RESPONSE OF YEARLING QUARTER HORSES TO VARYING CONCENTRATIONS OF DIETARY CALCIUM

By

AMBER DAHN MOFFETT

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Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Minerals are essential to many functions in the body ranging from the formation of structural components to enzymatic cofactors and energy transfer. Additionally, some minerals are integral parts of vitamins, hormones and amino acids. There are seven macrominerals (calcium, phosphorus, potassium, sodium, chloride, magnesium and sulfur) that horses require which can be obtained from pasture, roughage and/or grain. Mineral research has been of great interest to the horse industry, especially in regards to growing horses. Calcium (Ca) and phosphorus (P) are two minerals that have been extensively researched in humans, rats, horses, sheep and swine. Calcium and phosphorus metabolism are of great interest to the industry as horses are continually being pushed to grow faster and perform harder at a younger age. This push has sparked further evaluation of the initial Ca and P research performed in ponies by Schryver et al.(1970, 1971) and the requirement of Ca and P in exercised horses (Schryver et al., 1978; Buchholz et al., 1999), two-year olds in training (Nielsen, 1992; Nielsen et al., 1998, 1997, 1995) as well as growing horses (Hintz et al, 1986; Schryver et al., 1987; Gibbs et al., 1989).

Calcium and phosphorus requirements for growing horses were estimated from research performed in ponies (Jordan et al., 1975; Schryver et al., 1970a; Schryver et al.,

1971a,b). Krook and Maylin (1988) reported that a nutrient is generally required in inverse proportion to body weight. Therefore, the data reported from 200 kg ponies may not be applicable to 500 kg horses when expressed as milligram of nutrient per kilogram of body weight. Consequently, the objective of this experiment was to evaluate the effects of feeding various levels of calcium on mineral metabolism, bone density and parameters of growth in yearling quarter horses.

CHAPTER II

LITERATURE REVIEW

Calcium Absorption

Calcium is primarily absorbed in the small intestine of many species (Schryver et al., 1970b). Schryver et al. (1970; 1974b) utilized cannulated ponies to determine that the small intestine was the site of greatest absorption of Ca while little Ca was absorbed in the hindgut. The type of diet and amount of Ca in the diet had little effect on where Ca was absorbed (Schryver et al., 1970b). The upper half of the small intestine has the greatest capacity (40%) for Ca absorption while only 25% of the Ca entering the hindgut was absorbed (Schryver et al., 1970b, 1974b). Furthermore, very little (10%) of the Ca entering the large colon is absorbed.

Calcium is absorbed by passive or facilitated diffusion or by active transport (Wasserman, 1968). A protein called Ca-binding protein (CaBP) has been found in horses and actively transports Ca across the mucosa of the small intestine (Schryver et al., 1974b). Some factors affecting Ca absorption are the absolute or relative amount of Ca in the diet, the horse's vitamin D status, presence of oxalate, phytate, certain amino acids and carbohydrates, age of horse and extent of mineral digestion. Age apparently does not affect the horse's ability to efficiently absorb Ca as demonstrated by Schryver et al.(1974). The previous study indicated that young horses fed diets containing adequate

amounts of Ca absorbed half to two-thirds of the Ca from feedstuffs. According to Schryver et al. (1970) and Whitlock (1970), a horse's absorptive efficiency of Ca is higher at lower levels of Ca intake. Additionally, younger horses being fed two to three times their recommended levels of Ca have been reported to absorb almost 50% of the Ca present in the diet (Schryver et al., 1974b).

The lumen of the small intestine is bound by epithelial cells, which have striated borders composed of microvilli. On top of the microvilli is the brush border consisting of glycosaminoglycans. These structures probably play a significant role in Ca absorption. Many experiments in vivo and in vitro have been performed in chicks and rats, which have reported that the behaviors of the intestinal loops were not always identical (Irving, 1973). However, useful information has been obtained from in vitro studies and is a useful approach when its limitations are realized. Schacter and Rosen (1959) and Schacter et al. (1960a) first described an active transport mechanism for Ca absorption against a concentration gradient. This is relatively specific for Ca, not being found for other cations such as magnesium or potassium. Additionally this active transport was greater in samples from young rats versus older ones (Irving, 1973).

Bioavailability

Bronner (1994) more precisely defined digestibility as solubilization. According to Miller (1978), bioavailability refers to the availability of a mineral to an organism for use by the body. Total body retention, specific tissue incorporation or specific compound synthesis may be used to measure the bioavailability of an element from a basal ingredient or supplemental form. Because of this, bioavailability of Ca can be measured by Ca balance as well as incorporation into bone. Generally, the bioavailability of an

element from a particular source is determined by comparing its functional availability to a standard source. Standard sources allow the use of relative biological value (RBV) estimates for the expression of bioavailability of an element or source of an element. Calcium carbonate, which has a RBV of 100, is used as the standard for determining the RBV's of various sources of Ca (Miller, 1978).

Calcium Homeostasis

In terms of Ca homeostasis, a normal horse maintains plasma Ca levels between 11 and 12.5 mg/100ml (Schryver et al., 1974b). Plasma Ca is actually part of a larger Ca exchange pool. Calcium enters this pool by being absorbed through the intestinal mucosa as well as by bone resorption. Calcium can leave the exchange pool via urinary excretion, by endogenous intestinal excretion as well as Ca deposition into bone (Schryver et al., 1974b). To maintain Ca plasma concentrations at a constant level there must be a balance between Ca intake and retention. Little effect of Ca level was reported on Ca deposition and removal in horses (Schryver et al., 1970a; Whitlock, 1970) and in other species (Braithwaite et al., 1971; Phang et al., 1969). Horses differ from ruminants in the response of intestinal absorption and urinary excretion of Ca. According to Schryver et al. (1974b) at equivalent stages of maturity and intake of Ca, horses absorb more Ca and excrete more Ca as a proportion of absorbed Ca in the urine than sheep or calves (Schryver et al., 1974b).

The parathyroid gland is the most important endocrine gland in regards to Ca metabolism, since it helps maintain calcium homeostasis in the body (Irving, 1973). Parathyroid hormone (PTH) has a twofold action on bone. When blood Ca levels are below normal, PTH increases lysozomal enzymes and then increases osteoclast

production. At the same time, it is possible that osteoblast activity in bone is retarded as well as collagen synthesis diminished. Blood calcium level is the sole factor controlling PTH secretion which has a reciprocal relationship to the level of Ca in the blood (Irving, 1973). There is some evidence that PTH may influence absorption of Ca or phosphate in the gut, but this is a minor action compared to its effect on bone and kidney.

Blood calcium levels also affect the response of calcitonin. The fundamental action of calcitonin is to inhibit bone resorption Work performed in the early 1970's concluded that calcitonin played no major role in skeletal growth but the fall in blood calcium after hypercalcemia caused by PTH was calcitonin dependent (Irving, 1973).

Calcium Excretion and Retention

Urinary excretion of Ca is essential to Ca homeostasis in the horse. Diets high in P have been reported to increase urinary P excretion and decrease urinary excretion of Ca (Schryver et al., 1971a, 1971b, 1974b). The longer horses were fed a high Ca diet, the more Ca was excreted in the urine (Kemm, 1972). Kemm (1972) also reported increased ($P<.05$) urinary excretion of Ca in animals being fed a diet containing 1.6% Ca for 2 weeks or more with similar trends being seen with animals on a diet containing 0.8% Ca for extended periods of time. Other losses of Ca such as through sweat and lactation should be taken into account in determining Ca balance as well as for Ca requirements.

The fecal excretion of Ca is generally composed of endogenous intestinal secretion of Ca and unabsorbed dietary Ca. Typically, the amount of unabsorbed mineral present in feces is dependent upon intake and absorption (Schryver et al., 1974b). Balance trials have been performed in ponies and young horses using ^{47}Ca to estimate the amount of Ca secreted into the intestine, which is subsequently lost in the feces. Results

from these trials found, endogenous losses of Ca to be about 20 mg Ca/ kg body weight per day regardless of Ca intake even when animals were in a negative Ca balance (Schryver et al., 1970; Whitlock, 1970).

With 99% of the body's Ca found in bone, the rate of bone Ca deposition and removal is of great interest in growing animals. Braithwaite et al. (1971) reported that in sheep, that age appears to have a greater effect on bone Ca deposition than the amount of Ca in the diet. Buchholz et al. (1999) reported that supplementation of Ca and P during inactivity and subsequent aerobic activity affects the Ca and P balance in varying ages of horses. This increase in Ca balance resulted in increased bone densities after a sedentary period (Buchholz et al., 1999). Additionally, balance studies indicate increased retention of Ca as intake is increased, presumably by an increased skeletal mass (Schryver et al., 1974b, 1970a; Whitlock. 1970). Jordan et al. (1973) observed that the cortices of long bones was thinner in ponies fed a diet high in Ca (2.5%) for an extended period of time (30 months) when compared to ponies fed a normal diet. Whitlock (1970) reported that bone resorption and bone remodeling might be inhibited by a high Ca intake. Schryver et al. (1974b) also reported that bone Ca removal was inhibited by diets that contained high levels of Ca. However, it is not known whether this altered rate of bone turnover caused by the high Ca diet alters the integrity of bone itself. According to Schryver et al. (1974b) bending properties of femoral, radial and metacarpal cortices (Schryver et al, 1974a) and the whole metacarpal (Whitlock, 1970) showed no significant effects of dietary Ca when horses were fed diets adequate and low in Ca. On the other hand, Willoughby (1970) reported that foals fed a Ca diet below NRC requirements had lower

breaking strengths of the whole metacarpal bone than foals fed diets containing 0.75% Ca.

Calcium Requirements

Minerals are involved in a number of body functions ranging from the formation of structural components to enzymatic cofactors and energy transfer. Some of these minerals are important parts of vitamins, hormones and amino acids. Horses obtain a majority of their necessary minerals from grain, hay and pasture. Calcium makes up roughly 35% of bone structure (El Shorafa et al., 1979) and is involved in body functions such as muscle contraction and blood clotting. According to the NRC for horses (1989), the true absorptive efficiency of horses tend to decline from 70% in young horses to about 50% in mature horses. This variation in Ca absorption is due to the amount of Ca and P present in the feed (Schryver et al., 1970b). Because of this range in Ca absorption, NRC (1989) suggests using a 50% Ca absorption efficiency to calculate Ca requirements for any age of horse. Calcium requirements for growth and skeletal development are somewhat difficult to determine because the adequacy of dietary Ca for skeletal growth is not well defined. According to Schryver et al. (1974b), lean body tissue of the horse contains about 20g of Ca per kg of tissue. During growth, each kg of live weight gain requires 16g of Ca to be absorbed from the intestine to maintain a constant body mineral composition (Schryver et al., 1974b). The NRC (1989) suggests that horses, with a mature weight of 450kg, gain 100 kg or 1kg/day between 3 and 6 months of age and 100kg or 0.7kg/day from a yearling to a long yearling (18 months). This requires a young horse to absorb sufficient amount of Ca to meet obligatory losses in addition to minerals necessary for skeletal mineralization.

As previously stated, calculations for Ca requirements assume an absorptive efficiency of 50%, regardless of age. The NRC (1989) suggests the following equation to calculate the Ca requirement for growing horses not in training: Ca = 0.04BW + 32ADG. In this equation, body weight and average daily gain are reported in kg and kg of gain/day respectively. Additionally, 0.04 is derived from the following equation (500kg x 20mg/0.5 and 20mg/500kg = 0.04), which states that a 500kg horse would require 20g of Ca to meet obligatory losses assuming a 50% absorption efficiency. Schryver et al. (1971a, 1970) estimates endogenous losses of 20 mg of Ca/kg of BW per day. Requirements are generally calculated using data for obligatory losses, body composition and growth rates. Any difference in requirements is probably due to different absorption values or growth rates. Whitlock (1970) reported similar estimates of endogenous fecal Ca losses in young horses. The Ca required for growth and skeletal development in horses is slightly more difficult to determine than maintenance because the requirement of dietary Ca for proper skeletal development is not well defined (Schryver et al., 1970). Teeter et al. (1967) reported that the rate of body weight gain (ADG) has been used to estimate dietary Ca requirements and is actually of limited value in horses. Schryver et al. (1969) reported that ponies on low Ca diets gained weight and height at the same rate as those on high Ca diets. Bone formed during this time of lower Ca intake resulted in less Ca in the bone as well as weaker bones than those fed higher Ca diets. Due to this fact, retention of Ca is a better estimate than weight and height when determining optimal Ca levels required for proper skeletal development in growing horses.

Research since the NRC revision in 1989 have mainly been performed in young horses in training (Nielsen et al, 1995, 1997, 1998; Hiney et al., 2000) and in mature idle

horses (Buchholz et al., 1999). These studies have reported an increased need for Ca supplementation as young horses enter race training and as mature horses increase activity levels. The NRC (1989) suggests young horses (12 to 18 months) need 24-28g/d Ca assuming a 50% absorption rate. However, commercially available rations contain 3-5g Ca per pound of feed (Purina, Nutrena, ACCO and Buckeye, 2001). If NRC suggests 24-38g/d and commercial feed manufacturers are supplying 30-50 g/d, assuming 10 lbs/d feed intake, should the recommendations for Ca in growing horses be increased?

Radiographic Analysis of Bone Density and Biochemical Markers.

Bone metabolism is a dynamic and continuous remodeling process that is normally maintained in a tightly coupled balance between resorption of old or injured bone and formation of new bone. On a microscopic level, bone metabolism occurs as bone metabolism units (BMU) (Christenson, 1997). Christenson (1997) also states that global (overall) bone metabolism represents the cumulative behavior of several BMU's such that defects in the organization of bone or any imbalance leading to bone resorption can result in substantial changes in functional integrity over time. These changes can occur rapidly when the turnover rate is increased.

In human healthcare, many tools are available to diagnose and monitor diseases of bone ranging from histomorphometry and ultrasound to bone density measurements and biochemical markers of bone metabolism (Christenson, 1997). Many of the same procedures are used in various species of animals such as horses, chickens (Meakim et al., 1981; Nielsen et al, 1998a,b; Ott et al., 1985).

Based on the phases of bone cycle, markers of bone metabolism can be classified either as indicators of bone formation, bone resorption or overall bone turnover. Markers

for bone formation assess either osteoblastic synthetic activity or post release metabolism of procollagen while resorption markers reflect osteoclast activity and/or collagen degradation (Christenson, 1997). Alkaline phosphatase (ALP) is one marker associated with bone formation and is associated with cell plasma membranes. While ALP's exact function is unknown, it has a broad tissue distribution and appears to be involved in transport of substances from the intracellular compartment across the membrane to extracellular region (Christenson, 1997). In bone, ALP may also be involved in the breakdown of pyrophosphate, a potent inhibitor of Ca phosphate deposition at the extracellular level (Risteli, 1993). There are four ALP isoenzymes commonly present in circulation, each of which is specific for the respective liver, bone, placental and intestinal tissues they are associated with (Christenson et al., 1996). Bone-specific ALP is produced by osteoblasts in extremely high amounts during the formation phase of the bone cycle. Because of this, bone-specific ALP is an excellent indicator of global bone formation activity (Epstein, 1988; Whyte, 1983).

There are two immunoassay kits that have been cleared by the US Food and Drug Administration, Tandem-R Ostase™ assay (Hybritech, Inc., San Diego, CA) and Alkphase-B™ assay (Metra Biosystems, Inc., Mountain View, CA). The Ostase™ assay is based on two monoclonal antibodies in an IRMA format with results reported in ng/ml. Alkphase-B™ is an ELISA format where an immobilized monoclonal antibody picks up any bone-specific ALP in the serum. Paranitrophenyl phosphate substrate is converted by the captured bone-specific ALP and quantified spectrophotometrically.

Biochemical markers have been combined with radiographic analysis of bone density to evaluate mineral balance and estimate bone mineral content. Using a

procedure introduced by Meakim et al. (1981), bone mineral content has been studied in weanlings (Boren et al., 1986), yearlings (Ott et al., 1986) and young horses in training (Nielsen et al., 1995, 1997, 1998; Hiney et al., 2000). Radiographic evaluation typically utilizes dorsal-palmar and lateral-medial views of the left third metacarpal (MCIII) in order to determine radiographic bone aluminum equivalents (RBAE), which are measures of optical density (Meakim et al., 1981; Nielsen et al., 1998a,b; Hiney et al., 2000). An aluminum (AL) step wedge is exposed at the same time the radiographs are taken to standardize the readings from each radiograph. Radiographs are then scanned at the nutrient foramen of the MCIII using a video densitometer. Logarithmic regression is formed using the thickness of the steps from the AL step wedge to determine the RBAE in mm of AL from the maximum optical density readings of both cortices for each view of the metacarpal (Nielsen et al., 1998b).

Results from these observations are reported for each individual cortices (lateral, medial, dorsal, palmar) and the total bone (combination of all cortices). Nielsen et al. (1997, 1998a,b) reported no significant difference in RBAE for individual cortices between groups of exercising 2-year olds. However, there was a significant increase in total RBAE for those horses in the high exercised, high calcium group compared to the control. Use of RBAE to determine bone density and mineral balance has proven to be useful in young horses in training (Nielsen et al., 1997, 1998; Buchholz et al., 1999; Hiney et al., 2000) and can prove to be useful in other classes of horses in the future.

Developmental Orthopedic Disease

Many areas of the horse industry require young horses to perform at their maximum potential. As a result, early growth and development are of great importance

to halter competitors and horses that will enter race training as yearlings. Since the industry tends to dictate management practices for horses, horsemen realize that a young horse's marketing potential largely depends on significant early development. Because of this push toward younger, faster, stronger athletes, a broad range of skeletal limb deformities can occur during this time of rapid growth and skeletal development. Developmental Orthopedic Disease (DOD) is the term associated with these skeletal disorders that occur after birth and can pose severe problems in some aspects of the equine industry. Developmental Orthopedic Disease includes epiphysitis, osteochondrosis and osteochodrosis dissecans as well as angular limb deformities. Symptoms of DOD commonly seen in rapidly growing foals include enlargement and deformities of the ankles, knees, hocks as well as contracted tendons.

The causes of DOD are just as just as broad as the term used to classify a certain class of skeletal and developmental deformities in the horse. According to Lewis (1995), there are four reasons for the possible development of DOD. Rapid growth, which can be a prelude to DOD, results in weight load of the animal being beyond the developmental capacity of the skeletal structure. Trauma has a distinct link to the manifestation of developmental problems. Excess exercise as well as sudden increase in exercise has been reported to cause increased skeletal problems in growing horses. Horses may also be genetically predisposed to DOD either directly by sire/dam passing a specific disorder or indirectly by sire/dam passing the potential for rapid growth. Several areas of nutrition such as high energy/high protein, calcium and phosphorus imbalances and trace mineral inadequacies, have been investigated to research the connection between nutrition and incidence of DOD (Lewis, 1995). Two of the most critical minerals involved in proper

bone growth and development in young and growing horses are calcium and phosphorus. Thompson (1988) fed a Ca deficient diet (35% NRC requirement) was fed to growing weanlings which resulted in a significant decrease in the long bone growth and bone mineral content. Additionally, Knight et al. (1985) surveyed 19 breeding farms in Ohio and Kentucky and found that farms that had low or inverted Ca:P ratios in their rations had greater incidences of skeletal deformities than farms that fed NRC recommended ratios. The recommended ratio for horses is 2:1 according to the NRC (1989), which ensures adequate Ca absorption from the diet.

CHAPTER III

RESPONSE OF YEARLING QUARTER HORSES TO VARYING CONCENTRATIONS OF CALCIUM

INTRODUCTION

Calcium metabolism and requirements are of great interest to the horse industry, especially in growing horses. Calcium requirements for growing horses have been estimated from research performed in ponies (Jordan et. al, 1975; Schryver et. al, 1970a). Krook and Maylin (1988) reported that a nutrient is generally required in inverse proportion to body weight. Therefore, the data from 200 kg ponies may not be applicable to 500 kg horses when expressed as mg of nutrient per kg of body weight. The objective of this experiment was to evaluate the effects of feeding various levels of calcium on mineral metabolism, bone density and parameters of growth in yearling Quarter Horses.

MATERIALS AND METHODS

Experimental Design and Treatments. Fifteen yearling Quarter Horses were used in a split-plot designed experiment to evaluate the response of calcium (Ca) metabolism, bone density and parameters of growth to varying concentrations of dietary Ca. Horses were blocked by sex and weight, and then randomly assigned to one of three treatments (high, basal, and low). Horses were fed approximately 2.5% of their body weight per day in

TABLE 1. COMPOSITION OF TREATMENT DIETS, AS FED BASIS

	Treatments		
Ingredient (%)	**High**	**Basal**	**Low**
Ground Corn	40.50	40.10	39.50
Soybean Meal	14.00	14.20	13.60
Cottonseed Hulls	14.60	15.00	16.40
TM Salt	0.30	0.30	0.30
Limestone	0.50	0.40	0.20
Dicalcium Phosphate	0.10	---	---
Prairie Grass Hay	30.00	30.00	30.00
Nutrient			
DE, Mcal/kg[a]	2.80	2.81	2.79
CP, %	15.4	15.1	14.5
Ca, %	0.48	0.42	0.32
P, %	0.34	0.31	0.30

[a] Values calculated from NRC tables.

total ration. Experimental diets consisted of corn, soybean meal and cottonseed hulls fed in a 70:30 ratio with prairie grass hay (Table 1). Diets were formulated to be iso-nitrogenous and iso-caloric and to contain Ca levels at 115% (high, H), 100% (basal, B) and 85% (low, L) of NRC requirements. Additionally, all horses were allowed a minimum of 2 hours limited-free exercise per day in a 60' diameter round-pen. Routine vaccination (Eastern and Western Equine Encephlomylitis, Tetnus, Rhinonumanitis and Influenza) and deworming schedules were followed for the duration of the 25-week trial while farrier work was performed as needed.

Urine and Fecal Collection. The 25-week trial consisted of three 72-hour collection periods at day 0 (period I, 12 months of age), day 90 (period II, 15 months of age) and day 180 (period III, 18 months of age) during which complete urine and fecal collections were taken. Total urine was collected via urine harness from fillies and colts for 72-hours. A representative sample (4% of the total volume) was composited over time for each horse during each period. These composite samples were frozen at 0° C until

mineral analysis could be performed. Multiple fecal grab samples were also taken during the 72-hour collection period. Grab samples were immediately frozen for later mineral analysis.

Feed and Fecal Mineral Analysis. Individual fecal samples were allowed to thaw at room temperature for 24 hours and then placed in a 60° C drying oven for 72-hours. Dried samples were weighed, composited and then ground using a Regal grinder. For feed and fecal mineral analysis (Ca, P, Na, K, Mg and Cl), 1 gram of the composited sample was weighed out into pre-dried beakers, dried at 60° C for 24 hours and then weighed again to determine a final dry weight. Samples were ashed in a muffle furnace at 500° C for 4 hours then 3 ml of 6N HCL was added to the ash residue and evaporated to dryness at 100°-200° C hot plate. Minerals were extracted with an acid solution (1.5N HNO_3 + 0.5N HCL) and determined using Inductively Coupled Plasma Spectroscopy (ICAP 61, Thermo Jarrell Ash).

Urine Mineral Analysis. Urine Ca was analyzed by placing a 10μL drop of sample on a *Vitros* Ca slide while allowing the Ca to penetrate through the spreading layer into the underlying reagent layer. There, the Ca forms a complex with Arsenazo III dye, causing a shift in the absorption maximum. After incubation, the reflection density of the colored complex is measured spectrophotometrically at 680 nm. The amount of colored complex formed is proportional to the Ca concentration in the sample (Johnson & Johnson Clinical Diagnostics, Inc., 1996). Urinary P was analyzed by depositing a 10 μL drop of sample on a *Vitros* P slide and was evenly distributed by the spreading layer. P in the specimen forms a complex with ammonium molybdate. This complex is reduced by p-methylaminophenol sulfate to give a blue complex. The concentration of phosphorus in

the sample is determined by measuring the heteropolymolybdenum blue complex by reflectance spectrophotometry at 670 nm (Johnson & Johnson Clinical Diagnostics, Inc., 1996).

Bone-specific Alkaline Phosphatase. Blood samples were taken via jugular venipuncture in plain glass vaccutainers at twelve-hour intervals (7am, 1pm, 7pm) one day prior to initiation of collection periods. Samples were allowed to clot at room temperature, centrifuged and serum collected and frozen for later analysis. Serum was allowed to thaw and samples were analyzed for serum bone-specific alkaline phosphatase using the commercially available Alkphase-B kits (Metra Biosystems, Mountain View, CA).

Parameters of Growth. Weekly growth parameters were taken during the 25-week trial. These parameters included body weight, wither, hip, hock, shoulder and knee heights and heart girth circumference.

Wither Height – the vertical distance from the ground to the highest protruding thoracic vertebra in centimeters (cm).

Hip Height – the vertical distance from the ground to the furthest protruding point of the buttocks in cm.

Hock Height – the vertical distance from the coronary band on the posterior side of the hoof to the point of the hock (tubercalcis) in cm.

Shoulder Height – the vertical distance from the coronary band on the anterior side of the hoof to the point of the shoulder in cm.

Knee Height – the vertical distance from the coronary band on the anterior side of the hoof to the end of the distal radius in cm.

Body Weight – *the* weight determined at a single weighing 5 hours after the morning feeding and recorded to the nearest pound.

Heart Girth Circumference - the circumference of the thorax immediately posterior to the front leg in cm.

Radiographic Analysis and Bone Density. Radiographs (Lateromedial and Dorsal-palmar) of each yearling's left front cannon using conventional stationary equipment were performed one week prior to each collection period to evaluate for incidence of developmental orthopedic disease and determination of bone density. Metered readings for voltage and amperage were adjusted to 70 KPV and 100 mA, with a .033 s exposure time at a focal film distance of 101.6 cm. All films were processed in a rapid processor. An aluminum step wedge (AL) was simultaneously exposed as a reference standard for estimation of bone density for each radiograph.

Bone density was estimated at 3 anatomical sites approximately 1 cm below the nutrient foramen of MCIII using the Bio-Rad 700 densitomiter (Hiney et al., 2000). The three sites evaluated were: a) the longest path through the bone cortex on the medial side, b) the path midway between the two peaks, and c) the longest path through the bone cortex on the lateral side for both the anterior posterior and dorsal palmar views. All radiographs were standardized for background density and all steps of the AL were measured for density. A logarithmic regression was formed using the thickness of the steps of the AL to determine the radiographic bone aluminum equivalent (RBAE) in millimeters of aluminum from the maximum optical density readings of both cortices for each view. Total RBAE in mm^2 AL was determined by inverting the scans of both the dorsal-palmar/lateral-medial view and the AL to take into account volumetric changes

and changes in density (Nielsen et al., 1998b; Hiney et al., 2000). The total area under the curve of MCIII and the area under the step wedge curve corresponding to the steps with thickness of 14, 15, 20, 23 and 26 mmAL was 1270mm^2. By multiplying the scanned area of MCIII (mm*Optical Density) by 1270 and dividing the sum by the scanned area of the step wedge (mm*OD), the total RBAE in mm^2 AL of the MCIII was determined (Nielsen, 1998b).

Developmental Orthopedic Disease. All radiographs were further evaluated by a veterinary clinician with experience in evaluating clinical bone disorders, especially physitis and osteochondrosis of the distal radius. Radiographs for each horse during periods I, II and III were evaluated simultaneously for joint space and clarity of joint margination as well as for subchondral bone density, any signs of osteochondrosis, thickening of the cortex along the border of the metaphyseal region or increased lucency of the entire physis. Additionally, overall conformation and bone density along with trabecular patterns were observed. A soft tissue assessment of joint pouches to detect joint hydroarthrosis and the possibility of bone effuse were also made.

Statistical Analysis. Data was analyzed using general linear models procedure of SAS (1999) with horse, treatment and period as main effects. Least squares means were calculated for each treatment within a given period and the p-diff procedure of SAS was used to test for differences between treatment means. Polynomial regression analysis was performed on all growth data in order to determine best-fit models over time for each parameter measured. Indicator variables (dummy variables) were used to determine differences in intercepts and slopes between treatments. Orthogonal contrasts were used to evaluate balance data over time.

RESULTS AND DISCUSSION

Dry matter intake, fecal output and dry matter digestibility. Data for daily dry matter intake (DMI), fecal output (FO) and dry matter digestibility (DMD) is shown in table 2. During periods I, II and III of the trial, no difference (P>.05) was detected in DMI and FO for horses consuming diets H, B and L. Dry matter digestibility ranged from 58 to 69% across all periods and treatments. During periods I and II, DMD did not differ significantly between treatments. However, DMD was lower (P<.05) for horses consuming diet L (58%) compared to diets B (67%) and H (65%) during period III. Furthermore, urine volume was not significantly different across treatments for all three periods (Table 2). The dry matter digestibility values observed in the present study are higher than those reported by others (Cooper et al., 2000; Baker et al., 1998) who observed values ranging from 49 to 50% and 48 to 52% in yearling and mature horses, respectively. The difference in the digestibility values may be the result of taking total fecal collections in the present study compared to using chromic oxide as an external marker in the previous studies. Patterson et al. (2001) demonstrated that calculated values (utilizing chromic oxide) for fecal output are higher as compared to total fecal collections. Furthermore, the decreased DMD of horses consuming the low diet during period III may be due to the numerical decrease in DMI and the increased FO.

Calcium and Phosphorus balance. Data for Ca and phosphorus (P) intake, excretion and retention are shown in Table 3. Horses consuming diets H and B had a significantly higher intake and fecal excretion of Ca than horses on the low diet during periods I and II. During period III, similar results were

TABLE 2. EFFECTS OF VARYING LEVELS OF CALCIUM ON DM INTAKE, DAILY FECAL OUTPUT DM DIGESTIBILITY AND URINE VOLUME[a]

	Treatments			
Period I	**High**	**Basal**	**Low**	**SEM[d]**
DMI (g/d)	5862.96	6036.38	6043.66	323.75
F O (g/d)	2388.84	2547.76	2460.06	192.39
DMD (%)	59.45	57.74	59.13	2.14
BW, kg	321.20	336.40	335.40	17.91
Urine Vol (ml/d)	2960.20	2624.00	2946.60	525.34
Period II				
DMI (g/d)	6849.04	6938.96	6580.28	323.75
F O (g/d)	2078.20	2308.74	2355.22	192.39
DMD (%)	69.38	66.62	64.20	2.14
BW, kg	345.20	361.60	362.60	17.91
Urine Vol (ml/d)	3233.20	3690.20	5140.00	525.34
Period III				
DMI (g/d)	7864.40	8123.20	7533.00	323.75
F O (g/d)	2698.60	2660.60	3104.80	192.39
DMD (%)	65.40[b]	67.20[b]	58.00[c]	2.14
BW, kg	415.00	427.80	423.20	17.91
Urine Vol (ml/d)	4533.20	4266.80	4376.60	525.34

[a] Values are least square means.
[b,c] Means within a row with different superscripts differ (P<.05).
[d] Values are average standard errors.

observed for Ca intake while fecal excretion of Ca decreased significantly between the high (22.25 g/d), basal (17.21 g/d) and low (12.82 g/d) diets. Urinary excretion of Ca was not different (P>.05) between horses consuming diets H, B and L during all three periods. During period I, retention of Ca was higher (P<.05) in horses consuming diets H and B versus diet L. Furthermore, those horses fed diet H retained more Ca (P<.05) than horses fed diet L during period II. During period III, there was a tendency (P<.10) for horses consuming the basal diet to have a higher Ca balance than those on the low diet. Horses consuming the high Ca diet also had a numerically higher retention of Ca than those on the low, though not significant. Furthermore, Ca and P digestibility did not differ (P>.05) across all treatments during periods I, II and III.

TABLE 3. EFFECTS OF VARYING LEVELS OF CALCIUM ON MINERAL BALANCE[a]

Period I	Treatment			
Calcium	**High**	**Basal**	**Low**	**SEM[g]**
Intake g/d	28.13[b]	25.35[b]	19.33[c]	1.35
Fecal g/d	17.13[b]	15.47[b]	11.73[c]	1.33
Urine g/d	2.92	2.97	4.86	0.65
Balance g/d	8.07[b]	6.90[b]	2.74[c]	1.69
Phosphorus				
Intake g/d	19.93	19.09	18.13	1.05
Fecal g/d	15.28	15.97	14.22	1.56
Urine g/d	0.65	0.19	0.94	0.42
Balance g/d	3.99	2.92	2.96	1.77
Period II				
Calcium	**High**	**Basal**	**Low**	**SEM[g]**
Intake g/d	32.87[b]	29.14[b]	21.05[c]	1.35
Fecal g/d	18.56[b]	17.66[b]	12.51[c]	1.33
Urine g/d	1.45	1.54	1.04	0.65
Balance g/d	12.86[b]	9.93[bc]	7.49[c]	1.69
Phosphorus				
Intake g/d	23.28[b]	21.5[bc]	19.74[c]	1.05
Fecal g/d	16.41	17.35	12.95	1.56
Urine g/d	0.65	0.17	0.97	0.42
Balance g/d	6.22	3.97	5.81	1.77
Period III				
Calcium	**High**	**Basal**	**Low**	**SEM[g]**
Intake g/d	37.75[b]	34.12[b]	24.10[c]	1.57
Fecal g/d	22.25[b]	17.21[c]	12.82[d]	1.33
Urine g/d	3.85	3.30	4.46	0.65
Balance g/d	11.64[ef]	13.59[e]	6.82[f]	2.32
Phosphorus				
Intake g/d	26.80	27.60	25.60	1.05
Fecal g/d	16.82	14.97	14.44	1.56
Urine g/d	1.29[b]	2.25[bc]	2.53[c]	0.42
Balance g/d	8.62	10.38	8.63	1.77

[a] Values are least square means.

[b,c,d] Means within a row with different superscripts differ ($P<0.05$).

[e,f] Means within a row with different superscripts differ ($P<0.10$).

[g] Values are average standard errors.

TABLE 4. INTAKE, ABSORPTION, RETENTION, AND DIGESTIBILITY OF CALCIUM OVER TIME IN YEARLING HORSES CONSUMING VARYING CONCENTRATIONS OF CALCIUM[a]

Treatment	Period I	Period II	Period III	SEM[e]
High				
Intake, g/d	28.14[b]	32.88[bc]	37.75[c]	1.90
Absorption, g/d	11.00	14.32	15.49	1.99
Retention, g/d	8.08	12.87	11.64	2.04
Digestibility, %	39.75	42.98	40.36	4.83
Basal				
Intake, g/d	23.35[b]	29.14[c]	34.12[d]	0.73
Absorption, g/d	9.88[b]	11.48[b]	16.09[c]	1.68
Retention, g/d	6.90[b]	9.93[bc]	13.59[c]	1.77
Digestibility, %	38.75	38.89	49.55	5.34
Low				
Intake, g/d	13.34	21.06	24.10	1.72
Absorption, g/d	7.60	8.54	11.28	1.64
Retention, g/d	2.74	7.49	6.82	1.81
Digestibility, %	38.61	39.97	46.04	6.02

[a] Values are least square means.
[b,c,d] Means within a row with different superscripts differ ($P<0.05$).
[e] Values are average standard errors.

Concerning calcium balance, the increased retention of Ca in horses consuming diet H during periods I and II is similar to previous studies which have shown an enhanced Ca balance in horses consuming supplemental Ca (Buchholz et al., 1999; Nielsen et al., 1998; Schryver et al., 1970). This increased retention of Ca observed in the present study may be due to the increased ($P<.05$) intake of Ca in horses fed the high diet. Schryver et al. (1970) found that as Ca intake increased from 29 mg/kg BW on the low diet to 242 mg/kg BW on the high, retention of Ca increased from –7.5 mg/kg BW to 56 mg/kg BW, respectively.

Data from table 4, which demonstrates that horses consuming Ca at NRC levels (basal diet) experienced an increased ($P<.05$) absorption and retention of Ca during period III as compared to periods I and II. Furthermore, the average daily gain (ADG) of yearlings consuming the high, basal and low diets increased significantly between periods

II and III (Table 5). This increased gain may result in an increased requirement for Ca as horses move from a yearling (12 months, period I) to a long yearling (18 months, period III).

During period I, P intake, fecal and urinary excretion as well as balance were not different (P>.05) across treatments. Intake of P was significantly higher for horses consuming the high diet versus the low during period II while fecal and urinary excretion and retention of P were not significantly different between treatments. During period III, phosphorus intake, fecal excretion and balance were not different (P>.05) between treatments while urinary excretion of P was significantly higher for those consuming the low diet compared to the high.

Calcium intake, absorption, digestibility and requirements. The effects of varying concentrations of calcium on intake, absorption, digestibility and requirements of Ca in yearling horses are shown in Table 6. During period I, values calculated from the NRC for Ca intake were lower than observed values for horses on diets H and B. Calcium absorption values for horses on all diets were lower than calculated values. During period II however, horses on the basal and low diets had Ca absorption values lower than NRC while intake and digestibility of Ca were similar to the previous period. Horses consuming high, basal and low diets had lower observed Ca digestibilities than the 50% assumed by NRC during both of these periods. At day 180 (period III), the calculated NRC requirements for calcium ranged from 39.27 g/d (high) to 35.43 g/d (low). Calcium intake and absorption for horses on the high, basal and low diets were lower than those calculated from NRC. Horses consuming the basal diet during this time had calcium digestibility values similar to NRC recommendations while horses on the high and low

TABLE 5. RESPONSE OF AVERAGE DAILY GAIN (ADG) AND BODY WEIGHT (BW) OF YEARLING HORSES TO VARYING CONCENTRATIONS OF CALCIUM[a]

Item	High	Basal	Low	SEM[f]
Period I (12mo)				
ADG, kg/d	N/A	N/A	N/A	N/A
BW, kg	321.20	336.40	335.40	17.91
Period II (15mo)				
ADG, kg/d	0.31[d]	0.31[d]	0.34[d]	0.03
BW, kg	345.20	361.60	362.60	17.91
Period III (18mo)				
ADG, kg/d	0.71[be]	0.66[bce]	0.58[ce]	0.03
BW, kg	415.00	427.80	423.20	17.91

[a] Values are least squares means.
[b,c,] Means within a row with different superscripts differ (P<0.05).
[d,e] Means within a column with different superscripts differ (P<0.05).
[f] Values are average standard errors.

TABLE 6. INTAKE, ABSORPTION, DIGESTIBILITY AND REQUIREMENTS OF CALCIUM IN YEARLING HORSES CONSUMING VARYING CONCENTRATIONS OF CALCIUM[ab]

	Treatment			
Period I	**High**	**Basal**	**Low**	**SEM[e]**
Ca Intake (g/d)	28.13[c]	25.35[c]	19.33[d]	1.35
Ca Abs. (g/d)	11.20	9.80	7.60	1.52
Ca Dig. (%)	39.60	38.80	38.80	5.49
NRC Req. (g/d)	22.74	23.46	24.44	-----
NRC Abs. (g/d)	11.38	11.73	12.22	-----
Period II				
Ca Intake (g/d)	32.87[c]	29.14[c]	21.05[d]	1.35
Ca Abs. (g/d)	14.40[c]	11.60[cd]	8.80[d]	1.52
Ca Dig. (%)	43.00	39.00	40.00	5.49
NRC Req. (g/d)	23.71	24.46	25.53	-----
NRC Abs. (g/d)	11.86	12.23	12.77	-----
Period III				
Ca Intake (g/d)	37.75[c]	34.12[c]	24.10[d]	1.57
Ca Abs. (g/d)	15.49	16.90	11.28	1.52
Ca Dig. (%)	40.36	49.55	46.04	5.49
NRC Req. (g/d)	39.27	38.18	35.43	-----
NRC Abs. (g/d)	19.64	19.09	17.72	-----

[a] Values are least square means.
[b] NRC reqirement for calcium calculated as: 0.04BW + 32ADG.
[cd] Means within a row with different superscripts differ (p<0.05).
[e] Values are average standard errors.

diets reported lower digestibilities. Both intake and absorption values calculated in this study are above current NRC estimates of 27 g/d (intake) and 13.5 g/d (absorption) for long yearling horses (18 months) not in training. It appears from these data that horses consuming diets at NRC recommendations (basal diet) for Ca, as a percentage of total intake, may enhance absorption of Ca during period III to meet increased requirements related to the enhanced average daily gain during this time. One explanation for these results could be that, as horses move from 15 (period II) to 18 months of age (period III) and experience a significant increase in average daily gain, the demand for supplemental calcium increases. These results may be of concern as Ca intake in horses on the high and basal diets were below those calculated from the NRC and had no significant difference in bone density.

Bone-specific Alkaline Phosphatase. The response of bone-specific alkaline phosphatase (ALP) in yearling horses to varying concentrations of calcium is observed in Table 7. During period I, the values of ALP were higher for horses consuming diet H as compared to diet L. The concentration of ALP, during period II, increased ($P<.05$) across treatments between the high, basal and low diets. For period III, ALP concentrations were similar to levels reported in period I, however, no significant difference was detected between treatments. The increased concentration of ALP for horses on the high diet indicates an increase in bone formation activity compared to the horses on diet L. The significant decrease in ALP concentration may be an indicator of decreased bone formation activity during this period. Alkaline phosphatase concentrations increased numerically increased to levels similar to period I, which may be an indication of, increased bone formation activity.

TABLE 7. RESONSE OF BONE-SPECIFIC ALKALINE PHOSPHATASE IN YEARLINGS FED VARYING CONCENTRATIONS OF CALCIUM[a]

	Treatment			
Period I	**High**	**Basal**	**Low**	**SEM[e]**
ALP, U/L	72.28[b]	72.87[bc]	69.93[bc]	2.50
Period II				
ALP, U/L	37.47[b]	54.53[c]	61.93[d]	2.50
Period III				
ALP, U/L	64.27	69.13	68.07	2.50

[a]Values are least squares means.
[b,c,d]Means within a row with different superscripts differ (P<.05).
[e] Values are average standard errors.

Radiographic Bone Density. The effect varying levels of Ca on the bone density of yearling horses is reported in Table 8. The RBAE of the lateral cortex was similar (P>.05) between treatments for periods I, II and III. However, there was a tendency (P<.1) for horses on diet H to have a higher RBAE for the lateral cortex when compared to horses on diet B during period II. These results were similar to results from Nielsen et al. (1998a,b), which reported in 2-year old horses a tendency for the RBAE of the lateral cortex to increase in the high group (high Ca, high training level) while the control group (adequate Ca, controlled training level) remained unchanged. The RBAE of the medial, dorsal and palmar cortices were not different (P>.05) between treatments during any period. Nielsen et al. (1998a,b) observed similar results in 2-year olds in training in which no difference in RBAE was found between treatments (high versus control) Concerning Total RBAE, there was no significant difference between treatments for all periods. Similar results were reported in 2-year olds entering race training (Nielsen et al., 1998b).

Parameters of Growth. All mean values for growth parameters (body weight, wither, hip, hock, shoulder, knee height and heart girth circumference) did not differ (P>.05)

TABLE 8. EFFECTS OF VARYING LEVELS OF CALCIUM ON BONE DENSITY OF YEARLING HORSES[a,b]

	Treatments			
Period I	**High**	**Basal**	**Low**	**SEM[e]**
Lateral RBAE (mm AL)	19.40	21.20	22.60	1.87
Medial RBAE (mm AL)	19.60	21.60	22.20	1.85
Dorsal RBAE (mm AL)	19.00	18.60	22.40	1.84
Palmar RBAE (mm AL)	18.40	18.00	22.00	1.82
Total RBAE (mm^2 AL)	342.51	551.82	530.04	112.45
Period II				
Lateral RBAE (mm AL)	26.60[c]	21.60[d]	23.60[cd]	1.87
Medial RBAE (mm AL)	26.60	22.40	23.80	1.85
Dorsal RBAE (mm AL)	20.60	21.80	22.20	1.84
Palmar RBAE (mm AL)	19.40	21.40	21.80	1.82
Total RBAE (mm^2 AL)	346.09	424.07	512.75	112.45
Period III				
Lateral RBAE (mm AL)	24.40	22.20	23.80	1.87
Medial RBAE (mm AL)	24.00	21.70	23.60	1.85
Dorsal RBAE (mm AL)	20.20	21.60	22.40	1.84
Palmar RBAE (mm AL)	19.40	21.00	21.60	1.82
Total RBAE (mm^2 AL)	417.47	389.86	388.60	112.45

[a]Values are least squares means.
[b]Means do not differ (P>.05)
[c,d]Means within a row with different superscripts differ (P<.10)
[e] Values are average standard errors.

between males and females during this experiment (12 through 18 months of age). These findings agree with work in weanlings by Cooper et al. (2000) and with Cunningham and Fowler (1961). Cunningham and Fowler (1961) reported in growing Quarter Horses, that males and females tend to grow uniformly (all growth parameters) to 18 months of age. At this time, the growth rate of males continued to increase while females tended to slow in comparison until horses were five years of age. Additionally, Ott et al. (1989) reported that yearling Thoroughbreds gained an average of 0.64 kg/d with no significant differences between sexes during the 140-day trial.

The response of average daily gain (ADG) and body weight (BW) of yearling horses consuming varying amounts of Ca are reported in Table 5. There was no

significant difference in body weight across all diets during periods I, II and III. During the first 90 days of the trial (period I to period II), there was no significant difference in ADG across treatments. These results are similar to work performed by Gibbs et al. (1989) who varied protein, calcium and phosphorus levels and observed no significant difference in ADG, heart girth circumference and wither height for yearlings fed a oat-alfalfa and an concentrate-alfalfa diet. However, during the last 90 days (period II to period III), horses consuming the high diet had a higher ($P<.05$) ADG than those fed the low diet. This difference can be attributed to the increase ($P<.05$) in BW of those horses (321.20 to 415.00 kg) compared to the slight increase in BW of the low calcium horses (335.40 to 423.20 kg). Additionally, as horses moved from 15 to 18 months of age (long yearlings), there was a significant increase in average daily gains for all treatments. This increase in ADG does not agree with the NRC, which shows a decrease in ADG as horses progress from a yearling (12 months) to a long yearling (18 months). This increase in ADG may be attributed to a change in composition of gain, where horses had an increase in deposition of fat, however no analysis of gain composition was performed.

There was no significant difference in mean body weights between treatments (Table 9). No difference ($P>.05$) in the slope for the regression of age on body weight was detected between treatments (Figure 1). However, the intercept was significantly different for horses on the high diet as compared to the basal and low. Additionally, body weight increased ($P<.05$) quadratically in response to increasing age. These findings were similar to those found by Cooper et al. (2000) who observed body weights of weanlings increased quadratically ($P<.01$) in response to increasing age. Similarly, Boren et al. (1986) reported that weight gains of weanling Quarter Horses could best be

TABLE 9. MEAN RESPONSE OF GROWTH PARAMETERS IN YEARLING HORSES FED VARYING CONCENTRATIONS OF CALCIUM[ab]

	Treatment			
Growth Parameter	**High**	**Basal**	**Low**	**SEM**
Body Weight, kg	355.42	371.39	370.40	17.68
ADG, kg/d	0.39	0.40	0.41	0.015
Wither Height, cm	137.05	138.22	141.17	1.55
Hip Height, cm	141.47	143.64	145.22	1.66
Shoulder Height, cm	94.81	95.24	96.50	1.26
Hock Height, cm	51.58	51.96	53.54	0.75
Knee Height, cm	41.16	41.34	43.03	0.77
Heart Girth, cm	159.12	158.55	160.04	2.81

[a]Values are least squares means.
[b]Means do not differ (P>.05) between treatments.

FIGURE 1. RESPONSE OF BODY WEIGHT IN YEARLINGS CONSUMING VARYING AMOUNTS OF CALCIUM.

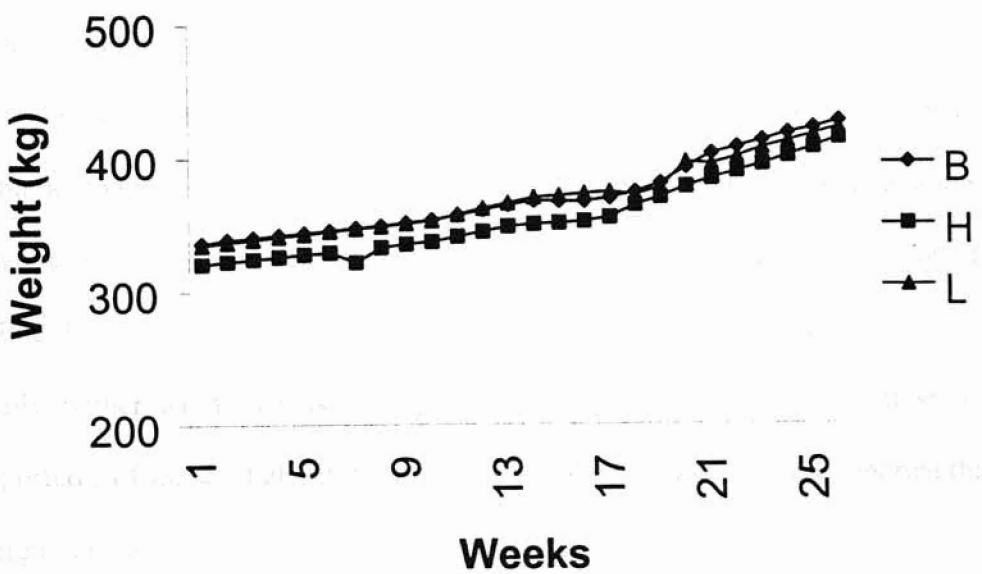
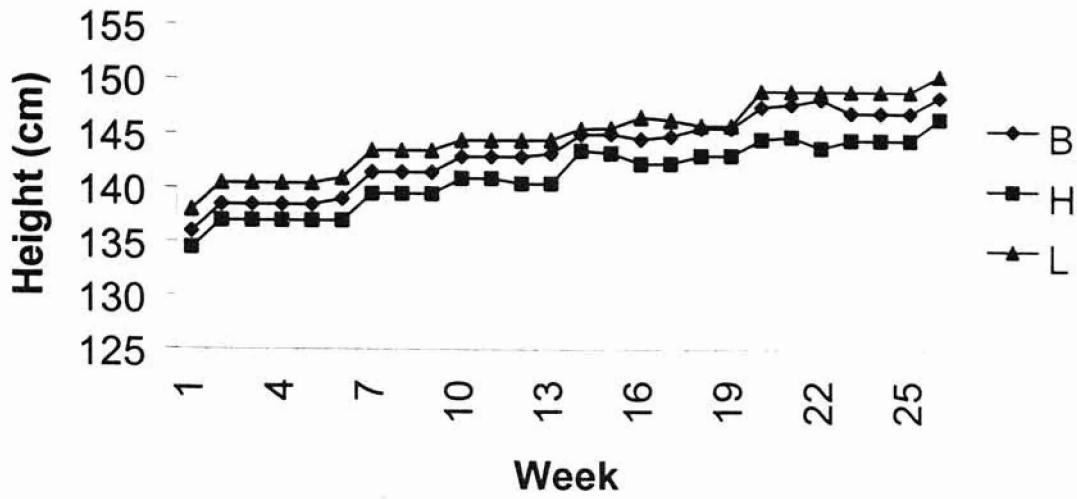


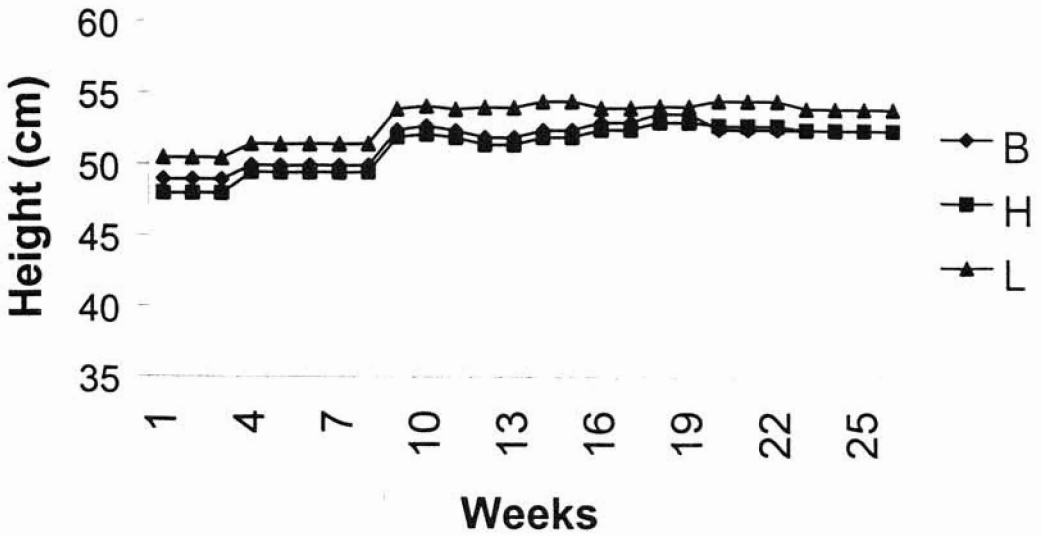
FIGURE 3. RESPONSE OF HIP HEIGHT IN YEARLINGS CONSUMING VARYING AMOUNTS OF CALCIUM.



No difference (P>.05) in slope was observed for hip height between treatments (Figure 3). However, initial hip height was significantly different between all three treatments. In addition, there was a significant quadratic effect of hip height in response to increasing age. This is further supported by work in weanlings by Cooper et al. (2000) in which hip height increased quadratically over time. This is in disagreement with Boren et al. (1986), which stated that weanling hip height, was best described by a third degree (cubic) polynomial.

There was no significant difference in mean hock height across all treatments for periods I, II and III (Table 9). There was a significant difference in the slope and intercept for the regression of age on hock height between horses consuming diets L and H (Figure 4). As well, a significant quadratic effect was observed over the duration of the trial. The results of hock height can be supported by Cooper et al. (2000) who observed height at the hock increased quadratically (P<.01) over time in weanlings.

FIGURE 4. RESPONSE OF HOCK HEIGHT IN YEARLINGS CONSUMING VARYING AMOUNTS OF CALCIUM.



There was no difference (P>.05) in mean shoulder height across treatments (Table 9). Regression analysis found no difference (P>.05) in the response of shoulder height over time (Figure 5). However, there was a significant difference in the intercept between horses on the low diet compared to the high and basal. Shoulder height demonstrated a significant quadratic response over the 25-week trial. The results for mean shoulder height as well as the significant quadratic response can be supported by work performed in weanlings (Cooper et al.,2000).

No significant difference was reported in mean knee height across all treatments (Table 9). The slope for the regression of age on knee height was significantly different for horses consuming the basal diet compared to those on the high and low (Figure 6). Additionally, the intercepts for all three treatments were significantly different from one another. The response of knee height over time experienced a significant linear effect. These results are in contrast to those reported by Cooper et al. (2000) who indicated that there was no significant linear or quadratic effects as well as no significant difference in

FIGURE 5. RESPONSE OF SHOULDER HEIGHT IN YEARLINGS CONSUMING VARYING AMOUNTS OF CALCIUM. CALCIUM

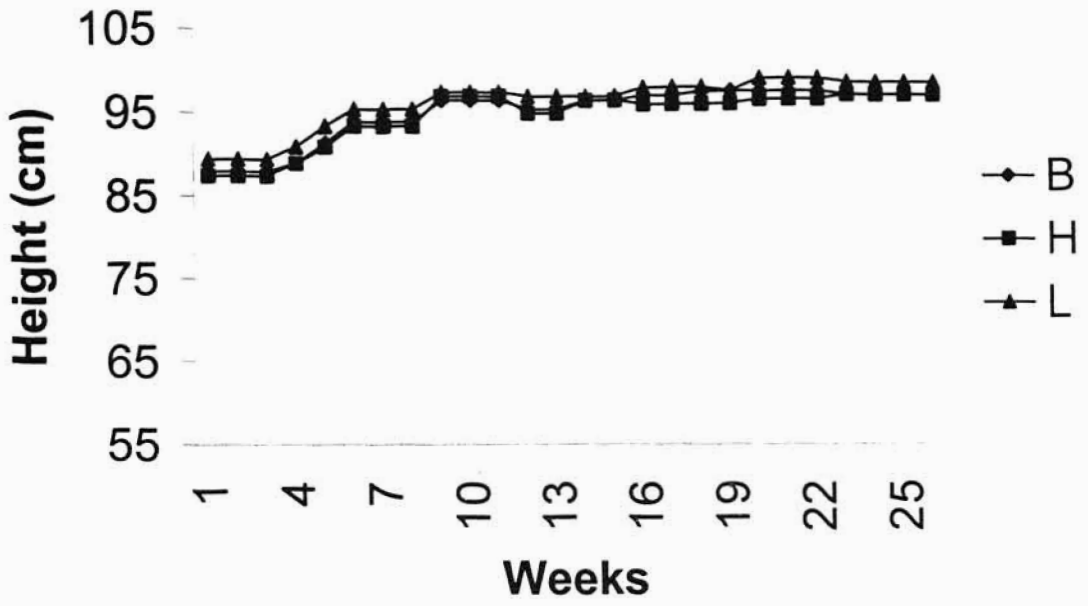


FIGURE 6. RESPONSE OF KNEE HEIGHT IN YEARLINGS CONSUMING VARYING AMOUNTS OF CALCIUM.

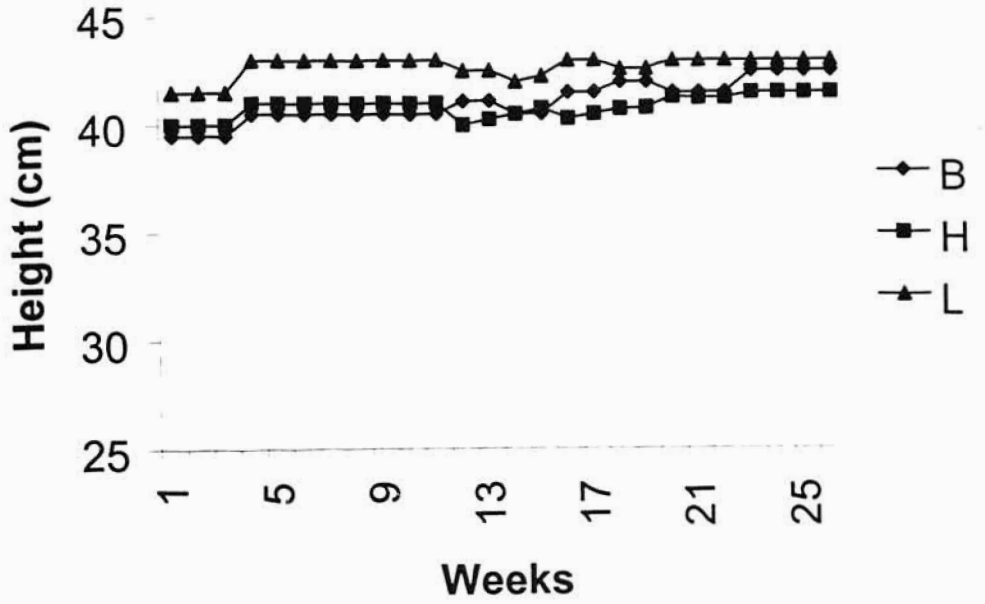
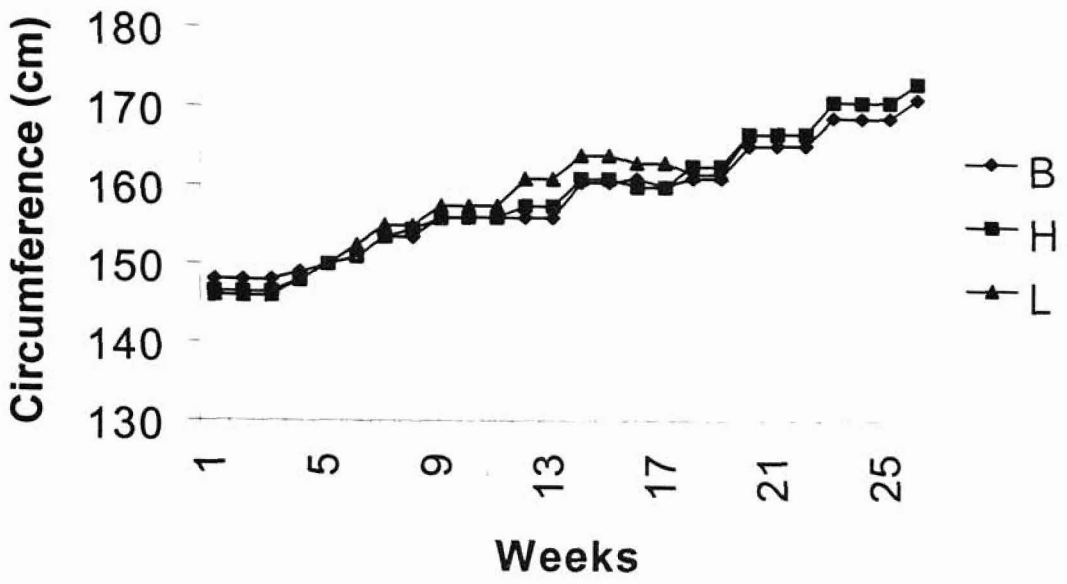


FIGURE 7. RESPONSE OF HEART GIRTH CIRCUMFERENCE IN YEARLINGS CONSUMING VARYING AMOUNTS OF



the intercept or slop of knee height in weanlings. In 1986, Boren et al., reported that weekly measurements for knee height could be best described as quartic polynomial and knee height increased as weanlings moved form 4 to 10 months of age. Additionally Cunningham and Fowler (1961) reported that in Quarter Horses that 82% of the length from the knee to the ground was present at birth and that knee height reached maturity at 6 months of age.

There was no significant difference in mean heart-girth circumference between all treatments for the entire trial (Table 9). These results are supported by work in yearlings, which found that yearling heart-girth circumference did not differ (P>.05) between treatments (Gibbs et al., 1989). The lack of differences was in agreement with data from several other yearling growth studies (Aber et al., 1975; Householder et al., 1976 and Ott et al., 1985). The regression of age on heart girth circumference found a difference

($P<.05$) in slope for horses on diet H and L as compared to diet B (Figure 7). In addition, a significant difference in initial heart-girth circumference was detected between diets B and L. These results are further supported by Cooper et al.(2000) in which weanlings had significantly different initial heart-girth circumferences as indicated by differences in intercepts. As well, slopes for the regression of age on heart-girth were different ($P<.01$). Heart girth circumference increased ($P<.05$) quadratically with increasing age.

CONCLUSIONS

Results from this study demonstrated that Ca balance was not significantly enhanced by feeding Ca above (115%) NRC recommended levels. On the other hand, when horses were fed low (85% of NRC) Ca diets the retention of Ca was significantly reduced. Despite this lowered Ca balance, no significant difference was detected between treatments in bone density or skeletal growth parameters. These data therefore support the current NRC recommendation for Ca intake in yearling horses and suggest that feeding Ca in excess of these recommended levels may not be of benefit to skeletal growth and mineralization.

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Directions for Determining RBAE (updated)

1. Go under Apple menu (left hand corner of screen) and open Multi-Analyst program.

2. Turn on densitometer (D) and wait until both orange lights are on.

3. Under **File** open **Acquire** and then D.

4. Place Radiograph on D with stepwedge in the front right corner (make sure bone is straight) (place a needle or a paperclip to mark the part of the bone you want to scan and one step on the stepwedge).

5. Under Parameter, change resolution to 24.

6. Under options, mark the negative box.

7. Hit **Preview**.

8. With the mouse, in one large box include all of the stepwedge and the area of bone of interest.

9. Change the resolution to 600.

10. Hit **Acquire**. *(only acquires what is inside the big box, takes a while)*

11. Enlarge screen by hitting graph image in lower left hand corner.

12. In upper left hand corner highlight the diagonal line (3rd icon down on the right) and drag over to the rectangular box and release. With the mouse box in the part of the stepwedge – usually a straight line down the side – you want to measure then create a second box around the part of the bone you want to measure (try not to make these boxes too wide). *(make sure you have a good scan of step; make sure you have ALL of bone, can leave just a little black on either side of bone just to be sure you have all of it)*

13. In upper left hand corner, hit the arrow.

14. Hold the shift key down and place the arrow on the corners of both boxes turning them red.

15. Go to **Analysis** and open **Extract Profiles (or hit open apple E)**. *(open apple E is the easiest way to do this step)*

16. This opens a new window – hold the shift key down to highlight both curves (one should be blue and one should be green).

17. Go to **Profile** and open **Scaling**.

18. Change the minimum Y-axis to 0.00, hit OK.

19. In upper left hand corner activate the box with a curve and a straight line through it.

20. Place this baseline at 0.05.

21. Go to **Profile** and open **Subtract Baseline**.

22. Under **File** push **Save As**. Save as d0-1 (day 0 horse 1). *(skip this step unless you have access to CD drive, didn't have access during this trial)*

23. Highlight only the profile for the stepwedge by removing the "X" from the bone curve.

24. Go to the upper left corner of the screen and highlight the curve with the dots, arrow and integration sign.

25. Go to the top step and drag under a good part of the top step.
    - an untitled box will appear.

26. Double click on the line of info that appears.
    - It will open an info box which will let you name that step (probably step 11).
    - The top step is usually step 11, but look for the needle – if you know what step it was placed on then you can count up to determine if the top step is number 11.
    - For horses steps 4-8 are usually where the bone density will fall so it is important to be able to have those steps.

27. Close the box by clicking on the upper left hand corner box.

28. Click back on the box with the curves. *(REMINDER: only close the info line boxes, you can't get results if you close the main boxes or save them, just go back and for between curves and scanned results boxes)*

29. Drag under each of the readable steps in order (highest to lowest), one at a time, (only in "good" parts of each step) until you can't read anymore.

30. Then measure the whole area under steps 8, 7, 6, 5 and 4 by dragging the cursor under all of them.

31. Go to the info screen, double click on that line and rename it as Total Steps.

32. Close the box by clicking on the upper left hand corner box.

33. Go back to the curve screen.

34. Click on the arrow in the upper left corner.

35. Click on the Profile of the bone and remove the profile of the steps by following the same process in step 24.

36. Click on the interprelation box (curve with dots, arrow and integration sign).

37. Drag under the whole bone from one side to the other. (check to see that it reads **100%** in info box, if not you can delete it **open apple B** and try again)

38. Click back on the box with the info lines, double click on the last line. Change it to Total Bone.

39. Close that box by clicking in the upper left hand corner.

40. Go back to the curve screen.

41. Drag under the highest point on the medial (left) cortex and then do the same for the lateral (right) cortex (the stepwedge is always placed on the lateral, or right side of the radiographs).*(this depends on what each radiologist has been informed to do, or has previously done; each view the step was in a different location; make sure it is consistent for each view and for each period)*

42. Double click on the information lines for the medial and lateral and name them.

43. Go to the screen with all the info and go to **Files**, drag down to **Export** and **Results**. *(REMEMBER: can't save these files in Biochemistry, print each screen so that you have a copy of the following: each individual curve on a separate sheet and your results; the step curve is important if they come out segmoidal, then you must use a different formula in the end calculation)*

44. Quit program and turn off densitometer. *(don't have to do that in Biochemistry, it stays on all the time)*

45. Open **Microsoft Excel** (upper right under puzzle pieces).

46. Under **File** hit open and proceed to open the desired Profile you just finished.

47. A screen will come up and you will need to click on Next, and the click on Next again, and the click on Finish. There are your numbers.

(ONLY do steps 45-47 if you can save the results on a disk)

48. The only columns necessary are **profile, width, height and area** columns. The others can be deleted by highlighting the column and then going **Edit, Delete**. *(will have to enter these numbers into spread sheet because don't have access to CD drive in Biochemistry)*

49. Now there should be 4 columns (A to D) in column E type in **mm Al**, in column F type **Ave OD**, in column G type **Ln OD** and in column H type **Total RBAE**.

50. Highlight the columns with exponential numbers and go to format cells and change them to number format with 4 decimal places (this makes graphing easier later on).

51. You can change the names of the steps in the profile column if you wish, as well as the medial (usually the taller side) and lateral readings, by clicking on the boxes (usually steps 11 through 2).

52. Click on **F3** and type the following equation **=d3/b3 or area divided by width** and then copy and paste it down the column. *(row number will depend on how you set up spread sheet, mine was F2 instead)*

53. The number you get is the **average OD** (optical density) of the step.
    -This should be slightly less than the number in column 5 (height) which is the maximum height.

54. In **mm Al** column, starting with the row corresponding to step 11, type in 35, hit return, type in 32 for step 10, and the 29, 26, 23, 20, 17, 14, 11, 8, 5 or until you run out of readable steps. In the same column, enter 1270 for the mm Al across from Total Steps.

55. Go down to the "Total Bone" row. In column H (Total RBAE) of that row, type in the following equation **=Area of the Total Bone *1270/area of the total**. This is your Total RBAE!!!!!!! *(area of total is the steps you have (ie 4-8, 5-8, 6-8, etc) and each radiograph will be different, try and make sure you have all of steps 4-8 makes life much easier)*

56. In the first row of column G **(ln OD)**, type the following equation **=ln (f3) or ln(ave OD for that row)**. Then copy and paste this formula down the column.

57. Next, highlight the numbers in the mm Al column that correspond to steps. Click copy and then paste these numbers below your data in column B.

58. Now, highlight the numbers in the Ave OD column that correspond to steps. Click copy and the paste special (as values) these numbers to the left of the mm Al numbers.

59. Highlight this group of numbers leaving out the titles and then click on the chart icon.

60. Select scatter graph with no line and click next twice.

61. On this screen you can enter the X and Y axis labels

62. X-axis = Ave OD and Y-axis = mm Al.

63. Click next and then finish.

64. The graph should now be on the screen – select **chart** from the menu and then **add trendlines.** ***(you have to click the actual data points to be able to add trendlines)***

65. Select **linear** and then under **options** check display **equation** and $\mathbf{r^2}$ on chart and click OK.

66. Look at graph and delete the points that do not fit on the "best fit" line (usually the highest or lowest 2 points). Ideally the r squared should be at least 0.99. *(you may have to delete values from your actual data sample, sometimes you can just delete from the graph itself; make sure you unknown is still somewhere in the grpah)*

67. Make sure that the graph represents the area that your unknowns are in.

68. Now use the equation on the graph to solve for y
    - go to the Total RBAE column and down the row with the first unknown (medial, lateral, dorsal, palmer...). Retype or copy and past the equation so it looks like **=60.43*(C_) + 17.45.** In this equation you are plugging in the column C (height) of the unknown to solve for total RBAE.
    -

69. Use the same equation to solve for all unknowns (can copy and paste it down).

70. Save the file name. If you don't want to over write the original be sure to save it in a different folder or rename it. *(You should end up with 1 file for each horse with 6 sheets (each view, each period))*

VITA

Amber Dahn Moffett 2

Candidate for the Degree of

Master of Science

Thesis: RESPONSE OF YEARLING QUARTER HORSES TO VARYING CONCENTRATIONS OF DIETARY CALCIUM

Major Field: Animal Science

Biographical:

Personal Data: Born in Fort Smith, Arkansas, November 1975, the daughter of Dustin and Elizabeth Moffett.

Education: Graduated from Muldrow High School, Muldrow, Oklahoma in May 1994; received Bachelor of Science Degree in Animal Science/ Biotechniques from Oklahoma State University in Stillwater, Oklahoma, December 1999; completed requirements for the Master of Science degree at Oklahoma State University in December 2001.

Professional Organization: Member of the American Registry of Professional Animal Scientists. Member of the American Quarter Horse Association.

Professional Experience: Teaching and Research Assistant, Department of Animal Science, Equine Section, Oklahoma State University, August 1999 to December 2001; Assistant Coach, OSU Horse Judging team January 1999-2000.