.95	12.37	11.02	0.92	0.34	0.42	0.09
Inside medial[13]	4.47	4.87	5.26	4.56	0.29	0.13	0.51	0.05
Inside lateral[14]	4.99	5.29	5.55	4.93	0.37	0.47	0.94	0.19
Inside area[15]	170.45	243.07	232.44	222.69	35.28	0.43	0.23	0.21
Bone width[16]	7.37	9.62	9.56	8.39	0.84	0.18	0.30	0.03
Medullary width[17]	3.45	4.67	4.22	4.48	0.63	0.41	0.22	0.41
Cortical width[18]	3.92	4.95	5.33	3.91	0.62	0.15	0.87	0.04

[1]Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]Medial = BMC_{RP} expressed in RBAE (mm Al) on the very most outside part of the bone on the medial side.

[3]Lateral = BMC_{RP} expressed in mm Al on the very most outside part of the bone on the lateral side.

[4]X1= Bone width measurement on outside of bone, mm.

[5]X2 = Bone width measurement on outside of bone, mm.

[6]Area = Area of bone, mm^2.

[7]Peak medial= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the medial side.

[8]Peak lateral= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the lateral side.

[9]Medullary low = BMC_{RP} expressed in mm Al at the least mineralized part of the bone.

[10]Length = length of bone, mm.

[11]Inside X1 = Bone width measurement on inside of bone, mm.

[12]Inside X2 = Bone width measurement on inside of bone, mm.

[13]Inside medial = BMC_{RP} expressed in mm Al on the most inside part of the bone on the medial side.

[14]Inside lateral Y2 = BMC_{RP} expressed in mm Al on the most inside part of the bone on the lateral side.

[15]Inside area = Area on the inside of bone, mm^2.

[16]Bone width = X2 – X1, mm.

[17]Medullary width = Inside X2 – inside X, mm.

[18]Cortical width = Bone width – medullary width, mm.

[19]n = 43

Table 29. Effect of stage of lactation on BMC_{RP} of caudal vertebrae 15 in dairy cattle.

Stage[1]	1	2	3	4	SEM[19]	*P* <	Linear	Quadratic
Medial[2]	3.06	3.03	4.03	3.46	0.42	0.95	0.63	0.67
Lateral[3]	3.03	3.06	3.61	3.12	0.47	0.71	0.61	0.56
X1[4]	4.62	5.36	4.48	4.16	0.55	0.32	0.23	0.30
X2[5]	11.68	13.60	13.28	11.80	0.85	0.20	0.99	0.03
Area[6]	328.57	388.07	455.60	349.75	48.21	0.17	0.43	0.07
Peak medial[7]	4.59	5.13	5.65	4.80	0.29	0.04	0.09	0.58
Peak lateral[8]	4.71	5.20	5.62	4.80	0.29	0.05	0.47	0.02
Medullary Low[9]	3.68	3.75	4.22	3.75	0.24	0.24	0.41	0.24
Length[10]	35.31	40.55	41.61	38.58	2.71	0.34	0.24	0.11
Inside X1[11]	6.90	7.76	7.33	6.39	0.60	0.23	0.33	0.11
Inside X2[12]	9.97	11.37	10.76	9.76	0.70	0.25	0.60	0.07
Inside medial[13]	4.15	4.23	5.05	4.26	0.30	0.06	0.26	0.13
Inside lateral[14]	4.30	4.83	5.31	4.50	0.30	0.06	0.29	0.02
Inside area[15]	149.76	166.93	190.80	156.80	23.67	0.52	0.58	0.25
Bone width[16]	7.07	8.24	8.80	7.64	0.77	0.36	0.39	0.11
Medullary width[17]	3.07	3.61	3.43	3.37	0.54	0.91	0.70	0.55
Cortical width[18]	3.99	4.63	5.37	4.27	0.58	0.27	0.43	0.11

[1] Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]Medial = BMC_{RP} expressed in RBAE (mm Al) on the very most outside part of the bone on the medial side.

[3]Lateral = BMC_{RP} expressed in mm Al on the very most outside part of the bone on the lateral side.

[4]X1= Bone width measurement on outside of bone, mm.

[5]X2 = Bone width measurement on outside of bone, mm.

[6]Area = Area of bone, mm^2.

[7]Peak medial= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the medial side.

[8]Peak lateral= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the lateral side.

[9]Medullary low = BMC_{RP} expressed in mm Al at the least mineralized part of the bone.

[10]Length = length of bone, mm.

[11]Inside X1 = Bone width measurement on inside of bone, mm.

[12]Inside X2 = Bone width measurement on inside of bone, mm.

[13]Inside medial = BMC_{RP} expressed in mm Al on the most inside part of the bone on the medial side.

[14]Inside lateral Y2 = BMC_{RP} expressed in mm Al on the most inside part of the bone on the lateral side.

[15]Inside area = Area on the inside of bone, mm^2 .

[16]Bone width = X2 – X1, mm.

[17]Medullary width = Inside X2 – inside X, mm.

[18]Cortical width = Bone width – medullary width, mm

[19]n = 43

Table 30. Effect of stage of lactation on BMC and BMD estimated via BMC_{DXA} in dairy cattle.

Stage[1]	1	2	3	4	SEM[14]	*P* <	Linear	Quadratic
BMCCV14[2]	1.44	1.42	2.18	1.37	0.27	0.09	0.58	0.15
BMCCV15[3]	0.91	0.87	1.65	0.92	0.24	0.05	0.34	0.15
BMCTOT[4]	4.96	4.78	7.47	4.84	0.94	0.10	0.49	0.20
BMDCV14[5]	0.48	0.50	0.53	0.47	0.02	0.20	0.76	0.10
BMDCV15[6]	0.45	0.44	0.51	0.45	0.02	0.09	0.43	0.24
BMDTOT[7]	0.47	0.49	0.53	0.47	0.02	0.24	0.60	0.14
BMC-UPPER[8]	11.98	12.42	11.61	11.79	1.75	0.99	0.83	0.94
BMC-MID[9]	43.61	40.57	35.89	40.69	4.02	0.58	0.36	0.33
BMCTOT1[10]	56.96	56.35	47.62	53.60	5.68	0.61	0.36	0.56
BMC-UPPER[11]	1.74	1.82	1.67	1.81	0.17	0.88	0.92	0.85
BMC-MID[12]	1.64	1.54	1.54	1.63	0.11	0.85	0.95	0.38
BMDTOT1[13]	1.64	1.57	1.54	1.64	0.12	0.91	0.98	0.50

[1] Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]BMCCV14= BMC_{DXA} of caudal vertebra 14, g

[3]BMC-MID = BMC_{DXA} of mid section of metacarpal, g

[4]BMCTOT= Total BMC_{DXA} of caudal vertebra 14 and15, g/cm^2

[5]BMDCV14= BMD_{DXA} of caudal vertebra 14, g/cm^2

[6]BMC-MID = BMD_{DXA} of mid section of metacarpal, g/cm^2

[7]BMDTOT1 = Total BMD_{DXA} of mid and upper metacarpal, g/cm^2

[8]BMC-UPPER = BMC_{DXA} of upper metacarpal, g

[9]BMCCV15= BMC_{DXA} of caudal vertebra 15, g

[10]BMCTOT1 = Total BMC_{DXA} of mid and upper metacarpal, g

[11]BMC-UPPER= BMD_{DXA} of upper metacarpal, g/cm^2

[12]BMDCV15= BMD_{DXA} of caudal vertebra 15, g/cm^2

[13]BMDTOT= Total BMD_{DXA} of caudal vertebra14 and 15, g/cm^2

[14]n = 43

Table 31. Effect of stage of lactation on breaking strength of the metacarpal in dairy cattle.

Stage[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Diameter, mm	34.87	34.67	35.16	35.34	0.37	0.76	0.38	0.74
Thickness, mm	10.29	10.07	9.62	10.20	0.19	0.39	0.51	0.19
Peak Load, k N	26.95	27.65	27.63	26.28	2.01	0.98	0.86	0.74
Peak Stress, N/mm^2	17.10	17.59	17.81	16.41	1.20	0.92	0.79	0.62
EnergyPkld,$N\text{-}mm^2$	35.9	44.53	35.83	30.63	4.32	0.42	0.32	0.31

[1]Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]n = 43

Table 32. Effect of stage of lactation on breaking strength of caudal vertebrae 14 in dairy cattle.

Stage[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Diameter, mm	6.86	7.01	8.12	6.59	0.37	0.19	0.89	0.15
Thickness, mm	2.06	1.80	2.37	1.89	0.11	0.08	0.94	0.53
Peak Load, N	2448.60	1861.22	2420.83	1738.50	160.1	0.04	0.10	0.85
Stress, N/mm^2	38.92	33.34	30.48	32.04	2.13	0.28	0.07	0.30
Energy Pkld, N-mm	5149.13	3169.03	4475.42	3202.53	380.46	0.02	0.05	0.56

[1]Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]n = 43

Table 33. Effect of stage of lactation on breaking strength of caudal vertebrae 15 in dairy cattle.

Stage[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Diameter, mm	6.09	5.88	6.77	5.40	0.34	0.20	0.55	0.29
Thickness, mm	1.79	1.80	2.12	1.72	0.12	0.35	0.87	0.27
Peak Load, N	1863.1	1471.18	1956.22	1379.53	161.85	0.16	0.31	0.72
Stress, N/mm^2	37.47	32.53	33.72	34.90	2.63	0.87	0.67	0.47
Energy Pkld, N-mm	3889.43	2396.07	3552.68	2046.97	358.87	0.02	0.04	0.99

[1]Stage of lactation defined by DIM. Stage 1 = < 90 DIM, Stage 2 = > 90 and < 150 DIM, Stage 3 = >150 and < 250 DIM and Stage 4 = > 250

[2]n = 43

Table 34. Effect of parity on $BMC_{CHEMICAL}$ of the metacarpal in dairy cattle.

Parity[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Ca, % Ash	41.09	40.91	40.98	41.15	0.26	0.87	0.81	0.44
Ca, % DM	25.60	24.75	23.35	24.29	0.96	0.35	0.17	0.26
P, % Ash	18.12	17.79	18.02	18.00	0.18	0.48	0.86	0.30
P, % DM	11.29	10.76	10.28	10.62	0.42	0.32	0.15	0.21
Mg, % Ash	0.59	0.59	0.56	0.56	0.01	0.20	0.05	0.91
Mg, %DM	0.37	0.36	0.32	0.33	0.01	0.08	0.02	0.38
% Ash	62.30	60.52	57.03	59.06	2.35	0.39	0.16	0.33

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.
[2]n = 43

Table 35. Effect of parity on $BMC_{CHEMICAL}$ of caudal vertebrae 14 in dairy cattle.

Parity[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Ca, % Ash	11.45	11.10	11.92	12.43	1.41	0.87	0.50	0.73
Ca, % DM	39.84	40.48	39.81	39.62	0.21	0.01	0.10	0.03
P, % Ash	4.36	4.81	5.10	4.75	0.83	0.92	0.92	0.87
P, % DM	17.18	17.20	17.05	17.10	0.22	0.96	0.67	0.94
Mg, % Ash	0.17	0.19	0.19	0.17	0.028	0.85	0.85	0.79
Mg, %DM	0.67	0.68	0.64	0.62	0.014	0.008	0.003	0.22
% Ash	28.3	27.5	30.0	31.4	0.036	0.82	0.41	0.71

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.
[2]n = 43

Table 36. Effect of parity on $BMC_{CHEMICAL}$ of caudal vertebrae 15 in dairy cattle.

Parity[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Ca, % Ash	11.45	11.10	11.92	12.43	1.41	0.87	0.05	0.73
Ca, % DM	39.84	40.48	39.82	39.62	0.21	0.01	0.10	0.26
P, % Ash	4.36	4.81	5.10	4.75	0.83	0.92	0.65	0.57
P, % DM	17.17	17.20	17.05	17.10	0.22	0.96	0.67	0.95
Mg, %Ash	0.17	0.19	0.19	0.17	0.028	0.85	0.99	0.38
Mg, % DM	0.67	0.68	0.64	0.63	0.014	0.008	0.003	0.22
% Ash	29.9	27.9	27.6	29.1	2.42	0.84	0.75	0.38

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.
[2]n = 43

Table 37. Effect of parity on BMC_{RP} in the metacarpal in dairy cattle.

Parity[1]	1	2	3	4+	SEM[18]	*P* <	Linear	Quadratic
Medial[2]	12.43	10.24	13.77	11.76	2.36	0.71	0.86	0.96
Lateral[3]	14.78	13.26	8.64	12.02	2.42	0.26	0.13	0.21
X1[4]	13.39	9.50	13.51	10.66	1.18	0.04	0.41	0.65
X2[5]	55.25	51.29	55.75	52.81	1.66	0.09	0.62	0.70
Area[6]	8654.60	8802.23	9373.78	9163.53	492.97	0.63	0.76	0.74
Peak medial[7]	22.45	21.00	23.06	22.07	0.88	0.30	0.23	0.65
Peak lateral[8]	21.24	21.23	21.40	21.26	0.73	0.10	0.93	0.91
Medullary Low[9]	14.45	17.43	18.19	17.74	0.93	0.81	0.55	0.63
Inside X1[10]	24.96	21.40	25.30	22.62	1.66	0.09	0.54	0.71
Inside X2[11]	44.31	39.85	44.60	41.55	1.71	0.06	0.55	0.60
Inside medial[12]	19.09	19.05	20.74	20.47	1.24	0.56	0.18	0.91
Inside lateral[13]	18.65	19.53	20.04	20.05	1.16	0.68	0.25	0.64
Inside area[14]	4359.42	4124.93	4456.94	4247.51	295.70	0.81	1.00	0.96
Bone width[15]	41.86	41.80	42.23	42.16	0.90	0.97	0.67	0.99
Medullary width[16]	19.35	18.44	19.30	18.94	1.16	0.90	0.93	0.77
Cortical width[17]	22.51	23.35	22.94	23.21	0.89	0.82	0.58	0.69

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.

[2]Medial = BMC_{RP} expressed in RBAE (mm Al) on the very most outside part of the bone on the medial side.

3Lateral = BMC_{RP} expressed in mm Al on the very most outside part of the bone on the lateral side.

[4]X1= Bone width measurement on outside of bone, mm.

[5]X2 = Bone width measurement on outside of bone, mm.

[6]Area = Area of bone, mm^2

[7]Peak medial= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the medial side.

[8]Peak lateral= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the lateral side.

[9]Medullary low = BMC_{RP} expressed in mm Al at the least mineralized part of the bone.

[10]Inside X1 = Bone width measurement on inside of bone, mm.

[11]Inside X2 = Bone width measurement on inside of bone, mm.

[12]Inside medial = BMC_{RP} expressed in mm Al on the most inside part of the bone on the medial side.

[13]Inside lateral Y2 = BMC_{RP} expressed in mm Al on the most inside part of the bone on the lateral side.

[14]Inside area = Area on the inside of bone, mm^2 .

[15]Bone width = X2 – X1, mm.

[16]Medullary width = Inside X2 – inside X, mm.

[17]Cortical width = Bone width – medullary width, mm

[18]n = 43

Table 38. Effect of parity on BMC_{RP} of caudal vertebrae 14 in dairy cattle.

Parity[1]	1	2	3	4+	SEM[19]	*P* <	Linear	Quadratic
Medial[2]	3.49	3.27	3.79	3.94	0.35	0.33	0.14	0.49
Lateral[3]	3.01	2.88	2.96	3.27	0.22	0.35	0.28	0.21
X1[4]	4.11	4.80	5.57	4.53	0.71	0.42	0.43	0.13
X2[5]	11.80	13.40	15.16	13.94	0.98	0.07	0.02	0.07
Area[6]	405.67	421.72	449.99	509.41	52.15	0.25	0.07	0.59
Peak medial[7]	5.18	4.97	5.59	5.78	0.40	0.29	0.10	0.53
Peak lateral[8]	5.12	5.28	5.48	5.69	0.29	0.32	0.08	0.91
Medullary Low[9]	3.81	3.66	4.24	4.28	0.24	0.09	0.02	0.62
Length[10]	39.49	44.13	45.22	43.29	2.78	0.32	0.21	0.14
Inside X1[11]	6.09	6.93	8.78	7.06	0.71	0.04	0.06	0.03
Inside X2[12]	9.84	11.48	12.45	11.90	0.92	0.12	0.04	0.13
Inside medial[13]	4.70	4.67	4.91	4.87	0.29	0.89	0.48	0.98
Inside lateral[14]	4.96	5.05	5.37	5.38	0.38	0.70	0.24	0.89
Inside area[15]	202.46	220.20	179.40	266.58	35.62	0.13	0.24	0.21
Bone width[16]	7.48	8.48	9.59	9.38	0.84	0.15	0.03	0.36
Medullary width[17]	3.76	4.55	3.67	4.84	0.63	0.23	0.30	0.71
Cortical width[18]	3.72	3.93	5.92	4.55	0.62	0.05	0.05	0.11

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.

[2]Medial = BMC_{RP} expressed in RBAE (mm Al) on the very most outside part of the bone on the medial side.

[3]Lateral = BMC_{RP} expressed in mm Al on the very most outside part of the bone on the lateral side.

[4]X1= Bone width measurement on outside of bone, mm.

[5]X2 = Bone width measurement on outside of bone, mm.

[6]Area = Area of bone, mm^2.

[7]Peak medial= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the medial side.

[8]Peak lateral= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the lateral side.

[9]Medullary low = BMC_{RP} expressed in mm Al at the least mineralized part of the bone.

[10]Length = length of bone, mm.

[11]Inside X1 = Bone width measurement on inside of bone, mm.

[12]Inside X2 = Bone width measurement on inside of bone, mm.

[13]Inside medial = BMC_{RP} expressed in mm Al on the most inside part of the bone on the medial side.

[14]Inside lateral Y2 = BMC_{RP} expressed in mm Al on the most inside part of the bone on the lateral side.

[15]Inside area = Area on the inside of bone, mm^2 .

[16]Bone width = X2 – X1, mm.

[17]Medullary width = Inside X2 – inside X, mm.

[18]Cortical width = Bone width – medullary width, mm

[19]n = 43

Table 39. Effect of parity on BMC_{RP} of caudal vertebrae 15 in dairy cattle.

Parity[1]	1	2	3	4+	SEM[19]	*P* <	Linear	Quadratic
Medial[2]	3.21	3.45	3.52	3.41	0.45	0.29	0.17	0.43
Lateral[3]	2.96	2.93	3.68	3.25	0.47	0.62	0.35	0.59
X1[4]	4.13	4.21	5.34	4.94	0.55	0.28	0.08	0.58
X2[5]	11.32	11.92	14.15	12.97	0.85	0.07	0.03	0.19
Area[6]	348.04	365.16	391.90	416.88	48.66	0.58	0.18	0.92
Peak medial[7]	4.85	4.84	5.11	5.37	0.30	0.30	0.09	0.58
Peak lateral[8]	4.87	4.86	5.20	5.40	0.29	0.27	0.07	0.64
Medullary Low[9]	3.63	3.66	4.13	3.97	0.25	0.33	0.09	0.62
Length[10]	6.25	6.66	8.21	7.25	0.60	0.09	0.04	0.15
Inside X1[11]	9.52	10.09	11.45	10.80	0.71	0.17	0.04	0.28
Inside X2[12]	36.05	39.46	40.66	39.88	2.74	0.49	0.20	0.33
Inside medial[13]	4.35	4.12	4.55	4.66	0.31	0.42	0.21	0.47
Inside lateral[14]	4.50	4.62	4.84	4.99	0.31	0.47	0.12	0.95
Inside area[15]	160.27	172.93	149.53	181.56	23.89	0.62	0.63	0.60
Bone width[16]	7.19	7.71	8.81	8.04	0.78	0.44	0.19	0.29
Medullary width[17]	3.27	3.42	3.24	3.55	0.54	0.94	0.73	0.85
Cortical width[18]	3.92	4.29	5.57	4.48	0.59	0.18	0.16	0.12

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.

[2]Medial = BMC_{RP} expressed in RBAE (mm Al) on the very most outside part of the bone on the medial side.

3Lateral = BMC_{RP} expressed in mm Al on the very most outside part of the bone on the lateral side.

[4]X1= Bone width measurement on outside of bone, mm.

[5]X2 = Bone width measurement on outside of bone, mm.

[6]Area = Area of bone, mm^2.

[7]Peak medial= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the medial side.

[8]Peak lateral= BMC_{RP} expressed in mm Al at the most mineralized part of the bone on the lateral side.

[9]Medullary low = BMC_{RP} expressed in mm Al at the least mineralized part of the bone.

[10]Length = length of bone, mm.

[11]Inside X1 = Bone width measurement on inside of bone, mm.

[12]Inside X2 = Bone width measurement on inside of bone, mm.

[13]Inside medial = BMC_{RP} expressed in mm Al on the most inside part of the bone on the medial side.

[14]Inside lateral Y2 = BMC_{RP} expressed in mm Al on the most inside part of the bone on the lateral side.

[15]Inside area = Area on the inside of bone, mm^2 .

[16]Bone width = X2 – X1, mm.

[17]Medullary width = Inside X2 – inside X, mm.

[18]Cortical width = Bone width – medullary width, mm

[19]n = 43

Table 40. Effect of parity on BMC_{DXA} and BMD_{DXA} in dairy cattle.

Parity[1]	1	2	3	4	SEM[14]	*P* <	Linear	Quadratic
BMCCV14[2]	1.58	1.51	1.61	1.71	0.28	0.94	0.58	0.15
BMCCV15[3]	1.09	0.92	1.13	1.21	0.24	0.78	0.34	0.15
BMCTOT[4]	5.53	5.14	5.57	5.80	0.95	0.95	0.49	0.20
BMDCV14[5]	0.48	0.48	0.51	0.50	0.02	0.65	0.76	0.10
BMDCV15[6]	0.45	0.46	0.48	0.47	0.02	0.63	0.43	0.25
BMDTOT[7]	0.48	0.48	0.51	0.49	0.02	0.69	0.60	0.14
BMC-UPPER[8]	9.47	13.06	10.69	14.58	1.7	0.07	0.83	0.94
BMC-MID[9]	33.41	44.04	37.83	45.49	4.07	0.06	0.36	0.33
BMCTOT1[10]	43.65	60.05	49.59	61.24	5.76	0.04	0.36	0.56
BMC-UPPER[11]	1.52	1.90	1.65	1.98	0.17	0.10	0.92	0.85
BMC-MID[12]	1.51	1.64	1.53	1.68	0.11	0.46	0.95	0.38
BMDTOT1[13]	1.48	1.67	1.53	1.72	0.12	0.28	0.98	0.50

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.

[2]BMCCV14= BMC_{DXA} of caudal vertebra 14, g

[3]BMC-MID = BMC_{DXA} of mid section of metacarpal, g

[4]BMCTOT= Total BMC_{DXA} of caudal vertebra 14 and15, g/cm^2

[5]BMDCV14= BMD_{DXA} of caudal vertebra 14, g/cm^2

[6]BMC-MID = BMD_{DXA} of mid section of metacarpal, g/cm^2

[7]BMDTOT1 = Total BMD_{DXA} of mid and upper metacarpal, g/cm^2

[8]BMC-UPPER = BMC_{DXA} of upper metacarpal, g

[9]BMCCV15= BMC_{DXA} of caudal vertebra 15, g

[10]BMCTOT1 = Total BMC_{DXA} of mid and upper metacarpal, g

[11]BMC-UPPER= BMD_{DXA} of upper metacarpal, g/cm^2

[12]BMDCV15= BMD_{DXA} of caudal vertebra 15, g/cm^2

[13]BMDTOT= Total BMD_{DXA} of caudal vertebra14 and 15, g/cm^2

[14]n = 43

Table 41. Effect of parity on breaking strength of the metacarpal in dairy cattle.

Parity[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Diameter, mm	34.61	35.67	34.59	35.48	0.42	0.43	0.42	0.90
Thickness, mm	9.74	10.38	10.00	10.05	0.22	0.43	0.67	0.28
Peak load, N	27.34	27.82	27.84	25.52	2.24	0.90	0.66	0.61
Stress, N/mm^2	17.96	17.24	17.94	15.78	1.34	0.47	0.43	0.66
Energy Pkld, N-mm	41.07	35.79	33.54	36.56	4.84	0.86	0.56	0.49

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.
[2]n = 43

Table 42. Effect of parity on breaking strength of caudal vertebrae 14 in dairy cattle.

Parity[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Diameter, mm	7.04	6.83	6.83	7.88	0.42	0.33	0.28	0.22
Thickness, mm	2.01	1.77	2.28	2.06	0.13	0.24	0.34	0.94
Peak load, N	1953.73	2110.76	2286.68	2117.98	182.73	0.81	0.51	0.46
Stress, N/mm^2	30.64	36.26	38.30	29.58	2.43	0.11	0.93	0.02
Energy Pkld, N-mm	3716.40	4259.67	4224.00	3796.05	434.16	0.83	0.93	0.36

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.
[2]n = 43

Table 43. Effect of parity on breaking strength of caudal vertebrae 15 in dairy cattle.

Parity[1]	1	2	3	4	SEM[2]	*P* <	Linear	Quadratic
Diameter, mm	5.86	5.90	5.78	6.60	0.39	0.51	0.33	0.41
Thickness, mm	1.90	1.67	1.97	1.90	0.13	0.64	0.68	0.60
Peak load, N	604.90	1738.37	1571.83	1754.95	184.70	0.91	0.78	0.91
Stress, N/mm2	32.33	38.48	33.88	33.93	3.00	0.69	0.99	0.40
Energy Pkld, N-mm	2715.60	3379.39	2773.86	3016.29	409.52	0.80	0.90	0.67

[1]Parity defined by number of lactations: Parity 1 = 1st lactation; Parity 2 = 2nd lactation; Parity 3 = 3rd lactation; Parity 4 ≥ 4 lactations.

[2]n = 43

FIGURE 1. ANATOMY OF BONE

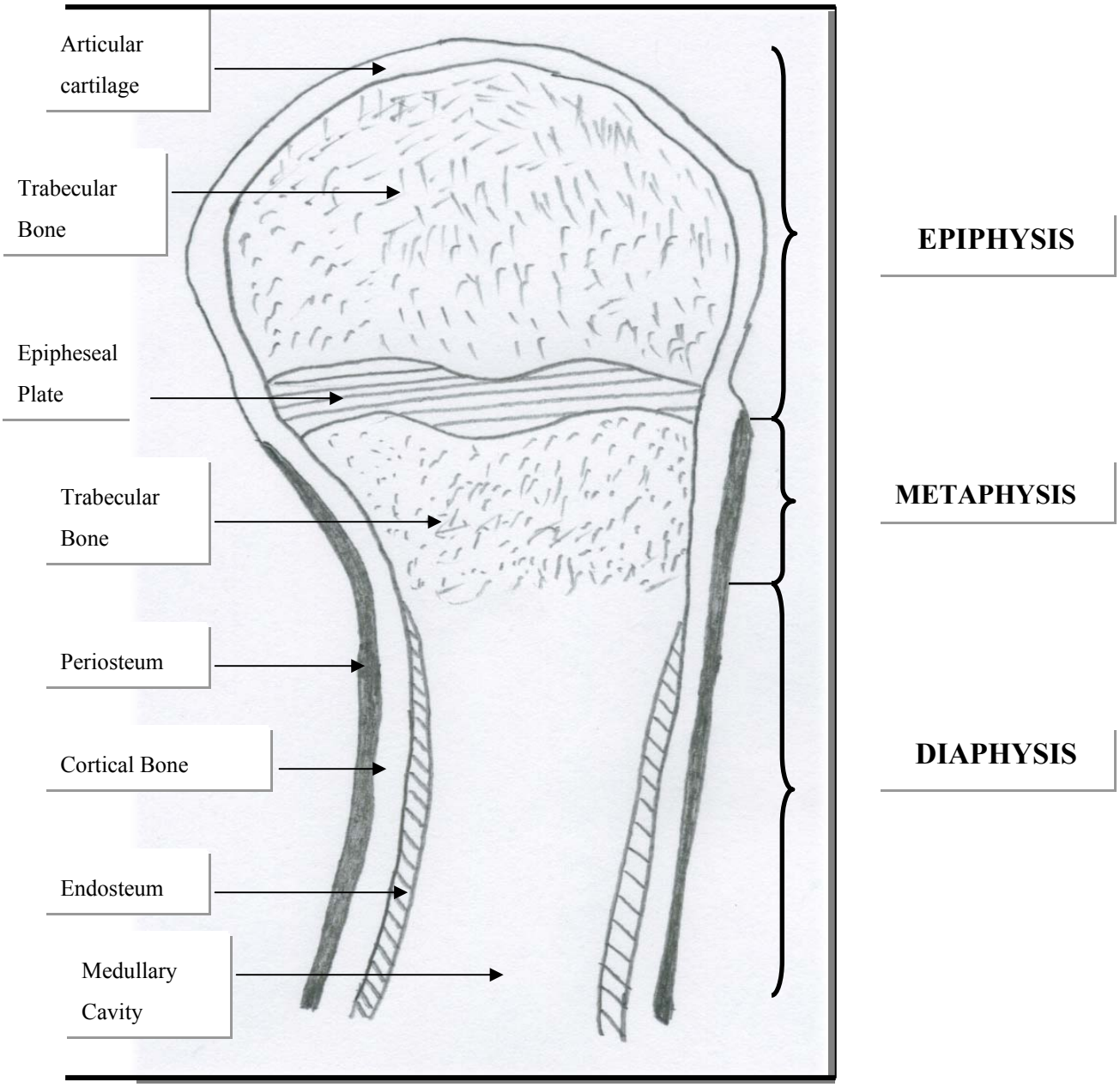


FIGURE 2. RADIOGRAPHIC PHOTOMETRY IN DAIRY CATTLE: CAUDAL VERTEBRAE (LEFT); METACARPAL (RIGHT)

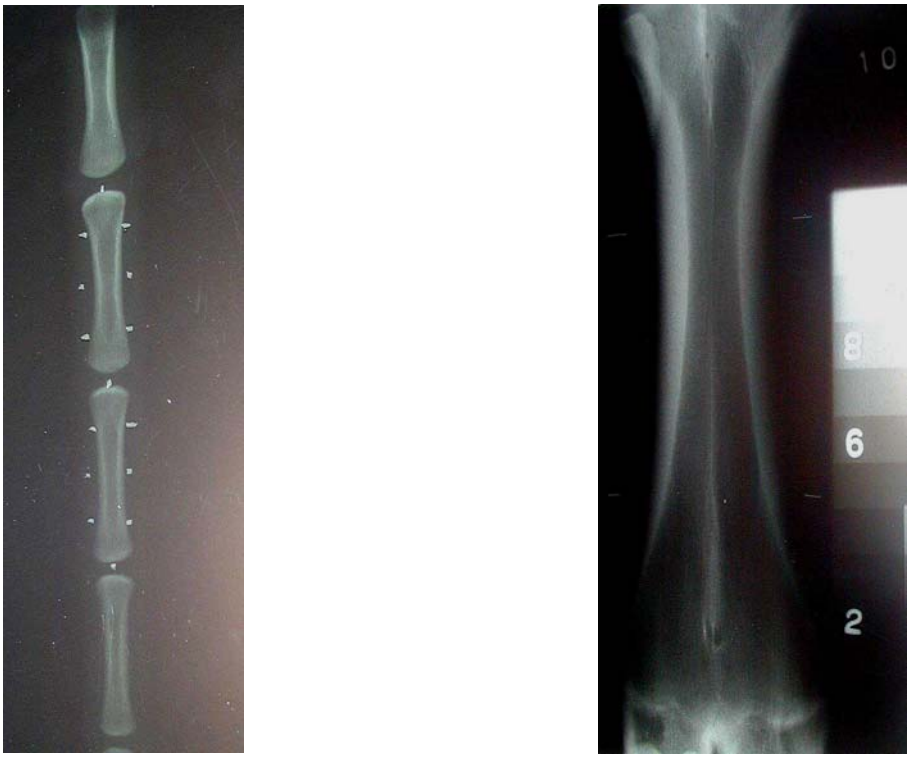


FIGURE 3. X-RAY INTERPRETATION OF A METACARPAL IN DAIRY CATTLE

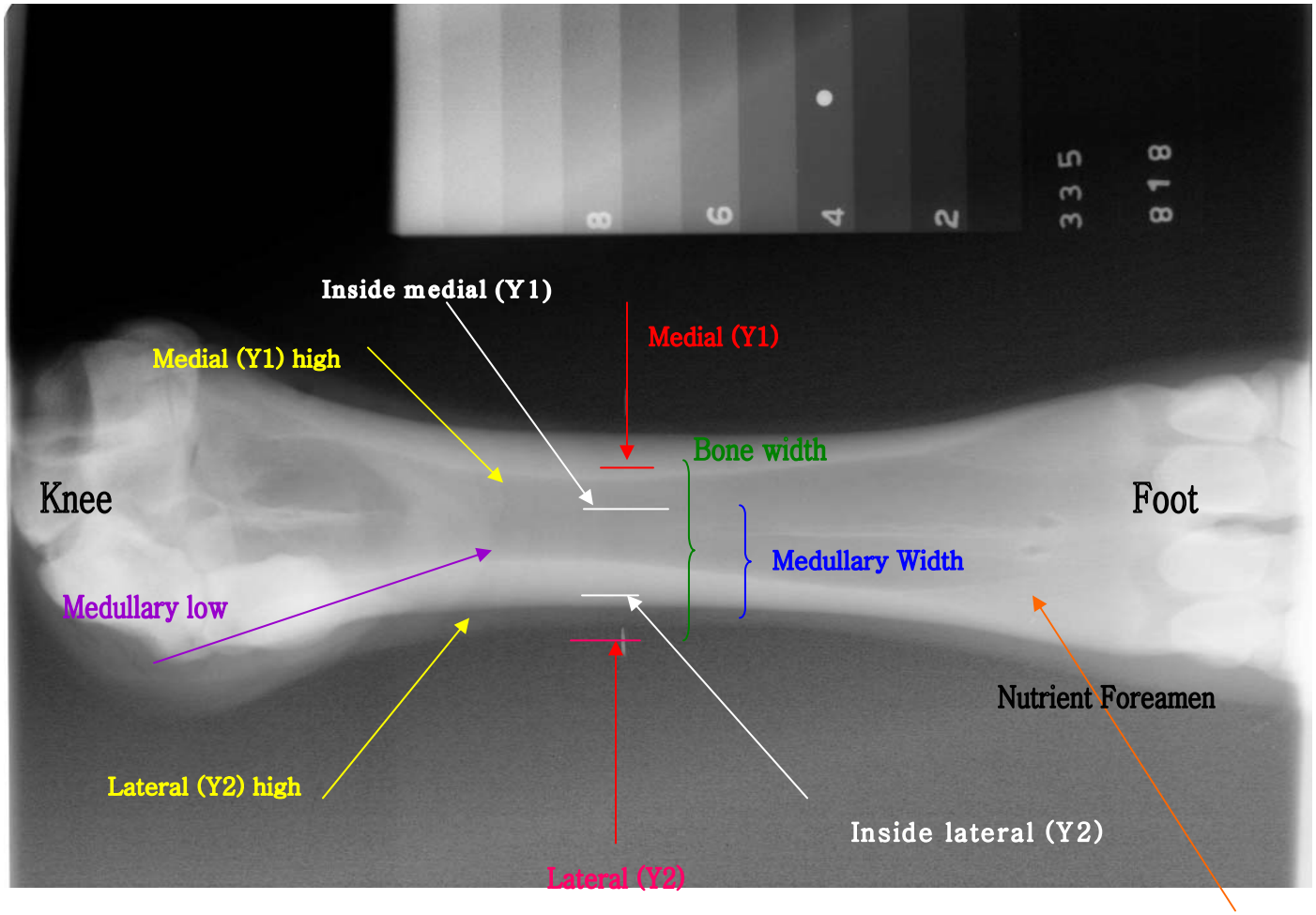


FIGURE 4. DXA SCAN OF A METACARPAL IN DAIRY CATTLE

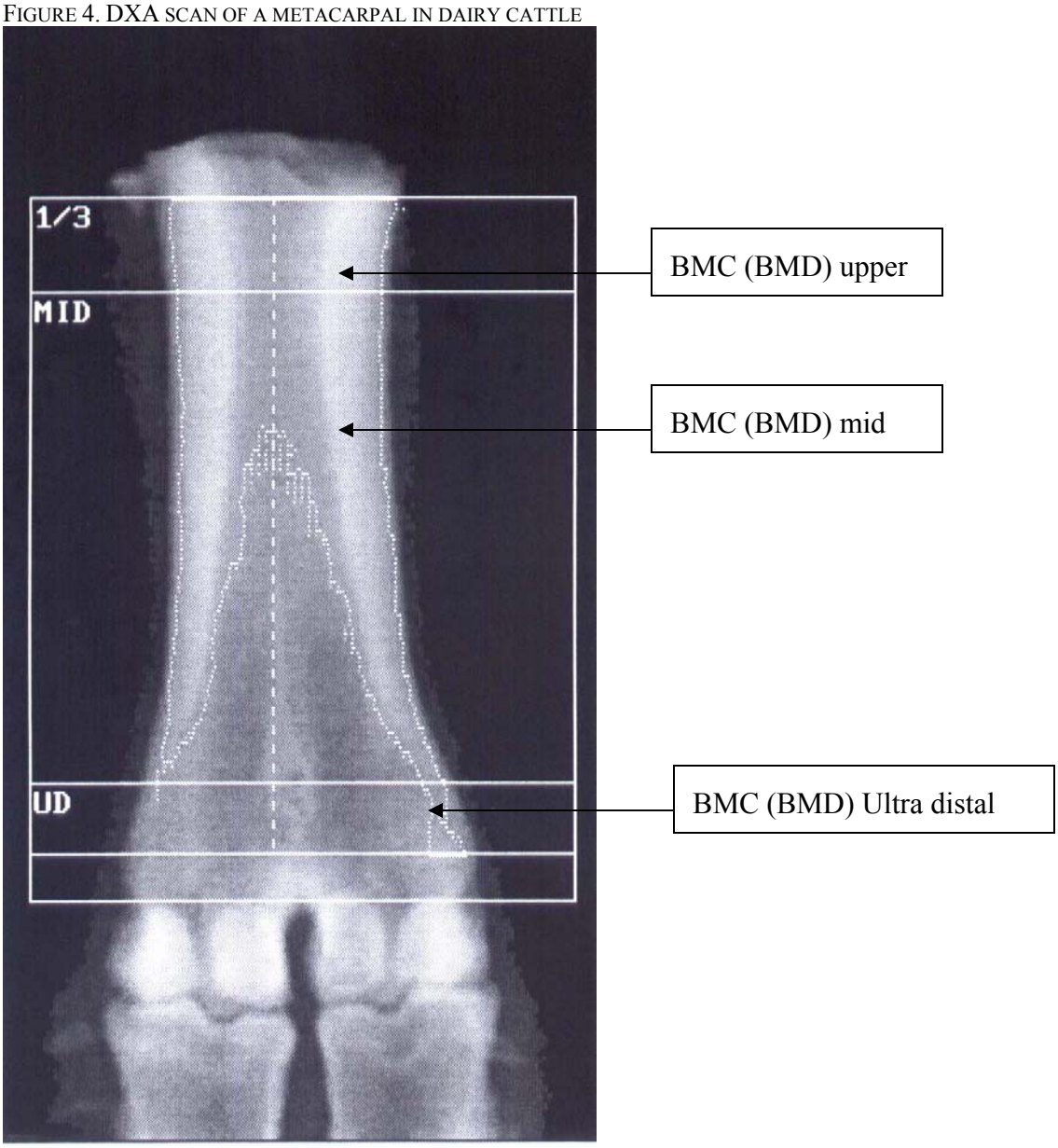


FIGURE 5. FORCE DEFORMATION CURVE

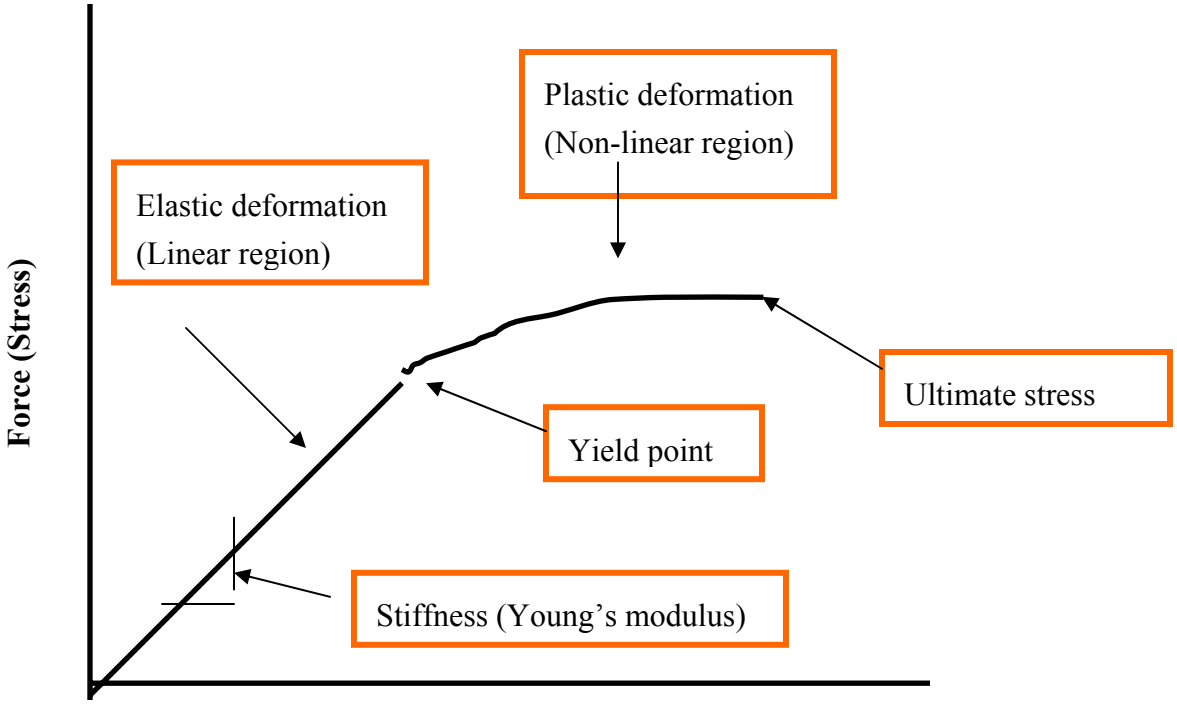
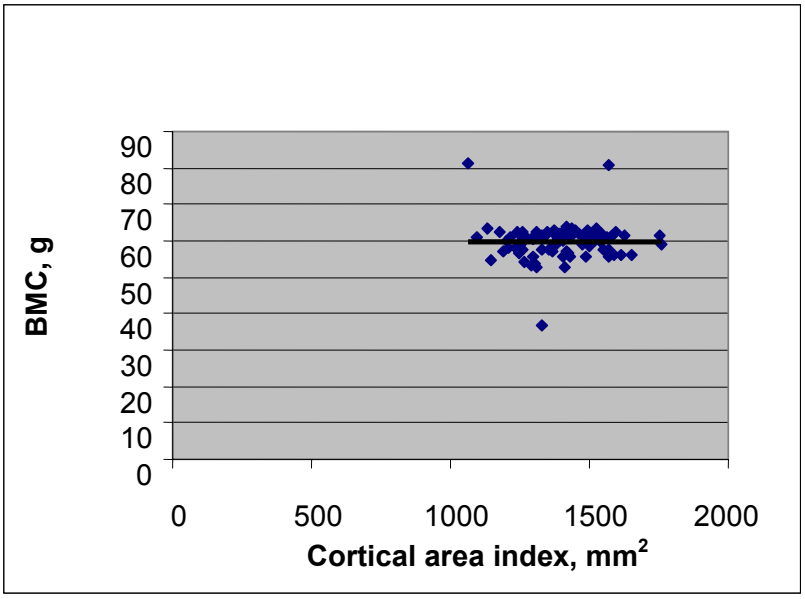
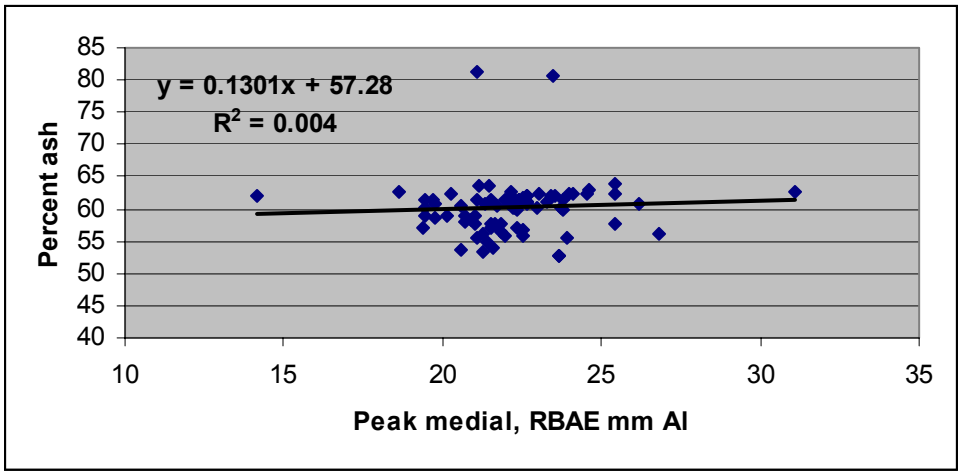


FIGURE 6. CORTICAL AREA INDEX VS. $BMC_{CHEMICAL}$ IN THE METACARPAL IN DAIRY CATTLE[1]



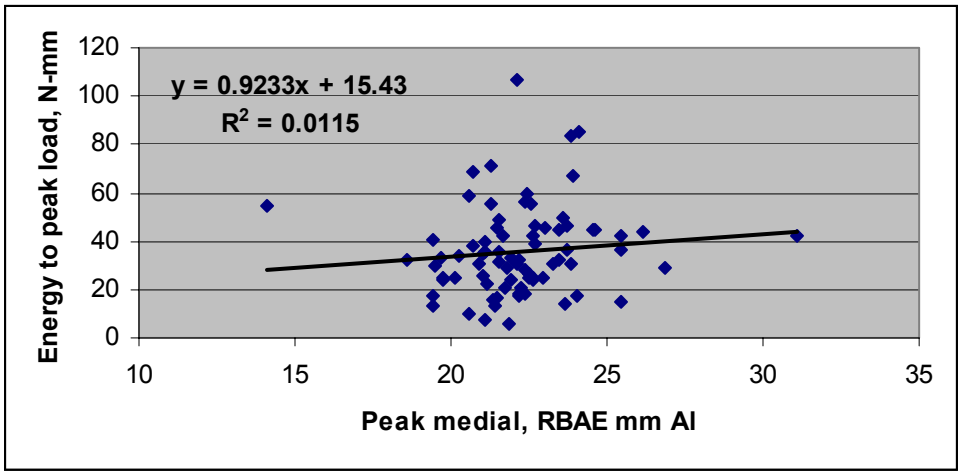
[1]n = 78

FIGURE 7. RELATIONSHIP BETWEEN $BMC_{CHEMICAL}$ AND BMC_{RP} IN THE METACARPAL OF DAIRY CATTLE[1].



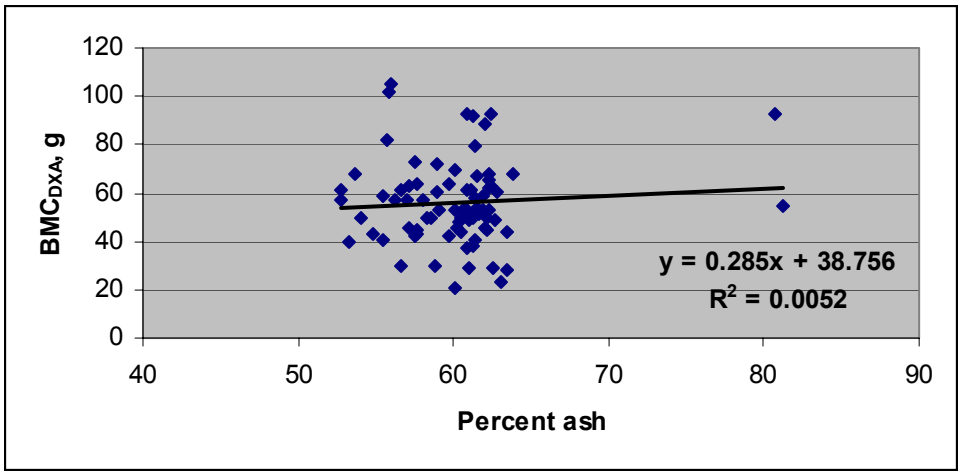
[1]n = 78

FIGURE 8. RELATIONSHIP BETWEEN BREAKING STRENGTH AND BMC_{RP} IN THE METACARPAL OF DAIRY CATTLE[1].



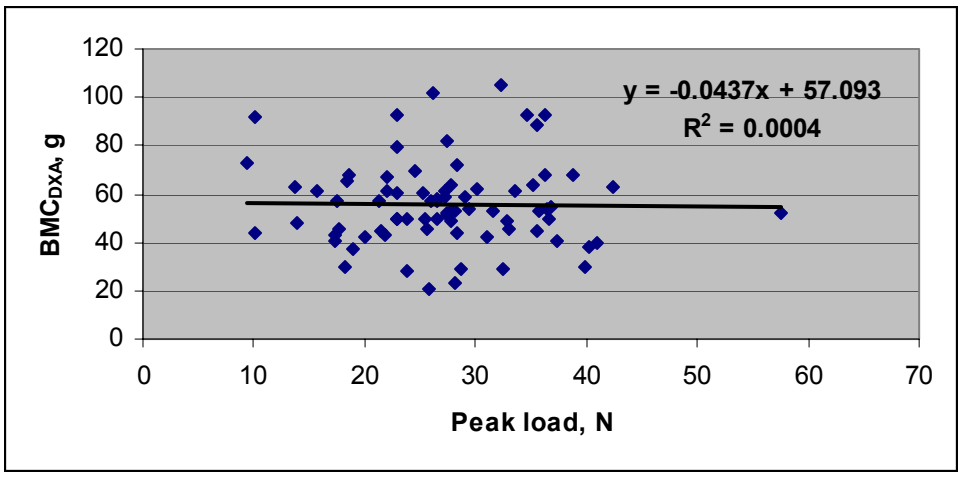
[1]n = 78

FIGURE 9. RELATIONSHIP BETWEEN $BMC_{CHEMICAL}$ AND BMC_{DXA} IN THE METACARPAL OF DAIRY CATTLE[1].



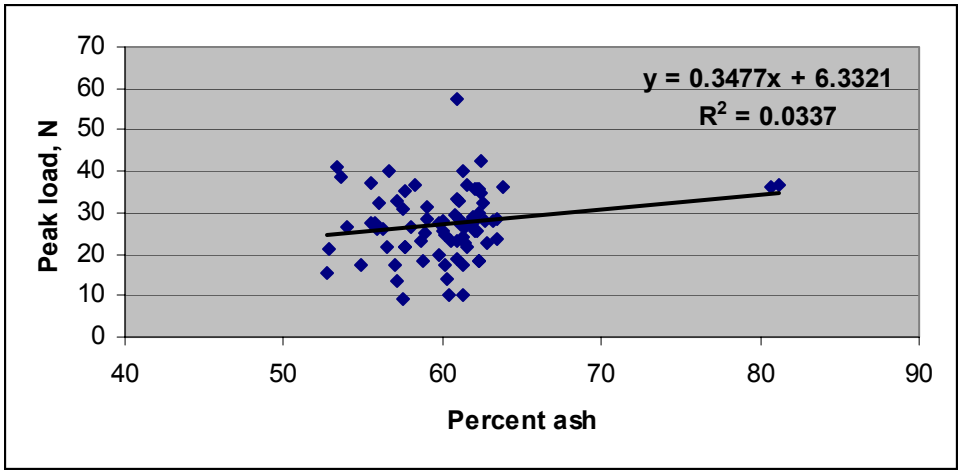
[1]n = 78

FIGURE 10. RELATIONSHIP BETWEEN BREAKING STRENGTH (PEAK LOAD) AND BMC_{DXA} IN THE METACARPAL OF DAIRY CATTLE[1].



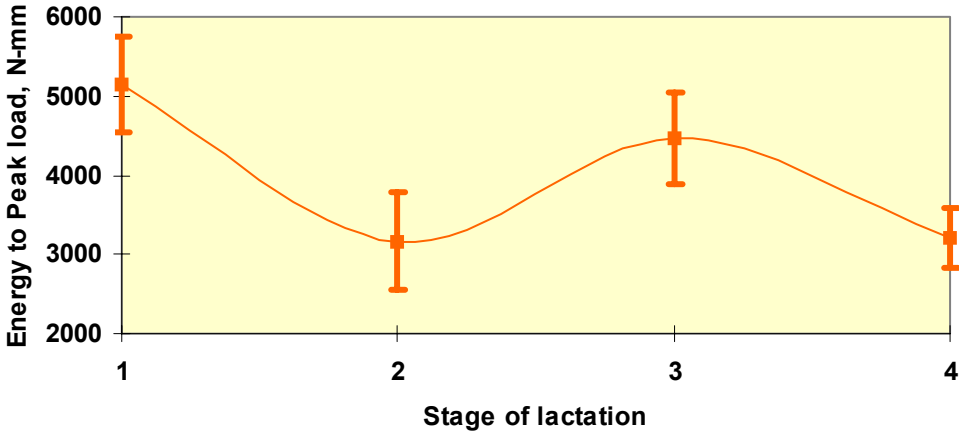
[1]n = 78

FIGURE 11. RELATIONSHIP BETWEEN $BMC_{CHEMICAL}$ AND BREAKING STRENGTH (PEAK LOAD) AND BMC_{DXA} IN THE METACARPAL OF DAIRY CATTLE[1].



[1]n = 78

FIGURE 12. EFFECT OF STAGE OF LACTATION ON ENERGY TO BREAKING STRENGTH (PEAK LOAD) IN CAUDAL VERTEBRAE 14 IN DAIRY CATTLE[1]



[1]n = 43

APPENDIX TABLES

Appendix Table 1. Effect of age on BMC_{DXA} and BMD_{DXA} in dairy cattle.

Age 3	4	5	6	SEM	*P* <	Linear	Quadratic
BMC-CV14	1.42	1.73	1.29	1.77	0.28	0.38	0.57	0.71
BMC-CV15	0.93	1.13	0.80	1.29	0.25	0.31	0.44	0.49
BMD-CV14	0.46	0.50	0.48	0.50	0.03	0.48	0.32	0.82
BMD-CV15	0.43	0.46	0.45	0.48	0.02	0.32	0.14	0.98
BMD-TOT	0.46	0.49	0.48	0.50	0.02	0.45	0.22	0.75
BMC-UPPER	9.64	11.21	12.45	14.76	1.90	0.12	0.03	0.81
BMC-MID	33.72	42.61	39.95	44.43	7.48	0.26	0.10	0.55
BMC-TOT1	44.53	56.55	53.19	60.29	6.40	0.24	0.09	0.64
BMD-UPPER	1.49	1.82	1.73	1.98	0.18	0.18	0.06	0.80
BMD-MID	1.51	1.61	1.56	1.67	0.12	0.70	0.38	0.96
BMD-TOT1	1.48	1.63	1.58	1.71	0.12	0.50	0.22	0.97

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

BMCCV14= BMC of caudal vertebra 14, g

BMCCV15= BMC of caudal vertebra 15,g

BMCTOT= Total BMC of caudal vertebra 14 and15, g/cm^2

BMDCV14= BMD of caudal vertebra 14, g/cm

BMDCV15= BMD of caudal vertebra 15, g/cm^2

BMDTOT= Total BMD of caudal vertebra14 and 15, g/cm^2

BMC-UPPER = BMC of upper metacarpal, g

BMC-MID = BMC of mid section of metacarpal, g

BMCTOT1 = Total BMC of mid and upper metacarpal, g

BMC-UPPER= BMD of upper metacarpal, g/cm^2

BMC-MID = BMD of mid section of metacarpal, g/cm^2

BMDTOT1 = Total BMD of mid and upper metacarpal, g/cm^2

Appendix Table 2. Effect of age on breaking strength of the metacarpal in dairy cattle.

Age	3	4	5	6	SEM	*P* <	Linear	Quadratic
Diameter, mm	34.89	35.41	34.71	35.43	0.41	0.46	0.37	0.84
Thickness, mm	9.74	10.00	10.47	10.07	0.21	0.43	0.24	0.21
Peak Load, kN	26.47	29.42	25.48	25.66	2.18	0.70	0.61	0.61
Stress, N/mm^2	17.45	18.40	16.22	15.90	1.31	0.66	0.37	0.70
Energy Pkld, N-mm	42.17	38.80	29.91	36.35	4.69	0.64	0.33	0.40

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

Appendix Table 3. Effect of age on breaking strength of caudal vertebrae 14 in dairy cattle.

Age	3	4	5	6	SEM	*P* <	Linear	Quadratic
Diameter, mm	6.84	7.11	6.99	7.73	0.41	0.55	0.29	0.65
Thickness, mm	1.93	1.85	2.24	2.05	0.13	0.45	0.30	0.71
Peak Load, N	1778.19	2256.88	2053.09	2166.97	172.90	0.49	0.34	0.40
Stress, N/mm^2	29.97	35.87	31.97	32.09	2.48	0.61	0.86	0.36
Energy Pkld, N-mm	3298.72	4647.51	3945.62	3765.52	404.33	0.32	0.77	0.14

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

Appendix Table 4. Effect of age on breaking strength of caudal vertebrae 15 in dairy cattle.

Age	3	4	5	6	SEM	*P* <	Linear	Quadratic
Diameter, mm	5.61	6.32	5.67	6.47	0.38	0.46	0.38	0.92
Thickness, mm	1.81	1.86	1.85	1.87	0.13	1.00	0.84	0.93
Peak Load, N	1319.30	1979.33	1447.46	1763.96	167.10	0.15	0.41	0.42
Stress, N/mm^2	29.75	37.18	33.79	35.64	2.87	0.55	0.40	0.44
Energy Pkld, N-mm	2222.91	3731.72	2636.28	3006.74	374.86	0.17	0.57	0.23

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

Appendix Table 5. Effect of age on BMC_{RP} in the metacarpal in dairy cattle.

Age	3	4	5	6	SEM	*P* <	Linear	Quadratic
Medial	12.82	9.34	10.27	12.75	2.49	0.47	0.74	0.30
Lateral	13.93	14.26	12.53	10.78	2.65	0.47	0.21	0.61
X1	13.27	9.99	12.27	11.11	1.64	0.29	0.45	0.40
X2	55.35	52.55	53.67	52.93	1.89	0.53	0.33	0.48
Area	8520.76	8883.20	9466.65	9099.75	524.37	0.55	0.19	0.37
Peak medial	21.98	21.29	23.33	22.09	0.96	0.42	0.46	0.71
Peak lateral	21.05	21.27	22.01	21.01	0.78	0.76	0.74	0.35
Medullary Low	16.93	17.37	19.28	17.70	0.96	0.31	0.19	0.18
Inside X1	24.52	22.02	23.93	23.16	1.70	0.56	0.70	0.51
Inside X2	44.84	41.33	41.73	41.64	1.93	0.33	0.16	0.25
Inside medial	18.79	19.16	20.37	20.59	1.32	0.48	0.14	0.94
Inside lateral	18.33	19.21	20.84	20.10	1.21	0.30	0.07	0.33
Inside area	4502.78	4344.79	4236.55	4120.80	313.64	0.67	0.24	0.93
Bone width	42.07	42.56	41.40	41.82	0.95	0.76	0.46	0.96
Medullary width	20.32	19.31	17.81	18.48	1.19	0.36	0.86	0.55
Cortical width	21.76	23.25	23.59	23.33	0.91	0.31	0.27	0.64

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

Medial = Bone mineral content (BMC) expressed in radiographic bone aluminum equivalents (RBAE; mm Al) on the very most outside part of the bone on the medial side.

Lateral = Bone mineral content (BMC) expressed in mm Al on the very most outside part of the bone on the lateral side.

X1= Bone width measurement on outside of bone, mm.

X2 = Bone width measurement on outside of bone, mm.

Area = Area of bone, $mm^{2.}$

Peak medial= Bone mineral content (BMC) expressed in mm Al at the most mineralized part of the bone on the medial side.

Peak lateral= Bone mineral content (BMC) expressed in mm Al at the most mineralized part of the bone on the lateral side.

Medullary low = Bone mineral content (BMC) expressed in mm Alat the least mineralized part of the bone.

Inside X1 = Bone width measurement on inside of bone, mm.

Inside X2 = Bone width measurement on inside of bone, mm.

Inside medial = Bone mineral content (BMC) expressed in mm Al on the most inside part of the bone on the medial side.

Inside lateral Y2 = Bone mineral content (BMC) expressed in mm Al on the most inside part of the bone on the lateral side.

Inside area = Area on the inside of bone, $mm^{2.}$

Bone width = X2 – X1, mm.

Medullary width = Inside X2 – inside X1, mm.

Cortical width = Bone width – medullary width, mm.

Appendix Table 6. Effect of age on BMC_{RP} of caudal vertebrae 14 in dairy cattle.

Age	3	4	5	6	SEM	*P* <	Linear	Quadratic
Medial	3.57	3.46	3.35	3.88	0.35	0.47	0.52	0.26
Lateral	3.00	2.93	2.81	3.27	0.22	0.21	0.39	0.13
X1	4.63	4.73	4.37	4.60	0.73	0.98	0.87	0.91
X2	12.30	13.56	12.84	14.00	1.04	0.45	0.25	0.95
Area	390.93	443.25	412.41	504.48	50.82	0.17	0.10	0.63
Peak medial	5.08	5.24	5.13	5.75	0.40	0.33	0.19	0.47
Peak lateral	5.13	5.49	5.01	5.64	0.28	0.16	0.31	0.56
Medullary Low	3.67	3.98	3.76	4.24	0.24	0.14	0.10	0.66
Length	40.80	44.83	39.48	43.39	2.75	0.35	0.81	0.98
Inside X1	6.46	6.99	6.89	7.26	0.78	0.82	0.42	0.90
Inside X2	10.46	11.44	10.64	11.92	0.96	0.45	0.31	0.84
Inside medial	4.58	4.85	4.63	4.88	0.29	0.72	0.52	0.97
Inside lateral	4.92	5.31	4.81	5.35	0.36	0.46	0.56	0.81
Inside area	208.60	223.48	180.35	256.59	36.10	0.27	0.45	0.30
Bone width	7.38	8.72	8.49	9.38	0.84	0.20	0.07	0.73
Medullary width	4.01	4.46	3.74	4.66	0.65	0.57	0.60	0.66
Cortical width	3.37	4.27	4.75	4.72	0.65	0.29	0.06	0.38

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

Medial = Bone mineral content (BMC) expressed in radiographic bone aluminum equivalents (RBAE; mm Al) on the very most outside part of the bone on the medial side.

Lateral = Bone mineral content (BMC) expressed in mm Al on the very most outside part of the bone on the lateral side.

X1= Bone width measurement on outside of bone, mm.

X2 = Bone width measurement on outside of bone, mm.

Area = Area of bone, $mm^{2.}$

Peak medial= Bone mineral content (BMC) expressed in mm Al at the most mineralized part of the bone on the medial side.

Peak lateral= Bone mineral content (BMC) expressed in mm Al at the most mineralized part of the bone on the lateral side.

Medullary low = Bone mineral content (BMC) expressed in mm Alat the least mineralized part of the bone.

Inside X1 = Bone width measurement on inside of bone, mm.

Inside X2 = Bone width measurement on inside of bone, mm.

Inside medial = Bone mineral content (BMC) expressed in mm Al on the most inside part of the bone on the medial side.

Inside lateral Y2 = Bone mineral content (BMC) expressed in mm Al on the most inside part of the bone on the lateral side.

Inside area = Area on the inside of bone, $mm^{2.}$

Bone width = X2 – X1, mm.

Medullary width = Inside X2 – inside X1, mm.

Cortical width = Bone width – medullary width, mm.

Appendix Table 7. Effect of age on BMC_{RP} of caudal vertebrae 15 in dairy cattle.

Age	3	4	5	6	SEM	*P* <	Linear	Quadratic
Medial	3.01	3.13	3.16	3.67	0.45	0.45	0.20	0.58
Lateral	3.14	2.92	3.48	3.18	0.48	0.83	0.70	0.92
X1	4.40	3.95	5.16	4.94	0.54	0.25	0.16	0.80
X2	11.73	11.80	12.72	13.08	0.90	0.43	0.13	0.84
Area	343.50	369.98	356.81	420.18	47.45	0.42	0.22	0.63
Peak medial	4.73	5.02	4.79	5.36	0.29	0.15	0.12	0.54
Peak lateral	4.83	5.01	4.82	5.39	0.28	0.18	0.16	0.40
Medullary Low	3.55	3.78	3.77	4.00	0.24	0.41	0.14	0.99
Inside X1	6.47	6.55	7.36	7.35	0.63	0.46	0.13	0.93
Inside X2	9.98	9.91	10.49	10.84	0.73	0.59	0.24	0.71
Length	37.21	40.59	34.79	39.86	2.64	0.25	0.82	0.69
Inside medial	4.33	4.30	4.09	4.67	0.30	0.31	0.46	0.21
Inside lateral	4.36	4.75	4.56	5.00	0.29	0.22	0.11	0.92
Inside area	164.90	170.25	150.34	179.30	23.77	0.74	0.79	0.54
Bone width	7.33	7.85	7.56	8.14	0.79	0.79	0.46	0.96
Medullary width	3.52	3.36	3.13	3.49	0.54	0.93	0.86	0.55
Cortical width	3.81	4.48	4.44	4.66	0.61	0.65	0.27	0.64

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

Medial = Bone mineral content (BMC) expressed in radiographic bone aluminum equivalents (RBAE; mm Al) on the very most outside part of the bone on the medial side.

Lateral = Bone mineral content (BMC) expressed in mm Al on the very most outside part of the bone on the lateral side.

X1= Bone width measurement on outside of bone, mm.

X2 = Bone width measurement on outside of bone, mm.

Area = Area of bone, $mm^{2.}$

Peak medial= Bone mineral content (BMC) expressed in mm Al at the most mineralized part of the bone on the medial side.

Peak lateral= Bone mineral content (BMC) expressed in mm Al at the most mineralized part of the bone on the lateral side.

Medullary low = Bone mineral content (BMC) expressed in mm Al at the least mineralized part of the bone.

Inside X1 = Bone width measurement on inside of bone, mm.

Inside X2 = Bone width measurement on inside of bone, mm.

Inside medial = Bone mineral content (BMC) expressed in mm Al on the most inside part of the bone on the medial side.

Inside lateral Y2 = Bone mineral content (BMC) expressed in mm Al on the most inside part of the bone on the lateral side.

Inside area = Area on the inside of bone, $mm^{2.}$

Bone width = X2 – X1, mm.

Medullary width = Inside X2 – inside X,, mm.

Cortical width = Bone width – medullary width, mm.

Appendix Table 8. Effect of age on $BMC_{CHEMICAL}$ of the metacarpal in dairy cattle.

Age	3	4	5	6	SEM	*P* <	Linear	Quadratic
Ca, % ash	41.00	40.95	41.07	41.14	0.25	0.92	0.61	0.77
Ca, %DM	24.56	25.73	23.11	24.46	0.89	0.19	0.44	0.91
P, % ash	18.14	18.02	17.75	17.96	0.17	0.45	0.26	0.27
P, %DM	10.86	11.32	9.98	10.67	0.39	0.10	0.24	0.73
Mg, % ash	0.59	0.59	0.56	0.56	0.01	0.10	0.02	0.79
Mg, %DM	0.36	0.37	0.32	0.33	0.01	0.30	0.03	0.83
% ash	59.90	62.83	56.30	59.46	2.18	0.18	0.39	0.95

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

Appendix Table 9. Effect of age on $BMC_{CHEMICAL}$ of caudal vertebrae 14 in dairy cattle.

Age	3	4	5	6	SEM	*P* <	Linear	Quadratic
Ca, % ash	11.10	11.23	12.39	12.25	1.47	0.84	0.42	0.91
Ca, %DM	39.60	40.41	39.90	39.70	0.22	0.01	0.78	0.008
P, % ash	4.15	4.80	5.35	4.67	0.83	0.77	0.52	0.34
P, %DM	17.24	17.15	17.39	16.96	0.22	0.35	0.52	0.34
Mg, % ash	0.17	0.19	0.19	0.17	0.028	0.82	0.87	0.34
Mg, %DM	0.68	0.67	0.63	0.63	0.015	0.005	0.001	0.66
% ash	0.28	0.28	0.31	0.31	0.036	0.80	0.37	0.96

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

Appendix Table 10. Effect of age on $BMC_{CHEMICAL}$ of caudal vertebrae 15 in dairy cattle.

Age	3	4	5	6	SEM	*P* <	Linear	Quadratic
Ca, % ash	11.10	11.23	12.39	12.25	1.47	0.84	0.38	0.82
Ca, %DM	39.61	40.41	39.90	39.70	0.22	0.01	0.91	0.86
P, % ash	4.15	4.80	5.35	4.67	0.83	0.77	0.08	0.92
P, %DM	17.24	17.15	17.39	16.96	0.22	0.35	0.46	0.91
Mg, % ash	0.17	0.19	0.19	0.17	0.028	0.82	0.0001	0.27
Mg, %DM	0.68	0.67	0.63	0.63	0.014	0.0052	0.04	0.35
% ash	0.28	0.28	0.31	0.31	0.036	0.80	0.99	0.86

Age defined as: Age 3 = 3 yrs. old; Age 4 = 4 yrs. old; Age 5 = 5 yrs. old; Age 6 = ≥ 6 yrs.

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