EFFECTS OF HOUSING, EXERCISE, AND DIET ON BONE DEVELOPMENT OF YEARLING HORSES

By

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To my family

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EFFECTS OF HOUSING, EXERCISE, AND DIET ON BONE DEVELOPMENT OF YEARLING HORSES

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A series of experiments to assess the influence of housing, exercise, and diet on bone development were conducted using yearling horses. The first experiment investigated the impact of housing utilizing pasture and dry lot housed groups. Pasture housed individuals maintained a numerically higher bone mineral content (BMC) and gained significantly ($P < 0.05$) more bone from d 56 to d 112. The second experiment expanded on the first by adding exercise to a portion of the dry lot housed yearlings. Although both pasture housed and dry lot housed yearlings with exercise developed greater BMC than did yearlings housed in dry lots without forced exercise, forced exercise did not result in additional BMC above that of pasture housed individuals. In addition, the group of pastured yearlings differed from both dry lot housed yearlings in bone geometry, modeling bone to the dorsomedial aspect of the third metacarpal (MC III). The actual long term impact of changes in geometry on the quality of the bone has not been established. The hypothesis of the third experiment was that a diet containing

calcium (Ca): phosphorus (P) ratio less than 1:1 would be detrimental to bone metabolism. Repeating the second experiment with a concentrate that was inadequate in Ca in relation to P (0.79:1), it was concluded that no negative effects were observed with the levels of Ca and P provided. Further investigations into bone and mineral metabolism are needed to more accurately define type and length of exercise and amount of Ca and P needed to maximize bone development in yearling horses.

CHAPTER 1
INTRODUCTION

Producing a marketable product at an early age for sale or competition results in a need to develop management techniques that optimize growth and development of the young horse. Housing, exercise, and diet directly impact skeletal strength and structure, essential elements for horses in competition. Influences during the period of rapid growth may impact the horse into its adult life and determine the longevity of its career (van Weeren, et al., 2000). Managing the young horse to maximize the skeletal strength and prevent associated injuries could lead to a decrease in economic loss from catastrophic injuries. Implementing changes in housing, exercise, and diet early in life may improve bone quality thus increasing the length of their athletic career.

Housing practices for young horses differ between regions of the country and segments of the industry, and are often based on space availability and needs of the facility. Confinement to stalls and its impact on young horses has been the subject of recent research specifically investigating bone mineral deposition (Hoekstra et al., 1999; Hiney et al., 2004), growth rates (van Weeren et al., 2000), and behavior (Rivera et al., 2002). Limiting exercise and loading of bone through increased confinement in stalls decreased skeletal strength in comparison to on pasture housed individuals (Barnevald and van Weeren, 1999). Therefore, the objective of the first experiment in this dissertation was to study the impact of group housing in a dry lot on bone mineral content.

The influence of exercise on bone development has also been well documented in the horse (McCarthy and Jeffcott, 1991; McCarthy and Jeffcott 1992; Firth et al., 1999). Response of the skeletal system to exercise varies depending on the amount, type and age of introduction (Torstveit, 2002 and Murray et al. 2001). The influence of biomechanical loading early in life, the changes produced by the loading on the overall quality of the bone, and the subsequent ability of the bone to respond to athletic demand and/or resistance to injury has not been determined nor has the long-term effects of exercising on young horses. Therefore, the objective of the second study of this dissertation was to determine if forced exercise of dry lot housed yearlings would sustain or exceed quantity of bone mineral deposition as compared to dry lot housed yearlings without exercise or pasture housed contemporaries.

Diet affects bone growth, particularly calcium (Ca) and phosphorus (P), which accounts for the inorganic compound of bone that is 65% of the total bone matrix (van der Harst, 2004). Adequate Ca and P intake is essential for proper bone growth. Inadequate intake of either mineral early in life impairs bone development and precludes achievement of peak bone mass essential for structural integrity later in life (Anderson, 1996). Excessive amounts of Ca in the diet do not appear to have detrimental effects in horses if the amount of P in the diet is sufficient (Jordan et al., 1975). However, a ratio of Ca to P less than 1:1 is considered to be detrimental to Ca absorption and can induce nutritional secondary hyperparathyroidism that results in skeletal malformations (Schryver et al., 1971). The hypothesis of the third study in this dissertation was that an inverse Ca:P ratio would result in impaired bone development and could possibly be exacerbated by exercise. The objective was to determine the extent bone mineral deposition was impaired by diet and exercise in the yearling horse.

CHAPTER 2
REVIEW OF LITERATURE

Bone Metabolism and Development

Metabolism

Bone formation and resorption are tightly coupled processes, together contributing to bone remodeling that are regulated by local and endocrine factors. Remodeling of bone is a continuous process by which bone increases in size as well as strength. Remodeling serves a repair function in bones subjected to mechanical stress. Bone is constantly being destroyed or resorbed by osteoclasts and then replaced by osteoblasts. The function of these two distinct cell types, the osteoblast, or bone-forming cells, and the osteoclast, or bone-resorbing cells are intimately linked.

The remodeling process involves five stages – quiescence, activation, resorption, reversal, formation, and again quiescence (Parfitt, 1984). The stages are so ordered that bone resorption always precedes bone deposition (Parfitt and Chir, 1987). Basic multicellular units (BMU) carry out the bone remodeling in singular clusters on the bone surface (Frost, 1987).

Activation is the least understood of the five remodeling stages since the biochemical signal for activation at a specific location is poorly understood. Several hypotheses have been developed yet no definitive answer has been revealed. It is known that the activation stage requires the recruitment of osteoclasts to the site where remodeling is to occur in order to begin the bone remodeling process. Osteoclasts infiltrate the cellular and connective tissue to reach the previously inactivated surface.

This allows for the second step, resorption, to occur by exposing the bone surface and creating a “clear zone” (Mundy, 1990). The rate and duration of bone resorption may be regulated by several factors including genetics, as well as local and/or systemic factors (Jaworski, 1984). The resorption phase lasts for approximately 1 to 3 weeks depending on the size of the activation site (Parffit and Chir, 1987).

The reversal period varies in length from 1 to 2 weeks and is again dependent on the size of the activation site (Parfitt and Chir, 1987). The bone forming cells, osteoblasts, are recruited to the site by an unknown biochemical signaling system possibly involving a strain-regulated mechanism (Smit and Beuger, 2000).

Bone formation is the fourth step and is a two part process. The osteoblasts form teams that produce and secrete the protein matrix of bone (Baron, 1990). Approximately 70% of the mineral is deposited during the first two weeks of mineralization (Pool, 1991), but the maximum density is not reached for 3-6 months (Parfitt and Chir, 1987). When the bone remodeling process is complete, the bone returns to quiescence, the fifth stage of bone remodeling.

Bone responds to patterns of loading or strain in order to achieve a balance between strength and mass (Rubin, 1984). A German scientist in the late nineteenth century named J. Wolff was the first to describe the ability of bone to alter its mass and shape to a load or mechanical strain (Frost, 2001). A translation of the original German to English reads (Rasche and Burke, 1962): “Every change in the form and function of bone or of their function alone is followed by certain definite changes in their internal architecture, and equally definite alteration in their external conformation, in accordance with mathematical laws.” In applying Wolff’s Law, the result of any increased activity above that normally experienced by an animal places unique strain on the bone which subsequently activates the bone modeling/remodeling process. Bone formation has been shown to be directly proportional to strain rate (Burr et al., 2002). Direct actions on bone cells by hormones, calcium, phosphorus, vitamin D, and genetics determine 3 – 10% of total strength, but mechanical usage effects on bone modeling and remodeling determine over 40% (Kiratli, 1996).

The bone modeling/remodeling process involves the addition of mineral to increase bone density or a change in the pre-existing shape by adding or removing bone. It has yet to be determined if density or shape of the bone is more important for strength. Similarly it is unknown if remodeling of bone is an age-related or an exercise regulated event. Whalen et al. (1993) considers the primary factor influencing the strength of long bones to be the moment of inertia or shape and not necessarily the overall density. Bending strength and modulus of elasticity was not different in horses ranging in age from 2 months to 4 years; thus, younger horses may not be mechanically deprived in comparison to its older equivalent (Bigot et al., 1996). A combination of all these factors is the most plausible explanation of how, when and why the bone remodeling takes place.

Development

Bone development in the growing horse is initiated *in utero* and continues until the animal is about five years of age, although most of the limb development is completed by thirty-six months of age in light horses. Initial ossification begins in the 9 week-old fetus with the development of the femur and tibia from cartilaginous processes. Bone mineral density of the third metacarpal (MC III) increases rapidly from day 15 to day 135 by 52% in pasture raised foals (Firth et al., 2000). After 6 months of age, foals experience an increase in periosteal apposition coupled with a decrease in bone mineral density that corresponds with replacement of primary bone with secondary osteons (Stover et al., 1992; Cornelissen et al., 1999). Removal of primary bone and incompletely filled secondary osteons leave resorption cavities that can be observed in yearling and two year-old horses (Stover et al., 1992) while others (Riggs and Boyd, 1999) reported that this event mainly occurs in 2 and 3 year-olds. Since skeletal maturity is not achieved until 4 – 6 years of age (Lawrence et al., 1994), opportunity for improvement of or injury to bone during the developmental period is an important consideration in young horses in training.

Rigidity and strength of the bone is determined by both the organic and inorganic fractions. Initially, the organic bone is built and then later mineralized. This mineralized tissue confers multiple mechanical and metabolic functions to the skeleton. Bone formation is implicated directly or indirectly in longitudinal bone growth, bone mineralization, and bone remodeling. The bone is unique in that a certain amount of activity is required to maintain bone health, in addition to meeting the nutritional requirements for continued growth and development.

Training

Mechanical loading is important in the adaptation of bone to training. Increases in bone mineral density or cortical bone volume due to exercise by immature horses has been reported by several researchers (McCarthy and Jeffcott, 1991; McCarthy and Jeffcott 1992; Firth et al., 1999). However, excessive loading or loading the bone to fatigue can produce traumatic failure or lead to progressive weakening of bone (Carter and Hayes, 1977). Therefore, physical training may increase bone density and bone mass but, the adaptive response of bone to exercise may depend on several factors including maturity, intensity of training and type of loading (Bennell et al., 1997).

Differing exercise protocols have produced varied results in bone mineral density, cortical thickness and subsequent resistance to stress. The magnitude of loading, type of activity, the rate of activity, and number of repetitions are all important elements in determining the effect of exercise on bone (Torstveit, 2002). Murray et al. (2001) documented an increase in bone thickness, increased bone modeling and reduced bone resorption in high intensity trained horses versus their lower intensity trained contemporaries. High intensity exercise protocol included three works per week at 7-14 m/s averaging 3250 m/work. Low intensity exercise underwent daily walking at approximately 1.7 m/s in both directions on a mechanical walker for a total of 40 min. This is supported by work conducted by Reilly and colleagues (1997) who determined that bone from the more intensely trained horses had higher impact strength. Burr et al. (2002) suggest that short periods with high load rates and sufficient rest between bouts are more effective osteogenic stimulus than a single sustained session of exercise. In another study, it was found that bone mineral density increased with duration of exercise at a constant speed to a point but beyond that no additional benefits were noted with longer duration; thus, concluding that bone adapts only to the current level of excise intensity required (Karlsson et al., 2001).

The influence of biomechanical loading early in life, the changes produced by the loading on the overall quality of the bone, and the subsequent ability of the bone to respond to athletic demand and/or resistance to injury has been the subject of interest for several researchers (van Weeren et al., 2000; Hiney et al., 2004; Brama et al., 2001). Unfortunately, it is unknown at this time what long-term effects of exercising may have on young animals as they have not been studied for periods over 24 months.

The changes produced in young animals have not been proven to persist for long intervals after cessation of the exercise. Barnevald and van Weeren (1999) found that increases in bone mineral density of forced exercised individuals did not persist 11 months after completion of the study when compared to their pasture housed contemporaries. Detraining effects including decrease in bone mineral density or bone mineral content have been well documented in other species (Yeh and Aloia, 1990; LeBlanc et al. 1990). However, it has been theorized that alterations made in bone geometry may be less susceptible to detraining effects than bone mineral density (Nelson and Bouxsein, 2001).

If training alters the bone geometry of a young animal in such a way as to prepare it for future athletic activity, then there may be a significant advantage in subjecting the animal to osteogenic stimulation early in its athletic career. However, exercise protocols that will effectively stimulate bone change without eliciting adverse effects have not been determined for young horses. Detrimental effects were seen in the soft tissue (tendons and cartilage) of foals subjected to an intense exercise protocol in comparison to there pasture and box stall raised contempories (van Weeren et al., 2000). The authors proposed that similar results in the increase of bone mineral content with forced exercise without the detrimental effects on tendon and cartilage quality may have been achieved with a less vigorous exercise.

Housing

Housing can play a significant role in the development of musculoskeletal system with the focus of many researchers on the influence of confinement and subsequent disuse (Hoekstra et al., 1999; Barneveld and van Weeren, 1999). Lack of exercise or disuse negatively impacts skeletal development and has been shown to cause a reduction in bone mass (Porr et al., 1998; Buckingham and Jeffcott, 1991). Relocation of foals from a pasture to stalled environment resulted in decreased osteocalcin concentrations inferring a decrease in bone formation (Maenpaa et al., 1988). In both weanlings and yearlings housed in stalls, a decrease in bone mineral content was found when compared to their pasture raised contemporaries (Hoekstra et al., 1999; Barneveld and van Weeren, 1999; Bell et al., 2001). Differing impacts of housing of the young versus mature equine has not been specifically studied; however, the young may be more sensitive to restriction of exercise, as was the case in a study in which 1 week old (young) rats had more bone loss than 3 week old (mature) rats after the cessation of a regular exercise program (Steinberg and Trueta, 1981).

The ability of bone in young, growing horses to recover from prolonged confinement has yet to be determined. The bones of a young animal may be more capable of recovery than a more mature individual (Tsuji et al., 1996). In foals housed in box stalls for the first 5 months of life, there was a reduced quantity of bone mineral density. Yet at 11 months of age, no differences were seen between the confined and pasture reared individual; therefore, older foals may be able to compensate for long periods of confinement (Cornelissen et al., 1999). Nonetheless, implementing strenuous training program on a stall reared foal without adequate acclimation may prove hazardous and have serious impacts on future athletic activity.

Calcium

Ninety-nine percent of the calcium in the body is found in teeth and bone and accounts for 1-2% of the total body weight (Cashman, 2002). The skeleton serves not only in a structural role but also as a reservoir for Ca. In times of deficiency or increased demand, Ca can be mobilized from the bone, but can result in weakened skeleton if removed in excess. In humans, inadequate intake of Ca early in life impairs bone development and precludes achievement of peak bone mass essential for structural integrity later in life (Anderson, 1996). Therefore, it is essential to maintain adequate intake and absorption throughout life and especially during times of rapid growth or stress (i.e. lactation and pregnancy).

Plasma Ca levels provide no indication of net Ca balance; therefore, it is not unusual for horses in a negative Ca balance to have normal plasma or serum Ca concentrations. Calcium homeostasis is regulated by hormones that act principally upon major organs involved with Ca metabolism: the small intestine, kidneys and skeleton. Parathyroid hormone and active hormone forms of vitamin D_3 are the most important hormones associated with Ca metabolism. Low blood calcium levels stimulate parathyroid hormone (PTH) secretion, which leads to production of the active form of vitamin D that result in resorbtion of Ca and phosphorus (P) from bone with a reflux of the element into the blood. PTH stimulates the production of calcitriol in the kidney, which increases Ca and P uptake in the digestive tract. An excess of Ca stimulates calcitonin, which decreases osteoclastic bone resorbtion, increases osteoblast activity and potentially increases overall Ca losses in the urine.

Dietary Ca is absorbed from the small intestine (Stadermann et al., 1992) and excreted primarily in the feces. The NRC (1989) uses the estimate of 50% absorption efficiency for all classes of horses. Absorption efficiency decreases with age yet it can be up to 70% for young horses. Dietary factors that affect Ca absorption include concentrations of Ca, P, oxalate, and phytate in the diet. Absorption efficiency decreases as Ca and/or P concentrations increases in the diet due to the competitive nature of Ca and P absorption in the small intestine. High dietary oxalate or phytate concentrations decrease Ca absorption. Other feed ingredients in the ration can also influence digestibility (Hoffman et al., 2000; Cooper et al., 2000). By comparison, stage of training may increase Ca digestibility (Stephens et al., 2004).

Calcium requirements for horses increase with increasing physiological stress such as pregnancy and lactation which are adjusted for in the NRC (1989). While the current NRC (1989) increases Ca requirements for exercise, these requirements are based on concomitant increases in energy requirements and do not specifically address exercise related adaptations to bone and muscle. As a result, the Ca requirements for exercise may not be adequate, depending on the composition of the diet. For example, as the diet becomes more energy dense, the amount of feed needed to meet the energy demands decreases and, therefore, the horse might not consume an adequate amount of Ca. In a number of studies, it has been shown that training, especially in a young horse, increases the Ca requirement above that currently suggested by the NRC (1989) (Gray et al., 1998; Nielsen et al.,1998; Stephens et al., 2004). Therefore feeding Ca in excess of the current NRC (1989) requirements could be beneficial in maintaining a positive Ca balance. According to the NRC (1989), Ca concentrations can be fed in excess without negative impacts if P levels are adequate.

Phosphorus

Phosphorus, like Ca, constitutes a major portion of the bone mineral content and is required for numerous energy transfer reactions associated with adenosine diphosphate (ADP) and adenosine triphosphate (ATP).

In the diet, phosphorus exists as one of two types: an organic sugar carbon compound such as inositiol phosphate (phytate) found in plants, or as inorganic salts (bound with calcium) such as calcium phosphates. Phytate phosphorus is less digestible than inorganic phosphate, but may be partially available due to phytase present in the lower gut (Schryver et al., 1971).

The dorsal colon and small colon are the major site of absorption and resorption of P. Absorption of phosphorus is dependent on the quantity of P in the ration, type of P fed, amount of total oxalates present in the diet, age of the horse, and physiological demand. The NRC (1989) states the true P absorption ranges from 35% for idle horses to 45% for lactating and growing horses. The higher P absorption of the latter is due to the routine supplementation of inorganic P to these groups of horses. There is substantial evidence that efficiency of P absorption can vary with demand by the animal (Stephens et al., 2004).

The requirement for phosphorus has been the subject of several research studies with emphasis placed on factors influencing retention efficiency. Inconsistencies in the effect of additional phosphorus above that recommended by the NRC (1989) could be due to possible interactions with other supplemented minerals (Nolan et al., 2001; Elmore-Smith et al., 1999). It does appear that exercise induces an increase in daily P retention (Nolan et al., 2001; Young et al., 1989). In addition, as with calcium, retention efficiency seems to decrease with age (Cymbaluk, 1990; Pagan, 1989).

Calcium and Phosphorus Ratio

The influence of the calcium-phosphorus ratio in the equine diet has historically been an important criterion for determining the value of any ration formulation. A ratio of Ca to P less than 1:1 is considered to be detrimental to Ca absorption and may result in development of nutritional secondary hyperparathyroidism. Nutritional secondary hyperparathyroidism can be induced by grazing predominantly tropical forages, grasses with high oxalate content, or rations with high concentrations of phosphorus (Krook and Lowe, 1964; Hodgson and Rose, 1994). Characteristics of nutritional secondary hyperparathyroidism are shifting lameness with severely affected individuals developing enlargement of the maxilla and mandible. The enlargement of the maxilla and mandible is due to the removal of Ca from the facial bones which replaces the lost mineral with fibrous connective tissue that serves as a mechanism of support. These events lead to the development of the clinical condition fibrous osteodystrophy better known as "big head" disease.

Excessive amounts of Ca in the diet do not appear to have detrimental effects in horses if the amount of P in the diet is sufficient (Jordan et al., 1975). The maximum tolerable amount of P, given adequate Ca, is 1% of the diet (NRC, 1989).

CHAPTER 3
EXPERIMENT 1: PASTURE VERSUS DRY LOT PROGRAMS FOR YEARLING HORSES

Introduction

Young horses are housed in various manners to facilitate the objectives of the particular facility. The effects of different housing situations have been evaluated investigating parameters such a bone mineral deposition (Hoekstra et al., 1999; Barnevald and van Weeren, 1999), growth rates (van Weeren et al., 2000) and behavior (Rivera et al., 2002). Increased confinement, which limits exercise and loading of the bone, has been shown to decrease skeletal strength (Barnevald and van Weeren, 1999). Stalled yearlings had a decrease in bone mineral content when compared to their contempories on pasture (Hoekstra et al., 1999). Increased access to free exercise and bone loading allowed the pasture yearlings adequate stimuli to increase bone mineralization and development (Hoekstra et al., 1999).

Group housing in a dry lot versus a pasture setting has not been exclusively studied. The objective of this study was to determine whether housing yearling horses in a dry lot situation would prove detrimental to bone mineralization and development when compared to their pasture-reared contemporaries. We hypothesized that group housing in a dry lot would hinder bone mineralization and development.

Materials and Methods

Management of Animals

Thirty Thoroughbred (n = 16) and Quarter Horse (n = 14) yearlings were randomly assigned within breed and gender to one of two experimental treatments: 1) dry lot or 2) pasture. Both treatment groups started the 112 d trial simultaneously. Two horses were unable to complete the study due to factors unrelated to this study and their data are excluded from the results. Horses were vaccinated, dewormed and provided with regular hoof care throughout the study. The University of Florida Institutional Animal Care and Use Committee approved the protocol for management and treatment of the animals.

Experimental Treatments

Dry lot housed yearlings were evenly distributed based on gender between four 430 m^2 paddocks and two 20235 m^2 pastures. The horses housed in the dry lots were allowed 107.5 m^2 per horse in two pens and the two remaining pens had 143 m^2 per horse. The fillies on pasture had 2529 m^2 per horse where as the colts and geldings had 2890 m^2 per horse.

Diets

The concentrate portion of the ration (Table 3.1) was formulated to meet or exceed the energy, protein, vitamin, macro mineral, and trace mineral requirements of yearling horses when fed with coastal bermuda grass hay or bahiagrass pasture(NRC, 1989). Both groups were fed concentrate to appetite at 700 and 1400 h for two 90 minute feeding periods daily in individual feeding stanchions. Orts were weighed back daily and adjustments to amount offered made in accordance with refusals. Dry lot housed yearlings received 1.5 kg/100 kg BW of Coastal Bermuda grass hay based on the average pen weight for the period. Pastured yearlings received 1.5 kg/100 kg BW of Coastal

Bermuda grass hay based on the average pen weight for 72 d until natural pasture was in season. Nutrient analysis for hay and pasture samples are presented in Table 3.1. Fresh water was available at all times.

Growth Measurements

Yearlings were measured for body weight, withers height, body length, hip height, body condition score, and heart girth at day 0, 28, 56, 84, and 112.

Bone Mineral Content

Radiographs of the dorsal/palmer aspect of the left third metacarpal were obtained on day 0, 56, and 112 and used to determine bone mineral content. Radiographs were obtained using an Easymatic Super 325 (Universal X-Ray Products, Chicago, IL) set at 97 pkv, 30 ma, and 0.067 sec. A ten-step aluminum stepwedge was taped to the cassette parallel to the third metacarpal and used as a standard in estimating the bone mineral content. While taking the radiographs, a 91.5 cm distance was maintained from the machine to the cassette. The films were processed with Kodak products and by Kodak development procedures. One centimeter below the nutrient foramen of the third metacarpal, a cross section of the cannon bone was compared to the standard using the image analyzer and bone mineral content was estimated by photodensitometry (Meakim et al., 1981; Ott et al., 1987).

Feed Analysis

At the beginning of each 28 d period and with each new batch of concentrate, samples of hay, pasture, and concentrate were collected and prepared for analysis. Concentrate and pasture samples were dried in an oven for 3 d at 60˚C then ground in a Wiley mill with a 1 mm screen. Hay samples were ground in a hammer mill, mixed, and a sub sample was then ground in a Wiley mill with a 1 mm screen. Feed samples were analyzed for Ca, Mn, Cu, Fe, and Zn concentrations by using the Perkin-Elmer Model 5000 Atomic Absoption Spectrophotometer (Perkin-Elmer Corp., Norwalk, CO). Crude protein was obtained by determining nitrogen after digesting the feed sample according to the procedure by Gallaher et al. (1975). The samples were then analyzed using the Alpkem autoanalyzer (Alpkem Corp., Clackemas, OR). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were all determined using the procedures outlined for use with an Ankom (1999) machine. High carbohydrate content of grains may interfere with the extraction of fats; therefore, the water soluble carbohydrate portion of the concentrate was extracted prior to being subjected to the Soxhilet procedure for fat extraction.

Statistical Analyses

Data were analyzed by analysis of variance for repeated measures using the general linear models procedure of SAS (Carry, NC) with treatment and time as the main effects. In addition, regression analyses were performed on correlations between bone mineral content and body weight. An $\alpha < 0.05$ was set as statistically significant.

Results

Withers height, girth, length, and hip height were not influenced by housing conditions (Table 3.2). However, final body weight ($P < 0.05$) and average daily gain ($P < 0.05$) were higher for pasture yearlings (Table 3.2). Regardless of treatment, each growth variable increased with age ($P < 0.05$; Table 3.2).

Pastured yearlings voluntarily consumed more concentrate than dry lot yearlings but, concentrate intake as a percentage of body weight was not different between treatments (Table 3.3). Average daily gain was higher ($P < 0.05$) for the pasture yearlings. Similarly, Ca and P intake was greater ($P < 0.05$) for pastured yearlings, but Ca and P intake on a mg/kg of BW basis was similar between treatments (Table 3.3).

Bone mineral content was correlated ($P = 0.01$) with body weight on days 0, 56, and 112 (Figure 3.1). Bone mineral content was similar between housing treatments at day 0 and d 56, but at d 112 pasture yearlings had greater ($P = 0.06$) bone density compared to dry lot yearlings (Figure 3.2). Change in bone mineral content was similar between treatments from d 0 to d 56, but the change from d 56 to d 112 was greater ($P < 0.05$) in pastured yearlings (Table 3.4). The overall change in bone mineral content, while not statistically significant, was numerically greater in pastured yearlings compared to those housed on dry lot (Table 3.4). No other significant differences could be detected between treatments.

Discussion

Pastured yearlings had greater total bone mineral content from d 56 to d 112 compared to that of the dry lot housed yearlings. Factors that could have influenced bone mineral content in pastured yearlings include greater concentrate intake, differences in nutrient composition between hay and pasture, body weight, and potentially an increased level of activity while on pasture.

Concentrate intake and subsequently Ca and P intake was greater for pastured yearlings compared to dry lot yearlings. Greater concentrate intake is likely responsible for the greater final BW and ADG observed for pastured yearlings. However, concentrate intake, as well as, Ca and P intake were similar for both types of housing when adjusted for BW. Ott and Asquith (1989) found minerals provided in proportion to energy were sufficient for adequate bone growth. Therefore, it is unlikely that greater consumption of Ca and P from the concentrate increased bone density of pastured horses over dry lot housed yearlings.

In addition to concentrate, a portion of the yearlings' nutrient requirements were met by forage. Dry lot housed yearlings were offered hay, where as pastured yearlings had access to hay and/or pasture. An argument could be made that pasture grasses provided slightly more Ca and P than hay, therefore contributing more of these minerals to bone growth in the pastured yearlings. However, based on hay consumption for dry lot yearlings (1 % BW), forage was a small component of the total diet (approximately 48 % of total daily intake by weight). Pasture consumption was not measured directly, but could be assumed to be similar to that of hay intake by dry lot yearlings on a dry matter basis. Therefore, while the pastures may have had greater mineral content, it is unlikely that the amounts eaten by pasture-reared yearlings would have contributed amounts significant enough to alter bone density.

The correlation between body weight and bone mineral content indicates that the greater load placed on the bone by weight results in denser bone. Pastured yearlings were 45 kg heavier at the end of the study, which would place a greater load on bone. Although activity level was not measured, pastured yearlings had more space to move about than dry lot yearlings. Therefore, it seems likely that a greater level of activity and increased speed, which has been shown to increase bone mineral content, in pastured yearlings could be responsible for the differences in bone density on the current study. According to Heleski et al. (1999), pastured horses spend most of their time interacting with one another, including sprints across the field, and grazing. Where as dry lot housed yearlings in a confined area were not able to place the same stress upon the bone resulting in decreased bone deposition.

With increased bone mineral content in pastured versus dry lot housed yearlings, the next phase of the experiment enrolled the dry lot housed yearlings in an exercise program to access if enough stimuli could be provided to instigate bone growth equal to that of the pastured yearlings.

Table 3.1. Concentrate formula and concentrate and forage nutrient content

	Concentrate	Coastal bermudagrass hay	Pasture grass
Formula, % as fed			
Oats, ground	40.00		
Corn, ground	27.30		
Soybean meal w/o hull	10.00		
Alfalfa meal, 17%	7.50		
Wheat bran	7.50		
Molasses	5.00		
Limestone, ground	1.00		
BioFos	0.50		
Salt	0.75		
Lysine, 98%	0.10		
TM premix[a]	1.00		
Vitamin premix[b]	0.05		
Analysis, DM basis, except DM			
DM, %	88.61	91.51	93.99
CP, %	15.25	7.24	11.46
NDF, %	29.50	77.66	70.61
ADF, %	12.02	37.53	31.03
Fat, %	3.08	0.58	2.11
Ca, %	0.87	0.37	0.61
P, %	0.57	0.23	0.29
Cu, ppm	45.39	4.43	4.65
Fe, ppm	262.00	93.00	140.00
Mn, ppm	112.00	44.67	157.50
Zn, ppm	113.00	36.67	32.25

[a] Trace mineral (TM) premix provided the following amounts of minerals per kilogram of concentrate: 25.4 mg Ca, 17.4 mg Fe, 47.3 mg Zn, 32.4 mg Mn, 25.3 mg Cu, 0.15 mg Co, 0.10 mg I, and 0.01 mg Se.

[b] Vitamin premix provided the following amounts of vitamins per kilogram of concentrate: 6600 IU vitamin A, 440 IU vitamin D_3, 206 IU vitamin E, 0.01 mg vitamin B12, 3.7 mg riboflavin, 11.7 mg niacin, 4.6 mg pantothenic acid, 66.9 mg choline chloride, 1.2 mg folic acid, 1.2 mg pyridoxine, and 2.1 mg thiamin.

Table 3.2. Influence of sex, breed, and treatment on growth and development of yearlings

	Sex		Breed		Treatment	
	Male	Female	TB	QH	Dry Lot	Pasture
Number	14	16	16	14	15	15
Weight, kg	362.6	362.1	362.1	362.6	357.9	366.7
Initial	318.4	324.7	323.0	320.8	320.5	323.5
Final	405.5	402.1	401.4	406.0	395.65[a]	441.4[b]
Gain	87.1	77.4	78.4	85.2	75.2	88.0
ADG	0.78	0.69	0.70	0.76	0.67[a]	0.79[b]
Girth, cm	158.6	160.2	160.1	158.8	159.0	160.1
Initial	151.5	155.0	154.4	152.5	153.0	153.9
Final	166.1	166.1	166.2	165.9	165.0	167.1
Gain	14.6	11.1	11.8	13.4	12.0	13.2
Withers height, cm	144.1	145.0	147.5	141.3	144.5	144.8
Initial	140.2	142.0	143.8	138.2	140.7	141.7
Final	147.1	147.5	150.2[a]	143.9[b]	147.4	147.2
Gain	6.9	5.6	6.5	5.7	6.8	5.5
Body Length, cm	142.6	143.4	143.8	142.3	142.4	143.8
Initial	137.7	138.9	139.5	137.1	138.3	138.6
Final	147.8	148.1	148.3	147.6	146.7	149.2
Gain	10.1	9.1	8.8	10.5	8.5	10.6
Hip Height, cm	148.5	149.4	151.3	146.4	148.7	149.3
Initial	144.7	146.7	148.0	143.3	145.5	146.2
Final	151.8	152.0	154.3	149.2	151.6	152.2
Gain	7.1	5.3	6.2	5.9	6.1	6.1

[a,b]Row means not sharing superscripts differ ($P < .05$).

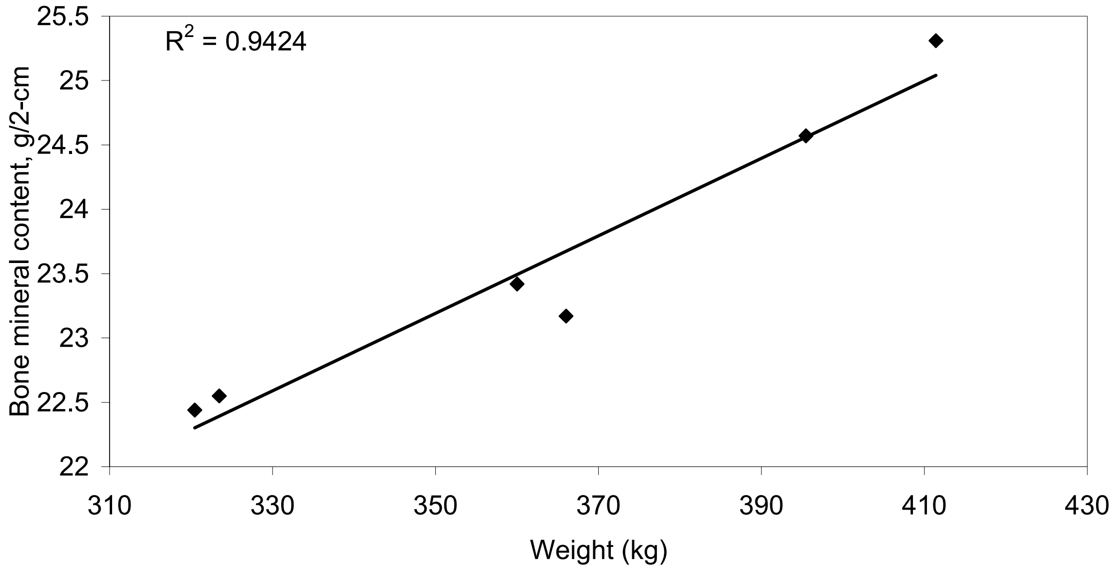

Figure 3.1. Regression between bone mineral content and weight (kg).

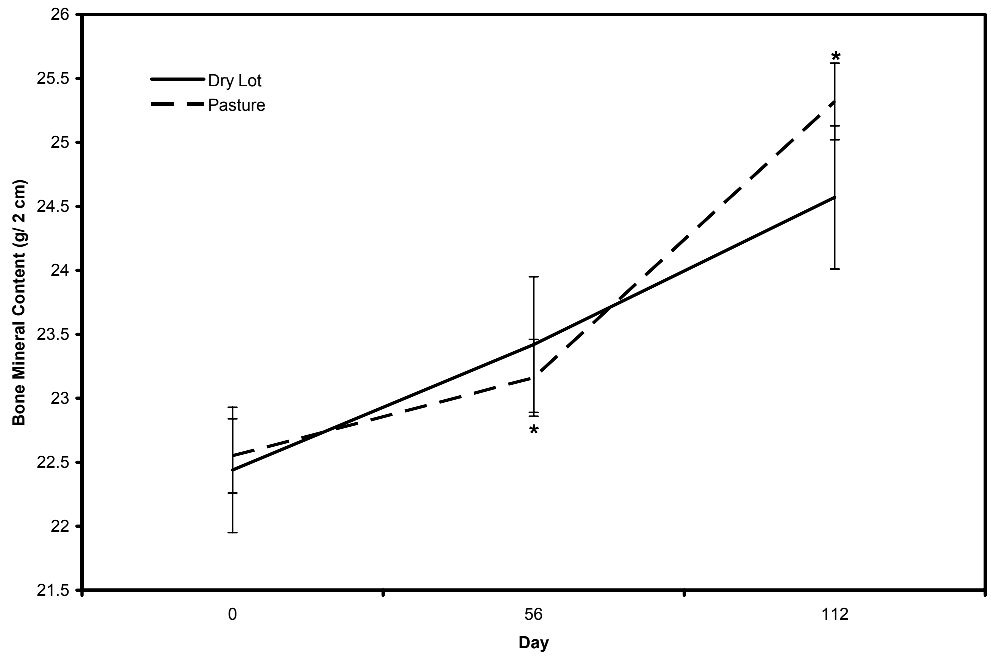

Figure 3.2. Bone mineral content in dry lot versus pasture housed yearlings.
* Pasture gain > dry lot gain (P < .05).

Table 3.3. Daily feed and nutrient intake by treatment.

Treatment	Dry Lot	Pasture[1]
Concentrate intake		
kg	4.14[a]	4.31[b]
% of BW	1.49	1.52
Hay intake		
kg	3.75	3.75
% of BW	1.01	.98
Calcium		
g	62.83[a]	66.55[b]
mg/kg BW/d	167.28	172.10
Phosphorus		
g	40.70	42.30
mg/kg BW/d	108.34	109.36
Ca:P Ratio	1.54	1.57

[a,b]Row means with different superscript differ ($P < .05$).

[1]Pasture intake estimated to be similar to hay intake of both dry lot.

Table 3.4. Influence of sex, breed, and treatment on bone mineral content in yearlings

	Sex		Breed		Treatment	
	Male	Female	TB	QH	Dry Lot	Pasture
Number	14	16	16	14	15	15
Bone mineral content (g/2 cm)						
d 0	22.0	22.9	23.5	21.3	22.4	22.5
d 56	22.7	23.7	24.3	22.1	23.4	23.2
d 112	24.4	25.4	25.7	24.0	24.6	25.3
Change in bone mineral content (g/2 cm)						
d 0 - d 56	0.71	0.87	0.82	0.78	0.98	0.62
d 56 - d 112	1.67	0.63	1.41	1.92	1.14[a]	2.15[b]
Total change	1.67	1.76	1.57	1.90	1.50	1.94

[a,b]Row means with different superscripts differ ($P < .05$).

CHAPTER 4
EXERCISE 2: EFFECT OF DRY LOT, DRY LOT WITH FORCED EXERCISE, AND PASTRURE PROGRAMS ON BONE CHARATERISTICS OF YEARLING HORSES

Introduction

Development of an adequate skeletal support system is important in determining the ability and longevity of horses' careers in competition. Housing conditions and exercise influence the quality and quantity of bone deposition in all horses and potentially most critical in the early stages of life (van Weeren et al., 2000). The skeletal system response to exercise can vary greatly depending on the amount, type and age of introduction (Rubin, 1984, Sherman et al., 1995, and Stover et al., 1992). Current research in humans suggests that conditioning of the skeleton at an early age prevents or mediates osteoporosis later in life. Management of the young growing horse to optimize the skeletal strength and prevent associated injuries could lead to a decrease in economic losses from catastrophic injuries and a loss of training time. Confinement resulting in inactivity decreases bone mineral content in weanling and yearlings with detrimental effects still observed after 56 d of conditioning (Hoekstra et al., 1999 and Bell et al., 2001). Long term effects of confinement on young growing horses have not been quantified. Some studies show young horses can compensate for some loss of bone mineral due to extended confinement if allowed adequate exercise (Barneveld and van Weeren, 1999). The intensity, duration, and age to introduce the exercise to provide adequate stimuli needed to reduce or negate the loss of bone mineral are unknown. The stimuli required for bone growth while minimizing the risk of injury difficult in young growing horses. Young bone and support structures are more pliable thus possibly more susceptible to adverse effects of prolonged or excessive loading.

The hypothesis of this experiment was that forced exercise would ameliorate the decrease in bone mass previously observed with confinement and would be equivalent to or increase above that of their pasture housed contemporaries.

Materials and Methods

Management of Animals

Thirty six Thoroughbred (n = 24) and Quarter Horse (n = 12) yearlings were randomly assigned within breed and gender to one of three experimental treatments: 1) dry lot housed (n = 12), housed on pasture (n = 12), or housed on dry lots with forced exercise (n = 12). All horses were vaccinated, wormed and provided with regular hoof care throughout the study. The University of Florida Institutional Animal Care and Use Committee approved the protocol for management and treatment of the animals.

Experimental Treatments

Dry lot housed yearlings were evenly distributed based on gender between four 430 m^2 paddocks and two 20,235 m^2 pastures. The horses housed in the dry lots were allowed 71.67 m^2 per horse and both pastures 3372.5 m^2 per horse.

Diets

The concentrate portion of the ration (Table 4.1) was formulated to meet or exceed the energy, protein, vitamins, macro minerals and trace minerals of yearling horses (NRC, 1989). Both groups were fed concentrate to appetite for two 90 minute feeding periods daily (700 and 1400 h) in individual feeding stanchions. Orts were weighed back daily and adjustments to amount offered made in accordance with refusals. Both dry lot groups received 1.5 kg/100 kg BW of Coastal Bermuda grass hay based on the average pen weight for the period. Pasture yearlings received 1.5 kg/100 kg BW of Coastal Bermuda grass hay based on the average pen weight for the first 56 d until natural pasture was in season. Nutrient analysis for hay and pasture samples are presented in Table 4.1. Fresh water was available at all times.

Exercise Program

Horses on the dry lot with exercise treatment were introduced and allowed to acclimate to the European free walker for one week then exercised four days a week in alternating directions. Time and distance were increased weekly until reaching the maximum of 15 minutes walking and 25 minutes trotting with a total distance of 8.5 km/d and 32 km/wk (Table 4.2).

Growth Measurements

Yearlings were measured for body weight, withers height, body length, hip height, and heart girth at day 0, 28, 56, 84, and 112.

Bone Mineral Content and Bone Geometry

Radiographs of the dorsal/palmer and medial/lateral aspects of the left third metacarpal were obtained on day 0, 56, and 112 and used to determine bone mineral content and cortical measurements. Radiographs were obtained using an Easymatic Super 325 (Universal X-Ray Products, Chicago, IL) set at 97 pkv, 30 ma, and 0.067 sec. A ten-step aluminum stepwedge was taped to the cassette parallel to the third metacarpal and used as a standard in estimating the bone mineral content. While taking the radiographs, a 91.5 cm distance was maintained from the x-ray machine to the cassette. The films were processed with Kodak products and by Kodak development procedures. One centimeter below the nutrient foramen of the third metacarpal, a cross section of the bone was compared to the standard using the image analyzer and bone mineral content was estimated by photodensitometry (Meakim et al., 1981; Ott et al., 1987).

The dorsalopalmar radiographic view was used to measure the width of the medial and lateral cortices, inner medullary cavity, and the outer cortical diameter (Figure 4.1 and 4.2). Using the method described by Hiney et al. (2004) a line graph was generated with photodensitometer values and, the highest point of the curve was measured for the width of each cortex (Figure 4.3). The medulary cavity width was determined by adding the measurement from each cortex and subtracting that value from the measured distance of the curve (or width of bone). The procedure was repeated for the lateromedial view for determination of the dorsal and palmar cortical widths, medullary cavity, and dorsopalmar width of the bone.

Feed Analysis

At the beginning of each 28 d period and with each new batch of concentrate, samples of hay, pasture, and concentrate were collected and prepared for analysis. Concentrate and pasture samples were collected as well. Feed and grass samples were dried in an oven for 3 d at 60˚C then ground in a Wiley mill with a 1 mm screen. Hay samples were ground in a hammer mill, mixed, and a sub sample was then ground in a Wiley mill with a 1 mm screen. Feed samples were analyzed for Ca, Mn, Cu, Fe, and Zn concentrations by using the Perkin-Elmer Model 5000 Atomic Absoption Spectrophotometer (Perkin-Elmer Corp., Norwalk, CO). Crude protein was obtained by determining nitrogen after digesting the feed sample according to the procedure by Gallaher et al. (1975). The samples were then analyzed using the Alpkem auto analyzer (Alpkem Corp., Clackemas, OR). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were all determined using the procedures outlined for use with an

Ankom (1999) machine. The high carbohydrate content of the grains may interfere with the extraction of fats; therefore, the water soluble carbohydrate portion of the concentrate was extracted prior to being subjected to the Soxhilet procedure for fat extraction (AOAC, 1995).

Statistical Analyses

Data were analyzed by analysis of variance for repeated measures using the general linear models procedures of SAS with treatment and time as the main effects. An $\alpha <$ 0.05 was set as statistically significant. Treatment means were compared using the Tukey test.

Results

Growth Measurements

Horses began the project at an average weight of 323 $\pm$ 4.8 kg and increased weight to 397 $\pm$ 5.2 kg for an average gain of 74 kg (Table 4.3). Wither height increased from 142.0 $\pm$ 0.6 cm to 147.9 $\pm$ 0.8 cm and hip height increased 146.2 $\pm$ 0.8 cm to 151.6 $\pm$ .7 cm from d 0 to d 112, which is an increase of 5 cm in both measurements. Girth increased from 153.0 $\pm$ 0.8 cm at day 0 to 165.2 $\pm$ 0.9 cm at day 112, which is an increase of 12 cm. Body length increased 11 cm from 138.0 $\pm$ 0.8 cm at day 0 to 148.9 $\pm$ 0.9 cm at day 112. All growth measurements increased from day 0 to day 112 ($P < .05$; Table 4.3). Treatment had no effect on any of the growth measurements. Similarly, no treatment x time interactions were detected for growth variables during the study.

Feed Intake

Treatment affected concentrate intake ($P < 0.05$) resulting in a difference in total calcium, phosphorus, and calcium: phosphorus ratio intakes (Table 4.4). Pastured yearlings had greater ($P < 0.05$) consumption of concentrate and higher ($P < 0.05$)

calcium: phosphorus ratio over that of dry lot yearlings with exercised yearlings falling between pastured and dry lot treatments. Treatment affected ($P < 0.05$) calcium and phosphorus intake (mg/kg of BW) with pasture yearlings having the highest intake and dry lot with the lowest (Table 4.4).

Bone Development

Pastured yearlings had greater ($P < 0.05$) gain in anterior cortical width between d 56 and d 112 than the other treatments (Table 4.5). In contrast, the posterior cortical width of pastured yearlings decreased over the course of the study below that of the dry lot housed and dry lot exercise groups ($P < 0.05$; Table 4.5). Anterior: posterior cortical ratio change was greater ($P < 0.05$) for pastured yearlings than both dry lot treatments (Table 4.6). No change in bone mineral content was detected throughout the study (Table 4.5).

Discussion

Pastured yearlings consumed more concentrate (both total kg and as percentage of body weight). As a result, pastured versus dry lot yearlings had higher intakes of both Ca and P and a wider Ca: P ratio. Exercise yearlings were intermediate between pastured and dry lot housed yearlings. Nonetheless, bone mineral density was not influenced by treatment. Therefore, the differences noted in Ca and P intake and the Ca: P ratio did not appear to effect bone mineral content.

Type and duration of activity has been proven to affect bone geometry and density (Hiney et al., 2004). Pastured yearlings changed bone geometry by increasing the dorsal cortical width and decreasing the palmar cortical width. This resulted in a greater change in dorsal: palmar cortical ratio indicating a geometric bone difference when compared to the dry lot treatments. These results are consistent with previous finding that indicate increased high intensity exercise remodels bone to accommodate higher strain rates produced in the dorsal aspect of the bone while removing bone from the palmar aspect, the least strained cortice (Hiney et al., 2004).

Table 4.1. Concentrate formula and concentrate and forage nutrient content

	Concentrate	Coastal Bermudagrass Hay	Pasture grass
Formula, % as fed			
Oats, ground	40.00		
Corn, ground	27.30		
Soybean meal w/o hull	10.00		
Alfalfa meal, 17%	7.50		
Wheat bran	7.50		
Molasses	5.00		
Limestone, ground	1.00		
BioFos	0.50		
Salt	0.75		
Lysine, 98%	0.10		
TM premix[a]	1.00		
Vitamin premix[b]	0.05		
Analysis, DM basis, except DM			
DM, %	89.04	92.85	89.49
CP, %	14.94	9.41	13.60
NDF, %	22.84	74.99	73.84
ADF, %	8.84	35.91	34.23
Fat, %	2.24	0.91	3.60
Ca, %	1.15	0.42	0.65
P, %	0.51	0.21	0.25
Cu, ppm	40.44	4.63	8.88
Fe, ppm	354.57	258.18	146.90
Mn, ppm	96.74	53.05	126.93
Zn, ppm	112.62	21.52	31.18

[a] Trace mineral (TM) premix provided the following amounts of minerals per kilogram of concentrate: 25.4 mg Ca, 17.4 mg Fe, 47.3 mg Zn, 32.4 mg Mn, 25.3 mg Cu, 0.15 mg Co, 0.10 mg I, and 0.01 mg Se.

[b] Vitamin premix provided the following amounts of vitamins per kilogram of concentrate: 6600 IU vitamin A, 440 IU vitamin D_3, 206 IU vitamin E, 0.01 mg vitamin B12, 3.7 mg riboflavin, 11.7 mg niacin, 4.6 mg pantothenic acid, 66.9 mg choline chloride, 1.2 mg folic acid, 1.2 mg pyridoxine, and 2.1 mg thiamin.

Table 4.2. Exercise Protocol of yearlings on the dry lot with forced exercise treatment

Week	Speed (m/s)	Time (min)	Distance (km)	Total distance per day (km)	Total distance per week (km)
1	Hand walk and acclimatize to exerciser				
2	2	20	2.4	2.4	9.6
3	2	10	1.2	3.9	15.6
	5	5	1.5		
	2	10	1.2		
4	2	10	1.2	5.4	21.6
	5	10	3		
	2	10	1.2		
5	2	10	1.2	6.9	27.6
	5	15	4.5		
	2	10	1.2		
6	2	7.5	0.9	7.8	31.2
	5	20	6		
	2	7.5	0.9		
7-16	2	7.5	0.5	8.5	34.0
	5	25	7.5		
	2	7.5	0.5		

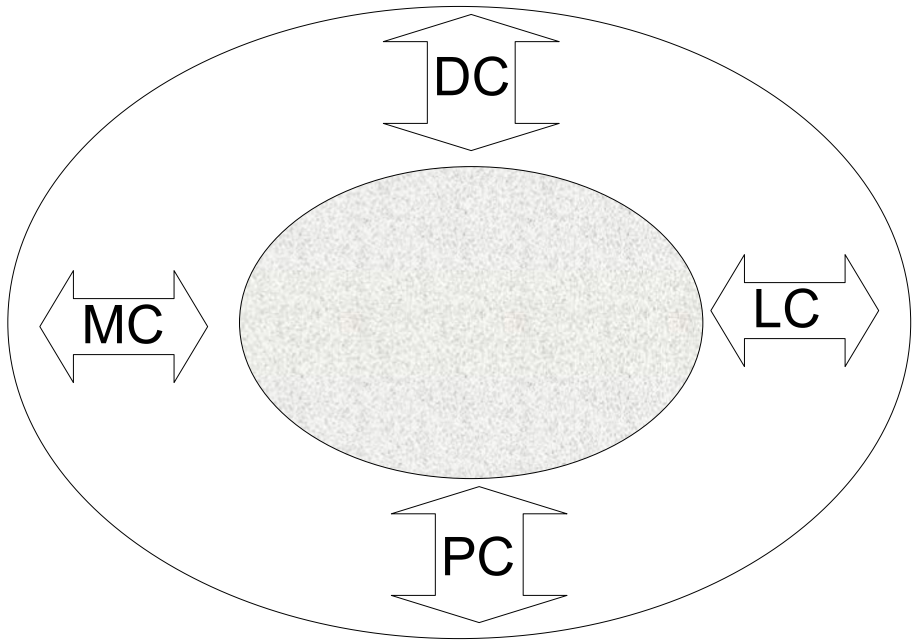

Figure 4.1. Schematic illustration of a cross-section of equine third metacarpal showing cortical measurements. DC = dorsal cortical width; PC = palmar cortical width; MC = medial cortical width; LC = lateral cortical width.

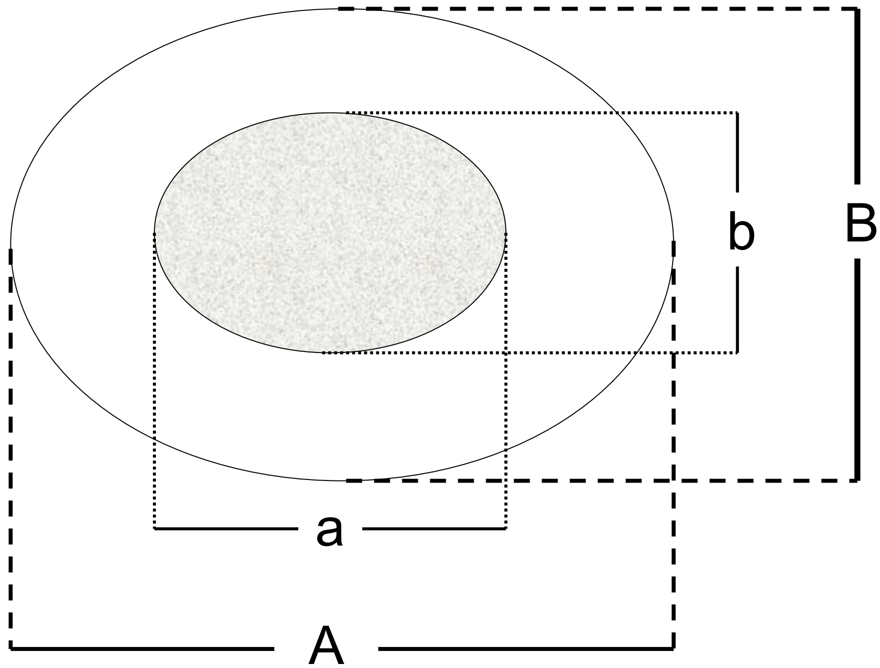

Figure 4.2. Schematic illustration of a cross-section of equine third metacarpal showing cortical measurements. A = lateromedial bone diameter; a = lateromedial medullary cavity; B = dorsopalmar bone diameter; b = dorsopalmar medullary cavity.

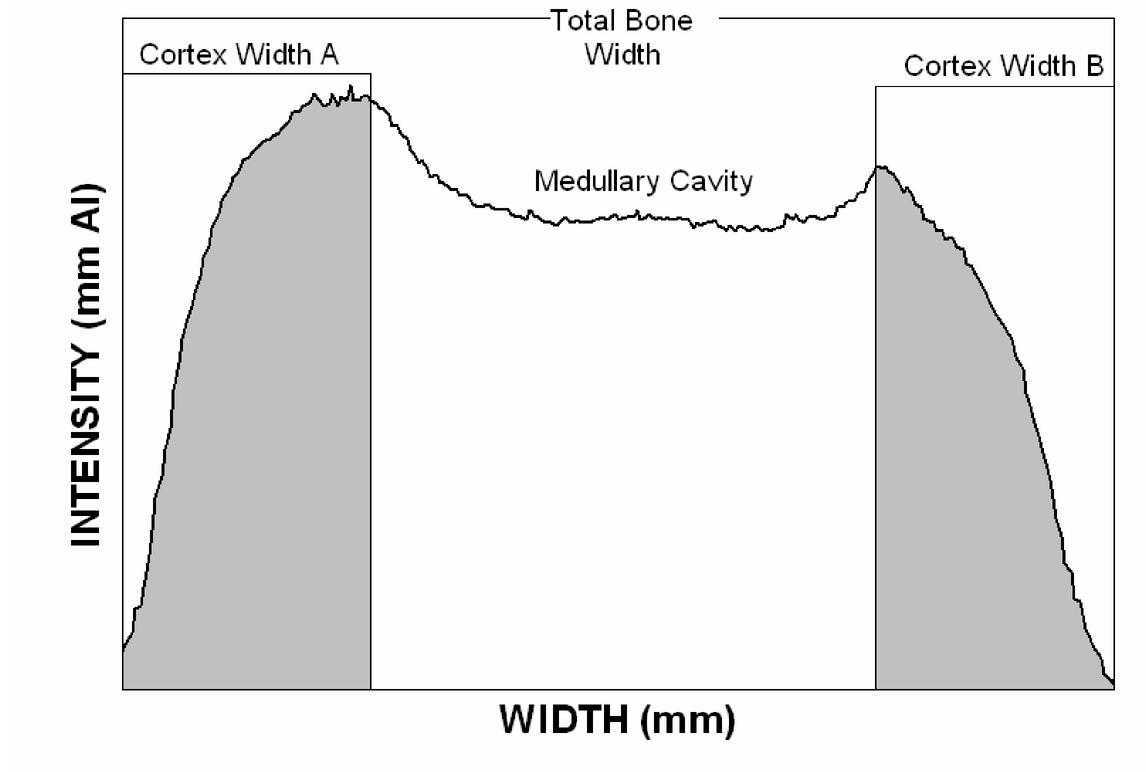

Figure 4.3. Schematic illustrations of cortical measurements as obtained from photodensitometer analysis of radiographs.

Table 4.3. Influence of sex, breed, and treatment on growth and development of yearlings

	Sex		Breed[1]		Treatment			Overall
	Male	Female	TB	QH	Dry Lot	Pasture	Exercise	
Number	18	18	18	18	12	12	12	36
Body weight[2], kg	356.8	363.8	355.7	368.4	362.8	360.6	357.4	360.3
Initial	326.7	319.8	320.2	328.6	322.7	321.9	325.2	323.2
Final	400.0	393.5	390.8	407.3	400.9	398.9	390.4	396.7
Gain	73.2	73.8	70.6	78.7	78.2	77.1	65.2	73.5
ADG	0.65	0.66	0.63	0.70	.70[a]	0.69[a]	0.58[b]	0.65
Girth[2], cm	154.4[a]	160.2[b]	158.7	160.1	159.8	159.2	158.7	159.2
Initial	151.7	154.4	152.7	153.7	153.6	151.7	153.8	153.0
Final	163.9	166.4	164.8	165.9	166.5	165.0	163.9	165.2
Gain	12.2	12.0	12.1	12.2	12.9	13.3	10.2	12.2
Withers height[2], cm	144.9	145.1	147.1	141.4	144.7	145.4	145.0	145.0
Initial	141.9	142.2	144.1	138.3	141.6	142.3	142.1	142.0
Final	148.0	147.8	149.8	144.6	147.4	148.7	147.6	147.9
Gain	6.2	5.6	5.7	6.3	5.8	6.4	5.5	5.9
Body length[2], cm	143.2	143.3	143.4	143.1	143.0	143.8	143.0	143.3
Initial	137.8	138.1	138.1	137.6	138.0	138.2	137.7	138.0
Final	149.0	148.8	149.0	148.6	148.2	149.2	149.3	148.9
Gain	11.2	10.7	10.9	11.0	10.2	11.0	11.6	10.9
Hip height[2], cm	148.9	149.3	150.8	146.0	148.8	149.6	148.9	149.1
Initial	145.9	146.5	148.0	143.1	145.9	146.8	145.8	146.2
Final	151.5	151.8	153.3	148.7	151.2	152.5	151.2	151.6
Gain	5.6	5.2	5.4	5.6	5.2	5.7	5.4	5.4

[a,b]Row means not sharing superscripts differ ($P < .05$).
[1]Breed effect for all measurements ($P < .05$) except girth and length.
[2]Means of measurement for overall experiment

Table 4.4. Daily feed and nutrient intake by treatment.

Treatment	Dry Lot	Pasture[1]	Exercise
Concentrate intake			
kg	5.03[a]	5.63[b]	5.21[a,b]
% of BW	1.31[a]	1.48[b]	1.39[c]
Hay intake			
kg	2.87	2.87	2.87
% of BW	0.75	0.76	0.78
Calcium			
g	60.97[a]	72.61[b]	62.89[a]
mg/kg BW/d	158.95[a]	191.40[b]	168.80[c]
Phosphorus			
g	32.29[a]	36.63[b]	33.24[a]
mg/kg BW/d	84.23[a]	96.56[b]	89.29[c]
Ca:P Ratio	1.88[a]	1.98[b]	1.89[a,b]

[a,b,c]Row means with different superscript differ ($P < .05$)

[1]Pature intake estimated to be similar to hay intake of both dry lot and exercise groups.

Table 4.5. Influence of sex, breed, and treatment on bone characteristics of yearlings

	Sex		Breed		Treatment		
	Male	Female	TB	QH	Dry Lot	Pasture	Exercise
Number	18	18	18	18	12	12	12
Bone mineral content (g/2 cm)							
d 0	21.47	21.38	21.69	20.96	20.79	21.37	22.10
d 56	20.12	20.25	20.39	19.83	20.11	19.99	20.44
d 112	22.46[a]	23.25[b]	23.27[a]	22.14[b]	22.91	22.77	22.89
Change in bone mineral content (g/2 cm)							
d 0 - d 56	-1.35	-1.13	-1.30	-1.13	-0.68	-1.38	-1.66
d 56 - d 112	2.34	3.00	2.87	2.31	2.81	2.77	2.45
Total change	0.99	1.87	1.57	1.18	2.12	1.39	0.79
Dorsal cortice (mm)							
d 0	9.74	10.29	9.81	10.38	10.51	9.70	9.84
d 56	9.93	10.03	9.87	10.16	10.27	9.51	10.16
d 112	9.91	10.28	9.91	10.42	10.11	10.31	9.87
Change in dorsal cortice (mm)							
d 0 - d 56	-0.17	0.19	0.07	-0.22	-0.25	-0.19	0.32
d 56 - d 112	0.26	-0.02	0.04	0.26	-0.16[a]	0.79[b]	-0.29[a]
Total change	-0.01	0.17	0.10	0.04	-0.40[a]	0.61[b]	.03[a]
Palmar cortice (mm)							
d 0	5.76	5.64	5.83	5.46	5.63	6.04	5.43
d 56	5.52	6.03	5.96	5.46	5.86	6.03	5.44
d 112	6.27	6.25	6.40	6.01	6.48	5.97	6.35
Change in palmar cortice (mm)							
d 0 - d 56	-0.24[a]	0.39[b]	0.12	0.00	0.23	-0.01	0.01
d 56 - d 112	0.75	0.28	0.45	0.55	0.62	-0.07	0.91
Total change	0.52	0.61	0.56	0.56	0.84[a]	-0.07[b]	0.91[a]

[a,b]Row means with different superscripts differ (P < .05).

Table 4.6. Influence of sex, breed, and treatment on dorsal: palmar cortical ratio of the third metacarpal in yearlings.

	Sex		Breed		Treatment		
	Male	Female	TB	QH	Dry Lot	Pasture	Exercise
Number	18	18	18	18	12	12	12
Dorsal: palmar ratio							
d 0	1.74	1.86	1.72	1.93	1.92	1.64	1.84
d 56	1.85	1.69	1.69^{a}	1.95^{b}	1.77	1.61	1.92
d 112	1.61	1.67	1.58	1.74	1.58	1.76	1.59
Change in dorsal: palmar ratio							
d 0 - d 56	0.12^{a}	-0.17^{b}	-0.04	0.00	-0.14	-0.02	0.08
d 56 - d 112	-0.24^{a}	-0.02^{b}	-0.11	0.55	-0.2^{a}	0.14^{b}	-0.34^{a}
Total change	-0.13	-0.19	-0.14	0.56	-0.34^{a}	0.12^{b}	-0.25^{a}

[a,b]Row means with different superscripts differ ($P < .05$).

CHAPTER 5
EXPERIMENT 3: MANAGEMENT PRACTICES INFLUENCE ON BONE DEVELOPMENT IN YEARLING HORSES FED INVERSE CALCIUM: PHOSPHORUS RATIO DIET

Introduction

A proper calcium-phosphorus ratio in the equine diet has long been considered essential for proper growth and development, especially for the skeletal system. Inadequate intakes of either calcium or phosphorus may result in bone demineralization and osteomalatic changes (Lewis, 1995). According to Cunha (1981), calcium and phosphorus are more efficiently util1itzed when present in certain ratios. Hintz (1996) suggest a range of ratios of 1:1 to 3:1; however, others suggest maintenance of adequate calcium intake may be more important than the calcium to phosphorus ratio (Wyatt et al., 2000). Jordan et al.(1975) observed that calcium: phosphorus ratios as high as 6:1 for growing horses may not be detrimental if phosphorus intake is sufficient. In contrast, excessive phosphorus intake due to an improper Ca:P ratio decreases a Ca absorption, causes skeletal malformations, and results in a state of nutritional secondary hyperparathyroidism (Schryver et al., 1971). This in turn causes mobilization of both calcium and phosphorus from bone that is replaced with fibrous tissue thus weakening the bone and causing osteodystrophia fibrosa.

The objective of this study was to determine the effect of an inverse calcium-phosphorus ratio on the growth and development of yearling horses in varying management conditions. The hypothesis for this study was that an imbalance in Ca:P ratio would negatively impact bone growth and development in yearling horses.

Materials and Methods

Management of Animals

Thirty one Thoroughbred (n = 18) and Quarter Horse (n = 12) yearlings were randomly assigned within breed and gender during this 112 d trial to one of three experimental treatments: dry lot housing (n = 9), housed on pasture (n = 9), or housed on dry lot with forced exercise (n = 11). One of the Quarter Horse geldings in the dry lot treatment had to be euthanized for reasons unrelated to this study prior to d 28. Data from this yearling was excluded from analyses. All horses were vaccinated, wormed and provided with regular hoof care throughout the study. The University of Florida Institutional Animal Care and Use Committee approved the protocol for management and treatment of the animals.

Experimental Treatments

Dry lot housed yearlings were evenly distributed based on gender between four 430 m^2 paddocks and two 20235 m^2 pastures. The horses housed in the dry lots were allowed 107.5 m^2 per horse in two pens and the two remaining pens had 143 m^2 per horse. The fillies on pasture had 4047 m^2 per horse where as the colts and geldings had 3372.5 m^2 per horse.

Diets

The concentrate portion of the ration (Table 5.1) was formulated to meet or exceed the energy, protein, vitamin, and trace mineral requirements of yearling horses (NRC, 1989). Calcium and phosphorus were included in the concentrate at an approximate 1:2 ratio. All groups were fed concentrate to appetite for two 90 minute feeding periods daily (700 and 1400 h) in individual feeding stanchions. Orts were weighed back daily and adjustments to amount offered made in accordance with refusals. Dry lot groups received a 60:40 concentrate: Coastal Bermuda grass hay based on the average pen intake for the period. Pasture housed yearlings consumed natural pasture in season (last 75 d of experiment). Nutrient composition of hay and pasture is presented in Table 5.1. Fresh water was available at all times.

Exercise

Yearlings on the dry lot with forced exercise treatment participated in a scheduled exercise program four times per week. After a 28 d acclimatization period, horses were introduced to the European free walker for one week then exercised four days a week in alternating directions. Time and distance were increased weekly until reaching the maximum of 15 minutes walking and 25 minutes trotting with a total distance of 8.5 km/d and 32 km/wk (Table 5.2).

Growth Measurements

Yearlings were measured for body weight, withers height, body length, hip height, and heart girth at day 0, 28, 56, 84, and 112.

Bone Mineral Content and Geometry

Radiographs of the dorsal/palmer and medial/lateral aspect of the left and right third metacarpal were obtained on day 0, 56, and 112 and used to determine bone mineral content and cortical width. Radiographs of the four views of the third metacarpal were obtained using an Easymatic Super 325 (Universal X-Ray Products, Chicago, IL) set at 97 pkv, 30 ma, and 0.067 sec. A ten-step aluminum stepwedge was taped to the cassette parallel to the third metacarpal and used as a standard in estimating the bone mineral content. While taking the radiographs, a 91.5 cm distance was maintained from the x-ray machine to the cassette. The films were processed with Kodak products and by Kodak development procedures. One centimeter below the nutrient foramen of the third metacarpal, a cross section of the cannon bone was compared to the standard using the image analyzer and bone mineral content was estimated by photodensitometry (Meakim et al., 1981; Ott et al., 1987).

The dorsalopalmar radiographic view was used to measure the width of the medial and lateral cortices, inner medullary cavity, and the outer cortical diameter. Using the method described by Hiney et al. (2004), a line graph was generated from values derived from the photodenitometer analysis and the highest point of the curve was measured for the width of each cortex. The medulary cavity width was determined by adding the measurement from each cortex and subtracting that value from the measured distance of the curve (or width of bone). The procedure was repeated for the lateromedial view for determination of the dorsal and palmar cortical widths, medullary cavity, and dorsopalmar width of the bone.

Feed Anaylsis

At the beginning of each 28 d period and with each new batch of concentrate, samples of hay, pasture, and concentrate were collected and prepared for analysis. Concentrate and pasture samples were dried in an oven for 3 d at 60˚C then ground in a Wiley mill with a 1 mm screen. Hay samples were ground in a hammer mill, mixed, and a sub sample was then ground in a Wiley mill with a 1 mm screen. Feed samples were analyzed for Ca, Mn, Cu, Fe, and Zn concentrations by using the Perkin-Elmer Model 5000 Atomic Absoption Spectrophotometer (Perkin-Elmer Corp., Norwalk, CO). Crude protein was obtained by determining nitrogen after digesting the feed sample according to the procedure by Gallaher et al. (1975). The samples were then analyzed using the Alpkem auto analyzer (Alpkem Corp., Clackemas, OR). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were all determined using the procedures outlined for use with an Ankom (1999) machine. The high carbohydrate content of grains may interfere with the extraction of fats; therefore, the water soluble carbohydrate portion of the concentrate was extracted prior to being subjected to the Soxhilet procedure for fat extraction.

Statistical Analyses

Data were analyzed using analysis of variance for repeated measures with general linear models procedures of SAS with treatment and time as the main effects. An $\alpha < 0.05$ was set as statistically significant. Treatment means were compared using the Tukey test.

Results

Physical Measurements

Horses began the project at an average age of 226 $\pm$ 6 d and an average body weight of 325 $\pm$ 4.8 kg and increased to 405 $\pm$ 5.7 kg for an average gain of 80 kg (Table 5.3). Average weights for the three treatments throughout the trial were different from each other ($P < 0.05$). Dry lot with exercise yearlings were consistently heavier than the pastured yearlings which were heavier than dry lot housed without exercise yearlings (Table 5.3).

Wither height increased from 142.6 $\pm$ 0.6 cm to 147.5 $\pm$ 0.6 cm and hip height increased 140.1 $\pm$ 0.8 cm to 149.3 $\pm$ 0.7 cm from d 0 to d 112, which is an increase of 5 cm and 9 cm respectively. Management did not affect either hip nor wither height.

Girth increased from 154.6 $\pm$ 1.0 cm at day 0 to 167.3 $\pm$ 1.0 cm at day 112, which is an increase of 13 cm. Body length increased 6 cm from 147.5 $\pm$ 0.6 cm at day 0 to 153.2 $\pm$ 0.6 cm at day 112. Dry lot yearlings who did not receive exercise had reduced ($P < 0.05$) girth and length compared to dry lot yearlings receiving exercise with pasture yearlings falling between. All growth measurements increased ($P < 0.05$) from day 0 to day 112 (Table 5.3).

Yearlings on dry lot without exercise had a lower ($P < 0.05$) average daily gain in comparison to pastured yearlings. There were no significant treatment by time interactions detected at the $P < 0.05$ level. All measurements except girth were different ($P < 0.05$) when blocked by sex. Breed had an effect on all measurements ($P < 0.05$).

Feed Intake

Management influenced ($P < 0.05$) concentrate intake (Table 5.4). Dry lot yearlings that were exercised had greater daily concentrate intake than dry lot yearlings that did not receive exercise (Table 5.4). Exercised yearlings also had greater ($P < 0.05$) concentrate intake relative to body weight than pastured yearlings (Table 5.4). Calcium intake (mg/kg BW) was lower ($P < 0.01$) for horses in the dry lot with and without exercise than those on pasture. Phosphorus intake (mg/kg BW) was not different among treatments. There were no treatment x time interactions for feed intake. All Ca: P ratios were below 1:1, but Ca :P was greater ($P < 0.05$) with pastured yearlings (Table 5.4).

Bone Development

Treatment affected bone density and geometry. Pastured yearlings developed greater density ($P < 0.05$) in the right and left third metacarpals (MCIII) based on the lateral/medial radiograph (Figure 5.1and Figure 5.2) and in the dorsal/palmar views of the left MCIII (Figure 5.3) at d 112. At both d 56 and d 112, the width of the right MCIII (dorsal/palmar view) was greater ($P < 0.05$) in the pastured yearlings compared to the other groups (Figure 5.4). Further, the gain of bone width in the dorsal cortice of the right MCIII from d 0 to d 56 for the pasture yearlings was greater than ($P < 0.05$; Table

5.5). There was also a trend ($P < 0.08$) for the dry lot non-exercised yearlings to increase the lateral cortical width at d 56 (Table 5.5).

Discussion

Inverse Ca: P ratios have been shown to negatively affect skeletal development (Schryver et al., 1971). In the current study, pastured yearlings, dry lot housed yearlings, and yearlings on dry lot with exercise all received a diet with an inverse Ca: P ratio (average 0.79:1). On average P was two times higher than required for yearlings at a moderate rate of growth (NRC, 1989). While Ca intake was substantially lower than P intake, it still exceeded (+10 g) Ca requirements (NRC, 1989).

Pastured yearlings received more total Ca, although it was inverted, had the greatest Ca: P ratio compared to both groups of dry lot housed yearlings. This, in part, could explain why pastured horses had greater bone mineral content at d 112. However, similar to experiment two, changes in bone geometry indicate that activity level may have more of an impact on bone development than diet alone. Pastured yearlings had greater width of bone, width of dorsal corice showing the most development. This type of bone modeling is consistent with findings by Hiney et al. (2004) showing that high intensity exercise remodels bone to accommodate higher strain rates produced on the dorsal aspect of the bone. Although the activity level of pastured yearlings was not determined in the current study, they did have more space to reach higher level of speed compared to dry lot housed yearlings both with and without exercise.

We hypothesized that a forced exercise program could provide adequate stimulus for enhanced bone development when yearlings were housed in confinement. However, bone mineral content and geometry were not affected by the exercise program used in this study. The exercise protocol utilized in this study may have not been strenuous enough to stimulate bone development equal to that produced by higher speeds achieved with pastured yearlings. Future research should strive to find the balance between exercise intensity that stimulates optimal bone remodeling while not over straining bone causing irrevocable damage.

Table 5.1. Concentrate formula and concentrate and forage nutrient content

	Concentrate	Coastal bermudagrass hay	Pasture grass
Formula, % as fed			
Oats, ground	40.00		
Corn, ground	27.30		
Soybean meal w/o hull	10.00		
Alfalfa meal, 17%	7.50		
Wheat bran	7.50		
Molasses	5.00		
BioFos	0.50		
Salt	0.75		
Lysine, 98%	0.10		
TM premix[a]	1.00		
Vitamin premix[b]	0.05		
Analysis, DM basis, except DM			
DM, %	88.89	93.18	89.54
CP, %	16.09	8.67	14.19
NDF, %	32.52	78.00	70.12
ADF, %	13.04	37.69	31.20
Fat, %	2.60	1.26	2.10
Ca, %	0.27	0.30	0.43
P, %	0.49	0.17	0.24
Cu, ppm	27.42	5.51	9.19
Fe, ppm	302.10	166.40	481.20
Mn, ppm	77.78	56.41	135.35
Zn, ppm	73.71	24.41	27.32

[a] Trace mineral (TM) premix provided the following amounts of minerals per kilogram of concentrate: 25.4 mg Ca, 17.4 mg Fe, 47.3 mg Zn, 32.4 mg Mn, 25.3 mg Cu, 0.15 mg Co, 0.10 mg I, and 0.01 mg Se.

[b] Vitamin premix provided the following amounts of vitamins per kilogram of concentrate: 6600 IU vitamin A, 440 IU vitamin D_3, 206 IU vitamin E, 0.01 mg vitamin B12, 3.7 mg riboflavin, 11.7 mg niacin, 4.6 mg pantothenic acid, 66.9 mg choline chloride, 1.2 mg folic acid, 1.2 mg pyridoxine, and 2.1 mg thiamin.

Table 5.2. Exercise Protocol of yearlings on the dry lot with forced exercise treatment

Week	Speed (m/s)	Time (min)	Distance (km)	Total distance per day (km)	Total distance per week (km)
4	Hand walk and acclimatize to exerciser				
5	2	20	2.4	2.4	9.6
6	2	10	1.2	3.9	15.6
	5	5	1.5		
	2	10	1.2		
7	2	10	1.2	5.4	21.6
	5	10	3		
	2	10	1.2		
8	2	10	1.2	6.9	27.6
	5	15	4.5		
	2	10	1.2		
9	2	7.5	0.9	7.8	31.2
	5	20	6		
	2	7.5	0.9		
10-16	2	7.5	0.5	8.5	34.0
	5	25	7.5		
	2	7.5	0.5		

Table 5.3. Influence of sex, breed, and treatment on growth and development of yearlings

	Sex[1]		Breed[2]		Treatment			Overall
	Male	Female	TB	QH	Dry Lot	Pasture	Exercise	
Number	16	13	17	12	9	9	11	29
Weight[3], kg	371.1	359.2	353.6	383.0	351.0^{a}	379.2^{b}	364.5^{c}	365.5
Initial	330.4	316.8	313.9	339.0	314.3^{a}	323.9^{b}	332.7^{c}	325.0
Final	409.1	394.0	390.4	424.6	386.7^{a}	404.1^{b}	419.6^{c}	405.2
Gain	78.7	77.2	76.5	85.6	72.3^{a}	80.2^{b}	86.9^{c}	80.2
ADG	0.70	0.69	0.68^{a}	0.76^{b}	0.65^{a}	$0.72^{a,b}$	0.78^{b}	0.72
Girth[3], cm	161.3	159.8	159.2	162.6	159.8	159.2	158.7	160.2
Initial	155.8	153.1	153.0	156.8	153.2	154.2	156.0	154.6
Final	167.5	167.2	166.0	169.3	164.7	167.4	169.4	167.3
Gain	11.7	14.0	12.9	12.5	11.5	13.1	13.5	12.7
Withers height[3], cm	146.1	144.3	145.0	143.5	145.7	144.9	145.5	144.7
Initial	143.3	141.8	143.8	141.0	143.2	142.8	142.1	142.6
Final	148.5	146.3	148.9	145.5	147.7	147.6	147.3	147.5
Gain	5.2	4.5	5.1	4.6	4.6	4.8	5.2	4.9
Body length[3], cm	145.7	143.6	144.1	145.7	143.0	143.8	143.0	144.5
Initial	141.0	139.0	139.7	140.7	138.0	139.6	142.2	140.1
Final	150.2	148.1	148.4	150.5	148.7	148.2	150.6	149.3
Gain	9.2	9.1	8.7	9.8	10.8	8.7	8.4	9.1
Hip height[3], cm	151.2	149.5	151.0	149.4	150.8	150.3	150.2	149.9
Initial	148.1	146.8	148.1	146.6	147.7	147.3	147.5	147.5
Final	154.0	152.3	154.0	152.2	153.5	153.0	153.3	153.2
Gain	6.0	5.5	5.9	5.6	5.8	5.6	5.8	5.7

[a,b]Row means with different superscripts differ ($P < .05$).
[1]Sex effect ($P < .05$) for all measurements except for girth.
[2]Breed effect for all measurements ($P < .05$).
[3]Means of measurement for entire experiment.

Table 5.4. Daily feed and nutrient intake by treatment.

Treatment	Dry Lot	Pasture[1]	Exercise
Concentrate intake			
kg	6.28[a]	6.59[a,b]	6.79[b]
% of BW	1.54[a,b]	1.50[a]	1.61[b]
Hay intake			
kg	3.62	3.62	3.62
% of BW	1.04	.99	1.01
Calcium			
g	26.30[a]	31.61[b]	27.57[c]
mg/kg BW/d	71.08[a]	78.96[b]	71.93[a]
Phosphorus			
g	34.13[a]	37.89[b]	36.43[c]
mg/kg BW/d	92.25	94.70	95.11
Ca:P Ratio	.77[a]	.83[b]	.76[c]

[a,b,c]Row means with different superscript differ ($P < .05$)
[1]Pature intake estimated to be similar to hay intake of both dry lot and exercise groups.

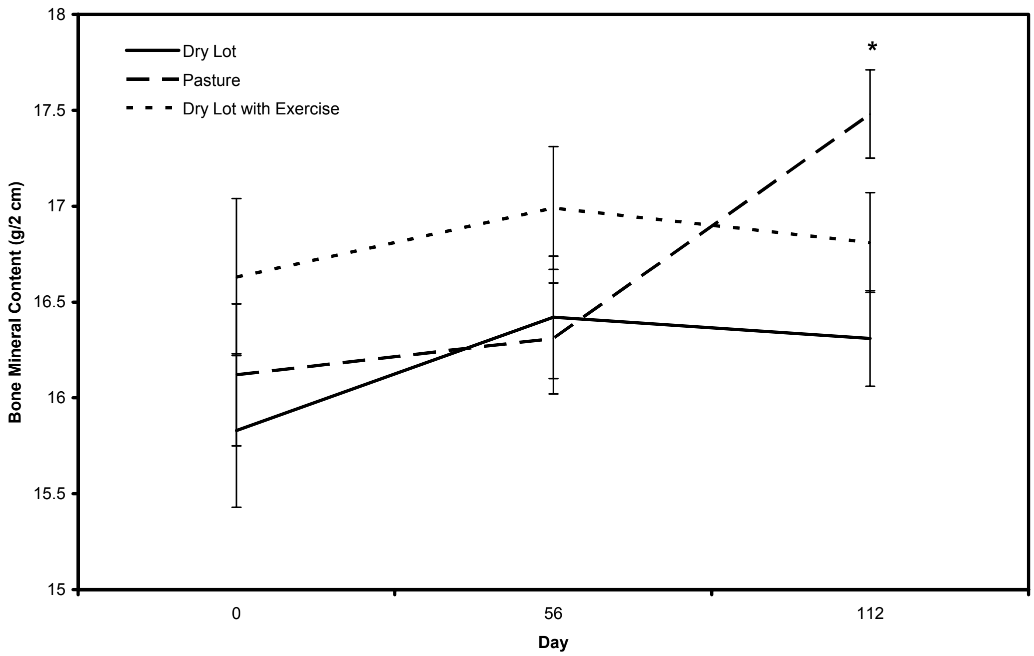

Figure 5.1. Bone mineral content of left third metacarpal, lateral/medial view.
* Pasture > dry lot and dry lot with exercise (P < .05).

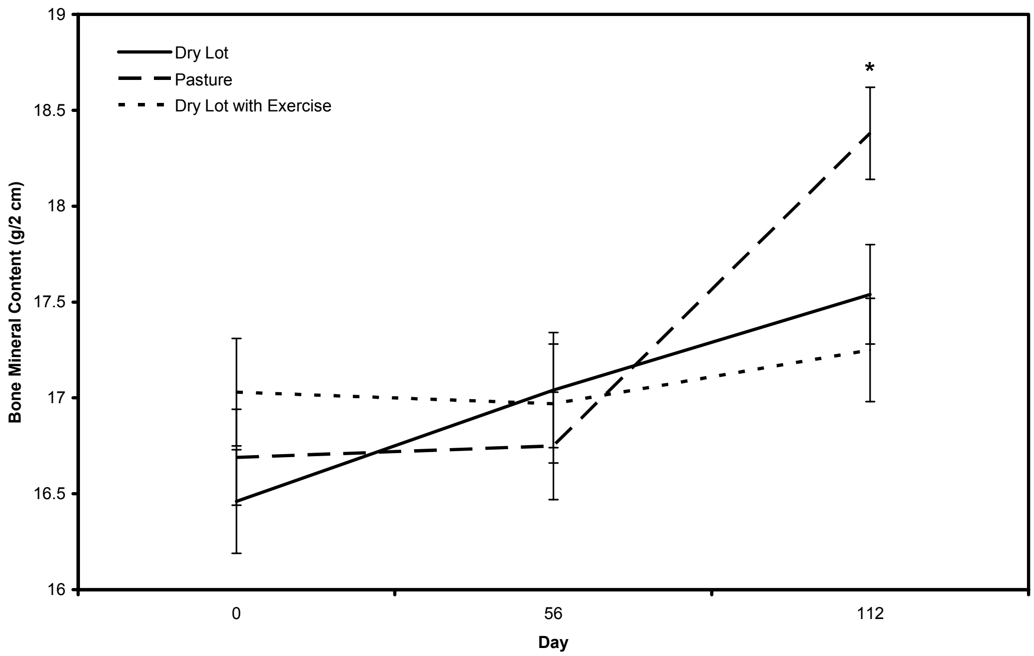

Figure 5.2. Bone mineral content of right third metacarpal, lateral/medial view.
* Pasture > dry lot and dry lot with exercise (P < .05).

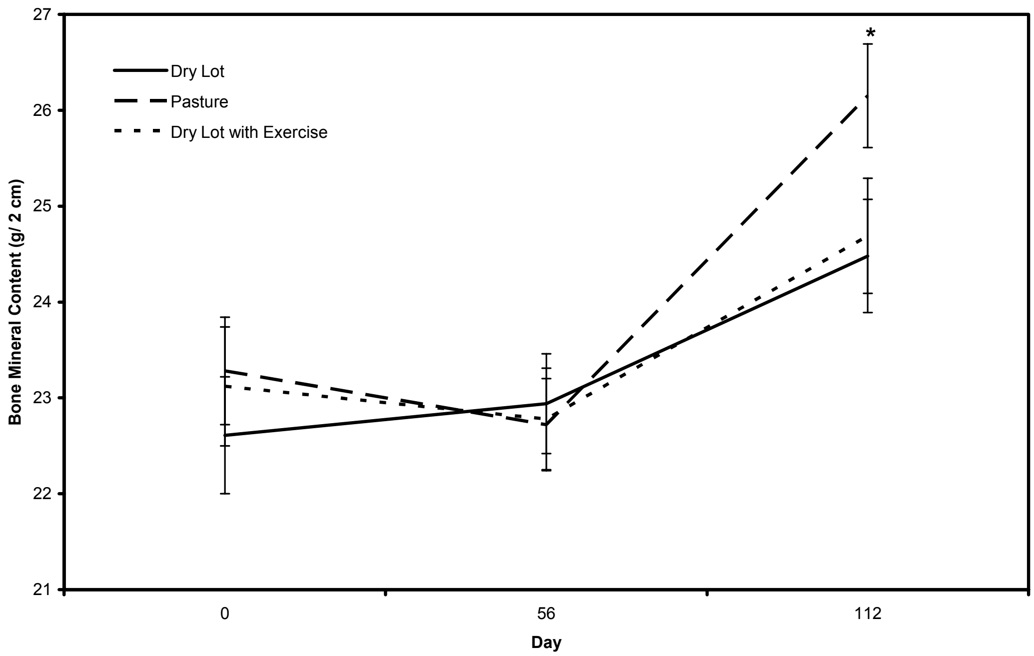

Figure 5.3. Bone mineral content of left third metacarpal, dorsal/palmar view. * Pasture > dry lot and dry lot with exercise (P < .05).

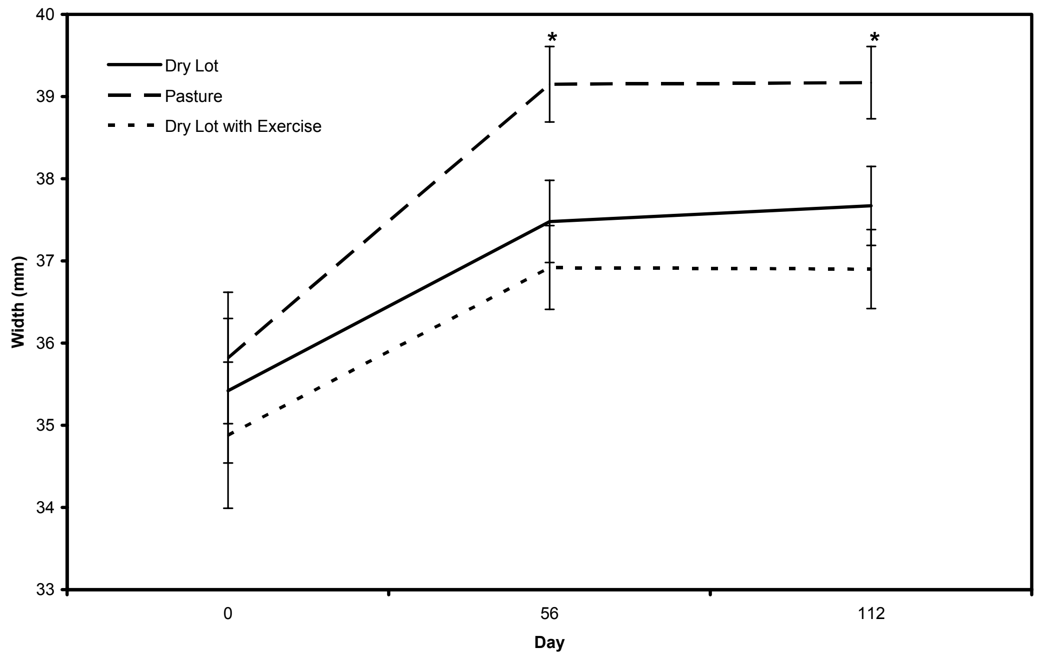

Figure 5.4. Bone width of right third metacarpal, dorsal/palmar view.
* Pasture > dry lot and dry lot with exercise (P < .05).

Table 5.5. Influence of sex, breed, and treatment on cortical widths of yearlings.

	Sex		Breed		Treatment		
	Male	Female	TB	QH	Dry Lot	Pasture	Exercise
Number	16	13	17	12	9	9	11
Right dorsal cortice (mm)							
d 0	9.52	9.81	9.31	10.15	9.85	9.22	9.99
d 56	9.70	10.12	9.58	10.33	9.85	9.89	9.92
d 112	9.75	9.93	9.54	10.25	9.75	9.94	9.77
Change right dorsal cortice (mm)							
d 0 - d 56	0.17	0.31	0.27	0.18	.00[a]	67[b]	-.07[a]
d 56 - d 112	0.05	-0.20	-0.04	-0.08	-0.10	0.05	-0.15
Total change	0.22	0.11	0.23	0.09	-0.10	0.72	-0.22
Left lateral cortice (mm)							
d 0	7.70	7.78	7.66	7.02	7.70	7.01	7.57
d 56	5.52	8.99	9.33[a]	8.69[b]	9.65[a]	8.77[b]	8.82[a,b]
d 112	6.27	8.40	8.78	8.17	8.43	8.63	8.51
Change left lateral cortice (mm)							
d 0 - d 56	2.04[a]	1.21[b]	1.66	1.67	1.95	1.76	1.25
d 56 - d 112	-0.48	-0.59	-0.55	-0.52	-1.22[a]	-0.14[b]	-0.32[a,b]
Total change	1.55[a]	0.61[b]	1.12	1.15	0.74[a]	1.61[b]	0.94[a]

[a,b]Row means with different superscripts differ (P < .05).

CHAPTER 6
CONCLUSIONS

Increases in bone mineral content (BMC) due to maturation of yearling horses has been reported with most occurring within the first year and a half of life (Nielsen et al., 1997, Nolan et al., 2001). Changes in housing, exercise, and diet have been found to impact the final quality and quantity of bone (Hoekstra et al., 1999; Porr et al., 1998; Cornelissen et al., 1999). These three experiments were conducted to determine the influence of housing, exercise, and diet on the bone development of yearling horses in order to maximize bone integrity.

In all studies, housing significantly influenced the quantity of bone with pasture housed horses maintaining a higher rate of deposition. Forced exercise did increase the BMC of dry lot housed yearlings but did not exceed that of their pasture housed contemporaries. Analyzing the cortices of the third metacarpal (MC III) indicated that pastured yearlings changed the geometry of bone when compared to both dry lot housed yearlings without or without exercise. It is not known at this time whether quantity of bone or geometry indicates overall strength and ability to withstand strain. Further research to elucidate the importance of these characteristics is needed to accurately access how effective exercise is in reducing the detrimental effects of limited exercise due to confinement. Inverse calcium (Ca) – phosphorus (P) ratio of 0.79:1 did not produce adverse effects in the criteria measured. This may indicate a need to revaluate the current NRC (1989) requirement for growing horses, as well as how much excess P is needed before adverse effects on bone development occur.

From these studies and previous research conducted with yearling horses, it appears the bone quantity and geometry can be influenced by both housing and forced exercise. Additionally, assessment of Ca and P metabolism in the growing horse is necessary to more accurately define the requirements.

The results of these experiments indicate the importance of housing yearlings on pasture allowing free exercise. Realizing pasture rearing yearlings is not always geographically or economically feasible; there is potential value in an exercise program to stimulate the skeleton of yearling horses to model bone.

LITERATURE CITED

Anderson, J. J. 1996. Calcium, phosphorus and human bone development. J. Nutr. 126(4)1153S-8S.

Barnevald, A., and P. R. van Weeren. 1999. Conclusions regarding the influence of exercise on the development of the equine musculoskeletal system with special reference to osteocondrosis. Equine Vet. Supp. 31:112-119.

Baron, R. 1990. Anatomy and ultrastructure of bone. In: Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. pp. 174-201. William Byrd Press. Richmond, VA.

Bell, R. A., B. D. Nielsen, K. Waite, D. Rosenstein, and M. Orth. 2001. Daily access to pasture turnout prevents loss of mineral in the third metacarpal of Arabian weanlings. J. Anim. Sci. 79:1142-1150.

Bennell, K. L., S. A. Malcolm, K. M. Kahn, S. A. Thomas, S. J. Reid, and P. D. Brunker. 1997. Bone mass and bone turnover in power athletes, endurance athletes, and controls: a 12 month longitudinal study. Bone 20:477-484.

Bigot, G., A. Bouzidi, C. Rumelhart, and W. Martin-Rosset. 1996. Evolution during growth of the mechanical properties of the cortical bone in equine cannon-bones. Med. Eng. Phys. 18(1):79-87.

Brama, P. A., R. A. Bank, J. M. Tekoppele, and P. R. van Weeren. 2001. Training affects the collagen framework of subcondral bone in foals. Vet. J. 162:24-32.

Buckingham, S. H. W., and L. B. Jeffcott. 1991. Osteopenic effects of forelimb immobilization in horses. Vet. Rec. 128:370-373.

Burr, D. B., A. G. Robling, and C.H. Turner. 2002. Effects of biomechanical stress on bones in animals. Bone 30:781-786.

Carter, D. R. and W. C. Hayes. 1977. Bone creep-fatigue damage accumulation. J. Biomechanics 22:625-635.

Cashman, K D. 2002. Calcium intake, calcium bioavailability, and bone health. Brit. J. Nutr. Supp 2:169-177.

Cooper, S. R., D. R. Topliff, D. W. Freeman, J. E. Breazile, and R. D. Geisert. 2000. Effect of dietary cation-anion difference on mineral balance, serum osteocalcin concentration and growth in weanling horses. J. Equine Vet. Sci. 20:39-44.

Cornelissen, B. P. M., P. R. van Weeren, and A. G. H. Ederveen. 1999. Influence of exercise on cortical bone mineral density of immature cortical and trabecular bone of the equine metacarpus and proximal sesamoid bone. Equine Vet. J. Suppl. 31:79-85.

Cunha, T. J. 1981. Bone development in horses: vitamins and minerals. Feedstuffs. 53(33):27-29.

Cymbaluk, N. F. 1990. Cold housing effects on growth and nutrient demand of young horses. J. Anim. Sci. 68:3152-3162.

Elmore-Smith, K. A., J. L. Pipkin, L. A. Baker, W. J. Lampley, J. C. Haliburton, and R. C. Bachman. 1999. The effect of aerobic exercise after a sedentary period on serum, fecal, and urine calcium and phosphorus concentrations in mature horses. Proc. 16th Equine Nutr. Phys. Symp. Raleigh, NC pp. 106-107.

Firth, E. C., C. W. Rogers, and A. E. Goodship. 2000. Bone mineral density changes in growing and training Thoroughbreds. AAEP Proceedings 46:295-299.

Firth, E. C., P. R. van Weeren, D. U. Pfeiffer, J. Delahunt, and A. Barnevald. 1999. Effect of age, exercise and growth rate on bone mineral density (BMD) in third carpal bone and distal radius of Dutch Warmblood foals with osteochondrosis. Equine Vet. J. Suppl. 31:74-78.

Frost, H. 1987. The mechanostat: A proposed pathogenetic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. Bone Miner. 2:73-85.

Frost, H. M. 2001. From Wolff's Law to the Utah Paradigm: Insights about bone physiology and its clinical applications. The Anatomical Rec.. 262:398-419.

Gallaher, R. N., C. O. Weldon, and J. G. Frutal. 1975. An aluminum block digester for plant and soil analysis. Soil Sci. Soc. Amer. Proc. 39:803-806.

Gray, J., P. Harris, and D. H. Snow. 1988. Preliminary investigations into the calcium and magnesium status of the horse. Anim. Clin. Biochem. 307-317.

Heleski, C. R., A. C. Shelle, B. D. Nielen, and A. J. Zanella. 1999. Influence of housing on behavior in weanling horses. In: Proc. 16th Equine Nutr. Phys. Symposium, Raleigh, NC. pp. 249-250.

Hiney, K. M., B. D. Nielsen, and D. Rosenstein. 2004. Short duration exercise and confinement alters bone mineral content and shape in weanling horses. J. Anim. Sc. 82:2313-2320.

Hintz, H. F. 1996. Mineral requirements of growing horses. Pferdeheikunde 12(3):303-307.

Hodgson, D. R., and R. J. Rose. 1994. The athletic horse: principales and practice of equine sports medicine. W. B. Saunders Co., Philadelphia, PA.

Hoekstra, K. E., B. D. Nielsen, M. W. Orth, D. S. Rosenstein, H. C. Schott II, and J. E. Shelle. 1999. Comparison of bone mineral content and biochemical markers of bone metabolism in stall- vs. pasture-reared horses. Equine Vet. J. 30:601-604.

Hoffman, R. M., L. A. Lawrence, D. S. Kronfeld, W. L. Cooper, D. J. Sklan, J. J. Dascanio, and P. A. Harris. 2000. Dietary carbohydrates and fat influence radiographic bone mineral content of growing horses. J. Anim. Sci. 77:3330-3338.

Jaworski, Z. 1984. Lamellar bone turnover system and its effector organ. Calcif. Tissue Int. 36:S46-52.

Jordan, R. M., V. S. Meyers, B. Yoho, and F. A. Spurrell. 1975. Effect of calcium and phosphorus levels on growth, reproduction, and bone development of ponies. J. Anim. Sci. 40:78.

Karlsson, M. K., H. Magnusson, C. Karlsson, and E. Seeman. 2001. The duration of exercise as a regulator of bone mass. Bone 28:128-132.

Kiratli, B. J. 1996. Osteoporosis: Immobilization osteopenia. Academic Press, Orlando, FL.

Krook, L., and J. E. Lowe. 1964. Nutritional secondary hyperparathyroidism in the horse. Pathol. Vet. 1(Suppl. 1):1-11.

Lawrence, L. A., E. A. Ott, G. J. Miller, P. W. Poulos, G. Piotrowski, and R. L. Asquith. 1994. The mechanical properties of equine third metacarpals as affected by age. J. Anim. Sci. 72:2617-2623.

LeBlanc, A. D., V. S. Schneider, H. J. Evans, D. A. Engelbretson, and J. M. Krebs. 1990. Bone mineral loss and recovery after 17 weeks of bed rest. Bone Min. Res. 5(8):843-850.

Lewis, L. D. 1995. Equine Clinical Nutrition: Feeding and Care. William and Wilkins, Baltimore, MD.

Maenpaa P. E., A. Pirskanen, and E. Koskinen. 1988. Biochemical indicators of bone formation in foals after transfer from pasture to stables for the winter months. Am. J. Vet. Res. 49(11):1990-1992.

Meakim, D. W., E. A. Ott, R. L. Asquith, and J. P. Feaster. 1981. Estimation of mineral content of the equine third metacarpal by radiographic photometry. J. Anim. Sci. 53(4):1019-1026.

McCarthy, R. N. and L. B. Jeffcott. 1991. Treadmill exercise intensity and its effects on cortical bone in horses of various ages. Equine Exercise Physiology 3:419-428.

McCarthy, R. N. and L. B. Jeffcott. 1992. Effects of treadmill exercise on cortical bone in the third metacarpus of young horses. Res. Vet. Sci. 52:29-37.

Murray, R. C., S. Vedi, H. L. Birch, K. H. Lakhani and A. E. Goodship. 2001. Subcondral bone thickness, hardness and remodeling are influenced by short-term exercise in a site specific manner. J. Ortho. Res. 19:1035-1042.

Nelson, D. A., and M. L. Bouxsein. 2001. Exercise maintains bone mass, but do people maintain exercise? J. Bone Miner. Res. 16(2):202-205.

Nielsen, B. D., G. D. Potter, L. W. Greene, E. L. Morris, M. Murray-Gerzik, W. B. Smith, and M. T. Martin. 1998. Response of young horses to training to varying concentrations of dietary calcium and phosphorus. J. Equine Vet. Sci. 18(6):397-404.

National Research Council. 1989. Nutrient Requirements of Horses (5th Ed.) National Academy Press, Washington, DC.

Nolan, M. M., G. D. Potter, K. J. Mathiason, P. G. Gibbs, E. L. Morris, L. W. Greene, and D. Topliff. 2001. Bone density in the juvenile racehorse fed differing levels of minerals. Proc. 17th Equine Nutr. Phys. Symp. Louisville, KY pp. 33-38.

Ott, E. A. and R. L. Asquith. 1989. The influence of mineral supplementation on growth and skeletal development of yearling horses. J. Anim. Sci. 67:2831 2840.

Ott, E. A., L. A. Lawrence, and C. Ice. 1987. Use of the image analyzer for radiographic photometric estimation of bone mineral content. Proc. Equine Nutr. And Physiol. Symp. Ft. Collins, CO. p 527.

Pagan, J. D. 1989. Calcium, hindgut function affect phosphorus needs. Feedstuffs. 61(35):1-2.

Parfitt, A. M. 1984. The cellular basis of bone remodeling: the quantum concept reexamined in the light of recent advances in the cell biology of bone. Calcificaiton Tissue Int. 36:S37-43.

Parfitt, A. and B. Chir. 1987. Bone remodeling and bone loss: understanding the pathophysiology of osteoporosis. Clin Obst. Gyn. 30(4):789-796.

Pool, R. 1991. Pathology of the metacarpus: normal adaptive remodeling of MCIII, dorsal metacarpal disease and condylar injuries. Proc. 13th Bain-Fallon Mem. Lect. 13:105-111.

Porr, C. A., D. S. Kronfeld, L. A. Lawrence, R. S. Pleasant, and P. A. Harris. 1998. Deconditioning reduces mineral content of the third metacarpal bone in horses. J. Anim. Sci. 76:1875-1879.

Rasch, P. J. and R. K. Burke. 1963. Kinesiology and applied anatomy. Philadelphia: Lea and Febiger.

Reilly, G. C., J. D. Currey, and A. E. Goodship. 1997. Exercise of young thoroughbred horses increase impact strength of the third metacarpal bone. J. Ortho. Res. 15(6):862-868.

Riggs, C. M. and A. Boyde. 1999. Effect of exercise on bone density in distal regions of the equine third metacarpal bone in two-year-old Thoroughbreds. Equ. Vet. J. 30:555-560.

Rivera, E., S. Bejamin, B. Nielsen, J. Shelle, and A. J. Zanella. 2002. Behavioral and physiological responses of horses to initial training: the comparison between pastured versus stalled horses. Appl. Anim. Behav. Sci. 78:235-252.

Rubin, C. T. 1984. Skeletal strain and functional significance of bone architecture. Calc. Tissue Inter. 36:S11-S18.

Schryver, H. F., H. F. Hintz, and P. H. Craig. 1971. Phosphorus metabolism in ponies fed varying levels of phosphorus. J. Nutr. 101:1257-1261.

Sherman, K. M., G. J. Miller, T. J. Wronski, P. T. Colahan, M. Brown, and W. Wilson. 1995. The effect of training on equine metacarpal bone breaking strength. Equine Vet. J. 27(2):135-139.

Smit, T. and A. H. Beuger. 2000. Is BMU-coupling a strain-regulated phenomenon? A finite element analysis. J. Bone Miner. Res. 15(2):301-307.

Stadermann, B., T. Nehring, and H. Meyer. 1992. Calcium and Magnesium absorption with roughage or mixed feed. Pferdeheilkunde. pp. 77-80.

Steinberg, M. E., and J. Trueta. 1981. Effects of activity on bone growth and development in the rat. Clin. Orth. Rel. Res. 156:52-60.

Stephens, T. L., G. D. Potter, P. G. Gibbs, and D. Hood. 2004. Mineral balance in juvenile horses in race training. J. Equine Vet. Sci. 24:438-450.

Stover, S. M., R. R. Pool, R. B. Martin, and J. P. Morgan. 1992. Histological features of the dorsal cortex of the third metacarpal bone mid-diaphysis during postnatal growth in Thoroughbred horses. J. Anat. 181:445-489.

Torstveit, M. K. 2002. Bone adaptation to mechanical loading. Tidsskr Nor Laegeforen 122:2109-2111.

Tsuji, S., F. Katsukawa, S. Onishi, and H. Yamazaki. 1996. Period of adolescence during which exercise maximizes bone mass in young women. J. Bone Min. Meta. 14:89-95.

van der Harst, M. R., P. A. J. Brama, C. H. A. van de Lest, G. H. Kiers, J DeGroot, and P. R. van Weeren. 2004. An integral biochemical analysis of the main constituents of articular cartilage, subchondral and trabecular bone. Osteoarthritis Cartilage. 12:752-761.

van Weeren, P. R., P. A. Brama, and A. Barneveld. 2000. Exercise at young age may influence the final quality of the equine musculoskeletal system. AAEP Proc. 46:29-35.

Whalen, R. T., D. R. Carter, and C. R. Steele. 1993. The relationship between physical activity and bone density. Orthop. Trans. 12:56-65.

Wyatt, C. J., M. E. Hernandez-Lazano, R. O. Mendez and M. E. Valencia. 2000. Effect of different calcium and phosphorus content in Mexican treatments on rat femur bone growth and composition. Nutr. Res. 20(3):427-431.

Yeh, J. K., and J. F. Aloia. 1990. Deconditioning increases bone resorption and decreases bone formation. Metab. 39:659-663.

Young, J. K., G. D. Potter, L. W. Greene, and J. W. Evans. 1989. Mineral balance in resting and exercised miniature horses. Proc. 11th Equine Nutr. Phys. Symp. pp. 79-82.

BIOGRAPHICAL SKETCH

Tonya Leigh Stephens was born on March 5, 1977, in Dublin, Texas. She attended grade school and high school in Comanche, Texas, and graduated in May 1995. After high school, she pursued a degree in Dairy Science at Texas A&M University in College Station, Texas. She graduated in December 1998 as a presidential endowed scholar and immediately began a Masters of Science degree specializing in equine nutrition and exercise physiology. While working on her degree with Dr. Gary Potter, Tonya was the assistant coach for the intercollegiate horse judging team in addition to her responsibilities as a teaching assistant for several labs and lectures. Her thesis dealt with mineral metabolism in young horses in race training and subsequent effect on bone. Her degree was conferred in May of 2002.

In August of 2001, Tonya relocated to Gainesville, Florida, to attend the University of Florida as a doctorial candidate in equine nutrition and exercise physiology under the guidance of Dr. Edgar A. Ott. Initially recruited to coach the intercollegiate judging team, Tonya also became to manager of the Horse Teaching Unit in August 2002. Effects of housing, exercise, and diet on the growth and development of bone in the yearling horse was the focus of her research throughout her doctorate program.