This is to certify that the

thesis entitled

Influence of Housing on the Third Metacarpus and Markers of Bone and Cartilage Metabolism in Weanling Horses

presented by

Renee Ann Bell

has been accepted towards fulfillment of the requirements for

M.S. degree in Animal Science

Brian D. Nielsen

Major professor

Date May 14, 1999

INFLUENCE OF HOUSING ON THE THIRD METACARPUS AND MARKERS OF BONE AND CARTILAGE METABOLISM IN WEANLING HORSES

By

Renee Ann Bell

A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Animal Science

1999

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ABSTRACT

INFLUENCE OF HOUSING ON THE THIRD METACARPUS AND MARKERS OF BONE AND CARTILAGE METABOLISM IN WEANLING HORSES

By

Renee Ann Bell

Eighteen Quarter Horse (Experiment 1) and 17 Arabian (Experiment 2) weanlings were used to determine the influence of housing on third metacarpal bone mass. In Experiment 1, weanlings were separated into paddock (P1, n = 6), stall (S1, n = 6), or exercised groups (X1, n = 6). Experiment 2 animals were separated into 3 treatment groups: pasture (P2, n = 6), stall (S2, n = 5), and partial pasture (PP2, n = 6). Radiographs of the left third metacarpal were taken every 28 d to determine radiographic bone aluminum equivalence (RBAE). Serum was collected every 14 d and analyzed for osteocalcin (OC), carboxyterminal telopeptide of type I collagen (ICTP) and keratan sulfate (KS). The P1 group demonstrated an increase in medial and total RBAE in Experiment 1 while S1 decreased in medial, palmar, and total RBAE. The X1 group decreased in dorsal and total RBAE. Keratan sulfate decreased in P1, S1, and X1 from d 0 to d 56. Group X1 decreased in ICTP from d 0 to d 42 while P1 and S1 decreased in ICTP from d 0 to d 56. Osteocalcin decreased in P1 from d 0 to d 56 and in X1 from d 0 to d 28 but was unchanged in S1. In Experiment 2, P2 increased in lateral, medial and total RBAE while PP2 increased in medial and total RBAE. Osteocalcin, ICTP and KS decreased in all groups. This study concludes that pasture rearing or 12-h daily turn-out is beneficial to maintaining and increasing bone mineral content in weanling horses.

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ACKNOWLEDGMENTS

This project was a great experience for me and helped me understand the horse industry and how active and exciting weanlings can be. There were a lot of people that helped and supported me throughout this project that I owe special thanks. Karen Waite is probably the best research assistant that one could ask for. She was there to help with assays, statistics, and general support. It all just comes down to Karen being a great friend!

The animal work for this project would not have been possible without the help and cooperation of Paula Hitzler and Shorty Parks. Thank you to Paula for letting me make my mistakes at the barn and letting a novice horse person deal with the weanlings. Also special thanks to my undergraduate students for assisting me every morning and night for four months. You made going out to the barn much fun and it was a pleasure working with you all.

For supporting and listening to me during my stressed out and neurotic moments I would like to send my gratitude to Ely Rivera, Kristine Lang, and Molly Nicodemus. Though it does not need to be mentioned I need to thank Lance Garlington, Jada Pierce, Candace Croney, Marquita Jones, and my parents for being there and giving me the confidence and faith to finish my degree.

Thank you to my committee, John Shelle, Michael Orth, John Caron, and Camie Heleski, for guiding me through my program.

Finally, thanks to Dr. Brian Nielsen for taking me at the last minute and convincing me to stay on with him to finish my degree. I was able to find what I really enjoy and to truly be happy and proud of my degree.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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INTRODUCTION

Young horses are often housed in stalls as opposed to being out on pasture with free access to exercise. Stalling may be detrimental to bone strength due to a lack of exercise. Exercise allows the bone to respond to changes ensuring bone mass capable of withstanding loads associated with training later in life (Price et al., 1995b). Without the necessary exercise, the extracellular matrix will be resorbed until bone is adapted to decreased loading. This could result in bone ill-prepared for the rigors of training. Wolff's Law states that cortical bone will remodel according to the strains placed on it (Woo et al., 1981). Remodeling allows bone to adapt to strains so bending is reduced and damage will be prevented (Carter, 1984). Numerous studies have demonstrated that depriving animals of exercise is detrimental to bone strength. Laying hens housed in battery cages had 54% weaker bones than those housed in percherys, demonstrating that bone strength is related to the amount of movement allowed (Knowles and Broom, 1990). Nielsen et al. (1997) found as bone mineral content of the third metacarpus decreased, the incidence of bone related injuries increased. McCarthy and Jeffcott (1992) found that yearling horses exercised on a treadmill demonstrated an increase in bone density. Hoekstra et al. (1998) found that yearling Arabian horses demonstrated a decrease in the rate of bone formation, an increase in bone resorption, and a decrease in bone mineral content when kept in stalls compared to control horses maintained on pasture. The hypothesis of this study was that stalling demonstrates a negative effect on bone mass. The first objective was to determine whether housing weanling horses in stalls is detrimental to bone and cartilage development compared to that in weanling horses

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maintained on pasture or in a paddock. The second objective was to determine if exercise or 12-h daily turnout of weanlings could eliminate a decrease in bone mass.

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CHAPTER 1

REVIEW OF LITERATURE

Connective Tissue Biology

Bone

Bone is the basic tissue that provides both protection and structure for the body. It aids in the distribution of forces, provides metabolic regulation of fluids, and acts as the reservoir for blood cell formation (Jee, 1988; Marieb, 1992). Limb bones, in particular, are responsible for withstanding repetitive loading and avoiding damage by adapting to the common loads of normal activity and growth (Lanyon, 1987). Bone is comprised of cortical (outer wall) and trabecular bone (central region containing marrow). Cortical bone makes up 80% of total bone (Hassager et al., 1994). Trabecular bone is composed of soft tissue (75% by volume) including the bone marrow while cortical bone is a dense solid mass (Jee, 1988). Though trabecular bone assists in resisting stresses placed on bone, cortical bone is primarily responsible for the strength of bone (Marieb, 1992).

Cortical bone contains osteons composed of lamella (Marieb, 1992; Jee, 1988). The osteons assist in creating structurally sound bone capable of twisting and withstanding stresses. The Haversian canal runs through each osteon supplying osteocytes with nutrients. Volkmann's canals run perpendicular to Haversian's canals connecting the vascular and neural fibers to the medullary cavity (marrow) (Marieb, 1992; Jee, 1988). Cortical bone consists of an amorphous ground substance which contains phosphoproteins, glycoproteins, γ-carboxyglutamic acid proteins (e.g. osteocalcin), proteoglycans, lipids, and peptides (Jee, 1988). Bone collagen fibers are

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embedded within the amorphous ground substance. Bone is made rigid by the presence of hydroxyapatite (mineral salts) held together by ground substance in between type I collagen fibrils (Jee, 1988; Marieb, 1992). Approximately 90% of the organic matrix in mineralized bone consist of type I collagen (Risteli et al., 1993).

The structure of long bones consists of the epiphysis, metaphysis, and diaphysis. In growing bone, the metaphysis and epiphysis are separated by the growth plate (Jee, 1988; Marieb, 1992). Growth plate cartilage regulates linear growth and the ultimate length of the bone (Fretz et al., 1984). The outer surface of long bones is covered by the periosteum. The periosteum is lined with mesenchymal stem cells which can be recruited to increase bone growth and assist in fracture repair (Jee, 1988).

Osteoblasts and osteoclasts are the cells responsible for bone formation and degradation, respectively. Osteoblasts synthesize and secrete unmineralized bone matrix (osteoid), type I collagen, non-collagenous matrix proteins, osteocalcin (OC), proteoglycans, and other bone forming macromolecules. Osteoblasts are generally located near bone surfaces. Osteoblasts possess receptors for parathyroid hormone and 1,25-dihydroxy vitamin D_3 and contain alkaline phosphatase in its plasma membrane (Jee, 1988). The life cycle of an osteoblast begins after differentiation of the progenitor cell and then further matures to participate in matrix formation. The last step is further maturation into bone-lining cells or osteocytes, participation in calcifying units, or death. Osteocytes are osteoblasts that have been entrapped in the mineralized matrix and are responsible for bone modeling and remodeling (Lanyon, 1987). These cells are sensitive to the distribution of loads, the rate of change, and the magnitude of strain placed on bone. Osteoclasts are housed in Howship's lacunae. Osteoclast maturation in the bone

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marrow is stimulated by 1,25-dihydroxy vitamin D_3. Osteoclasts create an acidic environment and secrete lysosomal enzymes to aid in the degradation of bone. The acidic environment solubilizes hydroxyapatite allowing the release of calcium into interstitial fluid (Jee, 1988; Marieb, 1992). Osteoclasts are also responsible for the breakdown of type I collagen (Allen et al., 1998).

Remodeling is the process in which immature or inferior bone is replaced by mature bone and old or damaged bone is replaced by new bone (Jee, 1988). During remodeling in mature bone, formation and resorption are balanced, but as the horse ages, resorption exceeds formation (Fraher, 1993). This process is necessary for the maintenance of biomechanically and metabolically competent bone. Remodeling is an adaptive response to commonly encountered strains to ensure structural soundness in order to avoid fatigue and failure (Lanyon, 1987). Carter (1984) defines remodeling as any alteration in the shape, size, and microstructure of bone. These alterations may involve any change in bone turnover. During training in horses, bones must undergo adaptive remodeling in order to resist the stress placed on their bones (Smith, 1991). This adaptation must be gradual to avoid resorption exceeding the rate of formation causing damage to occur (Carter, 1984). The complete remodeling cycle in which resorbed bone is replaced in full by new bone takes approximately 4 mo in humans (Jee, 1988). The 4 mo needed for complete remodeling includes 21 d for resorption and 101 d for formation (Jee, 1988). An increase in remodeling may lead to a loss of trabecular and cortical-endosteal bone while a decrease in remodeling conserves bone (Frost, 1987). An increase in remodeling leads to a loss in total bone due to bone being resorbed faster than it can be repaired.

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Modeling is the process in which bone develops into its mature adult form by altering the amount of bone present (Jee, 1998). Modeling occurs at different bone surfaces and rates during the growth process. In foals and weanlings, the rate of formation greatly exceeds the rate of resorption (Maenpaa et al., 1988; Fraher, 1993). Any alteration to this growing process may have a large impact on bone strength later in life (Nunamaker et al., 1990). Raub et al. (1989) states that exercise may be advantageous in growing bone due to its more responsive nature in its adaptation to stress than mature bone. Weaning may lead to a decrease in growth rate due to its stressful effects on the young horse (Warren et al., 1998). These stressful effects include the loss of nutrients from the mare's milk and a decline in feed intake (Warren et al., 1998). The alteration of the nutritional patterns causes a decrease in the nutrients required for bone formation. Though Warren et al. (1998) found that horses weaned at an earlier age had smaller third metacarpal bone circumference than those weaned at 6 mo, bone density analyses indicated no significant changes in bone in horses weaned at 4.5 mo or 6 mo of age.

Cartilage

Articular or hyaline cartilage, covers the ends of bones at the joints and serves as a cushion to limit the stress applied to the bone, providing a smooth gliding surface to ensure efficient movement (McDevitt, 1973). Synovial fluid lies in between joints interacting with the cartilage and assists in providing the joint a low coefficient of friction enhancing the ability of the viscoelastic cartilage to act as a cushion to absorb mechanical stresses (McDevitt, 1973).

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Mature cartilage is avascular, aneural, and lacks lymphatic vessels. Cartilage is composed of 70 to 80% water. The remaining dry weight consists of 50% collagen, 40% proteoglycans, and 10% non-collagenous proteins (Nixon, 1991). Type II collagen provides the cartilage tensile properties by forming a fibril network (Nixon, 1991). Type II collagen forms concentric rings around chondrocytes forming a chondron (Nixon, 1991; Hauser et al., 1996). Chondrocytes, making up only 5% of the tissue volume, are responsible for maintaining organization of the extracellular matrix (Roughley and Lee, 1994) and controlling cartilage turnover (Nixon, 1991). The extracellular matrix of cartilage is a collagenous matrix containing a ground substance of glycoproteins and proteoglycans (PG) (Roughley and Lee, 1994).

Proteoglycans are composed of a core protein with covalently bound glycosaminoglycan (GAG) chains (Hardingham and Fosang, 1992). Aggrecan is the major PG found in articular cartilage and is a product of chondrocytes. Aggrecan provides compressive stiffness to cartilage and helps in the distribution of loads (Roughley and Lee, 1994; Hardingham and Fosang, 1992). In the cartilage matrix aggrecan primarily exists as a PG aggregate. Proteoglycan aggregates are composed of up to 100 aggrecan molecules non-covalently bound to hyaluronan and stabilized by link protein (Roughley and Lee, 1994). Hyaluronan helps maintain the structural integrity of the extracellular matrix (Haapla et al., 1996). Proteoglycans have a half life of approximately 300 d, indicating that they do not undergo rapid turn-over in mature animals (Sandy, 1992). Proteoglycan content remains constant during turnover by replacing the old aggrecan molecules with newly synthesized molecules (Thonar and Glant, 1992).

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The GAG, attached to PG, are long chains of repeating disaccharide units (hexosamine linked by a glycosidic bond to a non-nitrogenous sugar). There are two types of GAG: sulfated, including chondroitin sulfate (CS), keratan sulfate (KS), dermatan sulfate, heparan sulfate, and heparin and non-sulfated including, hyaluronic acid. Heparin is the only glycosaminoglycan that is not present in cartilage (McDevitt, 1973; Hardingham and Fosang, 1992; Roughley and Lee, 1994). Glycosaminoglycans are negatively charged, due to the presence of carboxyl and sulfate groups, attracting positive ions which creates an osmotic imbalance thus causing water to be retained in the matrix, thus hydrating the cartilage (Mankin and Brandt, 1984; White, 1988; Hardingham and Fosang, 1992).

The lifespan of cartilage consists of two phases: 1) early "developmental" phase and 2) post-developmental "ageing" phase. The majority of changes cartilage undergoes occurs in the first phase. During this phase, articular cartilage increases in collagen content and keratan sulfate. During the second phase, the biochemical composition of cartilage undergoes less dramatic change (McDevitt, 1973).

During loading, cartilage is compressed causing water to be released by PG. Once loading is complete and the cartilage bears no weight, water is allowed back in returning the cartilage back to its original shape. Motion and force facilitates the diffusion of nutrients and removal of waste from the cartilage (Kincaid and Van Sickle, 1982). Loading allows nutrients to infiltrate the cartilage matrix from the synovial fluid (Richardson and Clark, 1993).

To maintain cartilage matrix composition, loading of the joint is necessary (Haapla et al., 1996). The affect of immobilization or lack of exercise on articular

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cartilage leads to the development of lesions similar to those of osteoarthritis (OA). During the early stages of OA, chondrocytes increase matrix PG synthesis in an attempt at repair however, PG depletion is often of greater magnitude (Hardingham and Fosang, 1992). Alwan et al. (1991) observed an increase of GAG in serum indicating a net loss of PG from cartilage in horses. The rate of PG released from the matrix is very high during the early stages of OA. Todhunter et al. (1997) found a tendency for plasma KS concentration in normal horses to be lower than KS concentrations in OA indicating increased PG degradation in OA horses.

Biochemical Markers of Bone and Cartilage Metabolism

Biochemical markers are utilized for non-invasive measuring of bone and cartilage turn-over. Markers are products of turn-over in either bone or cartilage, which serve particular functions in the matrices of these tissues. Markers can be detected in either blood or urine and may be used to determine the effects of pasture-rearing versus stall-rearing on bone growth in young horses.

Bone

Alkaline phosphatase -- Alkaline phosphatase (ALP) is an enzyme found in bone, liver and kidney (Price et al., 1995a). Bone specific variant of ALP (BALP) is a serum marker that differs from hepatic ALP by post-translational modifications of the gene (Delmas et al., 1993). The bone specific variant of ALP is thought to be located on the cell surface of osteoblasts acting as an osteoinductive cell marker and hence indicative of bone formation (Allen et al., 1998; Price et al., 1995a, Jee, 1988). Heikkinen et al.

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(1997) state that BALP is released primarily during matrix maturation and has low serum concentrations during mineralization. Because BALP is so closely related to hepatic ALP, Delmas et al. (1993) reported that BALP lacks sensitivity and is cumbersome to analyze due to techniques relying on activators, inhibitors, electrophoresis, and separation by specific antibodies. However, new methods now exist for analyzing BALP in serum by using radioimmunoassay or enzyme linked immunosorbent assay kits (Allen et al., 1998).

Type I collagen propeptides -- Collagen is synthesized from procollagen and then cleaved by extracellular propeptides releasing the amino- and carboxy-terminal ends called carboxy-terminal propeptide and amino-terminal propeptide of type I collagen (PICP and PINP respectively). Type I collagen is found in bone, skin, tendon, and ligament and hence, PICP and PINP are not specific for bone (Price et al., 1995a). Carboxyterminal propeptide of type I collagen decreases with increasing age in horses as remodeling decreases (Price et al., 1995a). Delmas (1993) reports that serum PICP is poorly correlated with OC and alkaline phosphatase indicating that this marker is not sensitive enough to be used as a marker of bone formation.

Osteocalcin -- Osteocalcin is a non-collagenous, vitamin K dependent, calcium-binding matrix protein. Osteocalcin acts as a link between the organic and inorganic matrices of bone and may account for 3% of bone protein (Fraher, 1993; Hope et al., 1993). Osteocalcin is synthesized primarily by osteoblasts and incorporated into the extracellular matrix of bone. Small fractions of OC are released into circulation during bone formation which can be detected by radioimmunoassay (RIA) or enzyme linked immunosorbent assays (ELISA) (Delmas, 1993; Kannus et al., 1996). Osteocalcin

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contains three residues of γ-carboxyglutamic acid (gla) which undergo conformational changes in the presence of Ca promoting OC binding to hydroxyapatite (Lepage et al., 1990; Lian and Gundberg, 1988; Price, 1982). Osteocalcin concentrations are dependent upon age and time of day. Lepage et al. (1990) determined OC concentration in the serum of 50 female Standardbreds of varying ages. Mean serum OC concentration were lower in older horses, indicating a decrease in bone turnover with increasing age. In addition to age-related changes, OC undergoes circadian variation so that serum concentrations are dependent on the time of sample collection. Lepage et al. (1991) reported that the lowest serum concentrations occurred between 1900 and 2000 and maximum concentrations were between 2000 and 0500 in Standardbreds. From 0700 to 1800 levels remained constant. Lepage et al. (1991) concluded that photoperiod is directly associated with OC serum levels. During bone turn-over, formation and resorption occur simultaneously which causes the release of markers of both formation and resorption. Because formation and resorption are coupled during turn-over, some studies have indicated the use of OC as a measure of bone turn-over as well as bone formation (Heikkinen et al., 1997; Kannus et al., 1996).

Hydroxyproline -- Hydroxyproline represents 13% of the amino acid content of collagens. Since the majority of total body collagen is found within bone, hydroxyproline is thought to be a marker of bone resorption (Delmas, 1993). Approximately 90% of the hydroxyproline released during bone resorption is metabolized, circulates in plasma, is reabsorbed in the kidneys, and is then degraded to CO_2 and urea. This whole process only leaves approximately 10% of hydroxyproline in the peptide-bound form to be released in the urine (Delmas, 1993). Because only a small

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percentage of hydroxyproline is released in the urine, it is not a very sensitive or effective marker of bone resorption.

Pyridinoline and Deoxypyridinoline -- Pyridinoline (Pyr) and deoxypyridinoline (Dpd) are cross-links that stabilize collagen. Pyridinoline and Dpd provide stability to collagen by cross-linking 3 hydroxylysine or lysine residues between chains of collagen creating a cyclic structure (Valimaki et al., 1994). Pyridinoline is not specific for bone but is also found in cartilage and other connective tissues (Delmas, 1993) while Dpd is found primarily in bone with minute amounts in aorta, dentine, and ligaments (Gomez et al., 1996). Both Pyr and Dpd can be detected in urine and serum and measured as markers of bone resorption (Gomez et al., 1996). Both markers are more specific and sensitive than hydroxyproline making them good markers of bone resorption. Both markers exhibit diurnal variation with the higher values at night and the lowest values during the day, making urine collections very cumbersome and often involve 24-h collections (Valimaki et al., 1994; Delmas, 1993). However, Hoekstra (1998) found very similar results between 24-h collection analysis and spot samples adjusted for creatinine concentrations.

Carboxyterminal telopeptide of type I collagen -- Carboxyterminal telopeptide of type I collagen (ICTP) is released due to the breakdown of pyridinium cross-link of type I collagen within the extracellular matrix of bone (Allen et al., 1998). Osteoclasts release proteases that degrade type I collagen into amino acids and peptide fragments releasing ICTP into serum (Allen et al., 1998; Price et al., 1995a). Carboxyterminal telopeptide of type I collagen (ICTP) was used in the present study as a marker of bone degradation. Plebani et al. (1996) and Kylmala et al. (1995) identified ICTP as being the most reliable

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and sensitive marker of bone resorption. However, Hassager et al. (1994) found no correlation between serum ICTP and histomorphometric measurements of bone turnover. This may be a result of only measuring cancellous bone and not cortical bone, since cancellous bone is only 20% of total bone and has a different turn-over rate than cortical bone. Valimaki et al. (1994) found that ICTP is closely correlated with urinary Pyd (r = .667) and Dpd (r = .452). As turnover decreases with increasing age, serum ICTP decreases (Price et al., 1995a). Price et al. (1995a) found that horses less than 1 yr old had higher concentrations of ICTP than horses older than 1 yr.

Cartilage

Hyaluronan -- Hyaluronan (HA) assists in stabilizing the extracellular matrix of cartilage and can be used as a marker of cartilage degradation (Thonar et al., 1995b). Sharif and Dieppe (unpublished data) found that OA patients demonstrating high serum concentrations of HA progressed to more severe joint problems than patients with low concentrations of HA (Thonar et al., 1995b). Leipold et al. (1989) studied KS and HA serum concentrations in dogs with induced OA and found that KS increased 10 fold while HA increased only 2 fold. This indicates that HA may be a less sensitive marker of cartilage metabolism than KS.

Keratan sulfate -- Keratan sulfate is a GAG with repeating disaccharide units of galactose and N-acetyl glucosamine (Roughley and Lee, 1994). Keratan sulfate is the second most abundant GAG on aggrecan after chondroitin sulfate. When aggrecan is proteolytically cleaved, KS is released into the synovial fluid and eventually reaches the bloodstream. Keratan sulfate is primarily found in cartilage so that serum KS most likely

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reflects changes in metabolism of PG, suggesting that serum KS can be used as a biochemical marker of proteoglycan degradation (Campion et al., 1991; Thonar et al., 1986). Keratan sulfate concentrations are partly dependent on age and are shown to decrease with age in humans. During the first 4 yr of life in children, serum KS concentrations are high and remain elevated until 12 yr of age and then decrease at 13 yr of age (Thonar et al., 1988). Plasma KS concentration peaked at 10 wk of age in foals (Todhunter et al., 1997). Leipold et al. (1989) concluded that high levels of serum KS in young dogs (< 1 yr) are due to the higher rate of synthesis of KS. Okumura et al. (1997) found that the mean serum KS concentration during the first 3 mo of life were five times higher than the KS concentration in yearlings or older horses. Between 3 to 5 mo of age, serum KS concentration decreased rapidly.

Keratan sulfate in synovial fluid indicates the rate of degradation of articular cartilage within a particular joint while KS in blood measures the average rate of aggrecan degradation in the body (Thonar et al., 1995a). When cartilage begins degrading, serum KS concentration increases (Alwan et al., 1990). This increase may either confirm degradation is occurring or identify a predisposition to osteoarthritis (Thonar and Glant, 1992). Williams et al. (1988) injected rabbit knee joints with chymopapain to induce cartilage degradation and found an increase of serum KS indicating the acute loss of PG from the joint. Spector et al. (1992) suggested that KS is not an accurate measure of cartilage degradation or OA due to its high variability possibly associated with sex, age, and injury. Serum KS concentration may not be an accurate measure of cartilage degradation because KS reflects both normal and diseased cartilage PG and that synovial joints account for only 15% of all cartilaginous tissue.

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Though KS is a variable marker, it is the most sensitive marker found to represent cartilage degradation (Liepold et al., 1989).

Measurements of Bone Mineral Content

Non-invasive measurements of bone density are used with biochemical markers to provide accurate measures of bone turnover.

Ultrasonography -- Ultrasonography measures the velocity of sound through a bone using an ultrasonic beam and two transducers to determine bone mineral content in horses (Jeffcott and McCartney, 1985). Jeffcott and McCartney (1985) found that ultrasonography is an accurate, inexpensive method to measure of bone mineral content. The disadvantages to using ultrasonography, compared to radiographic photometry, are that it is more labor intensive and requires direct contact with the limb. With smaller bones (i.e. weanlings) the transducers need to be held on the limb longer in order to have greater signal capability of the oscilloscope. It is not feasible to have a horse, specifically a weanling, to stand perfectly still for more than a few seconds unless tranquilized.

Single photon absorptiometry -- Single photon absorptiometry measures the degree of attenuation of a low energy photon beam to bone and tissue (Jeffcott et al., 1986). Though single photon absorptiometry provides a precise measurement of bone density, it is expensive and requires the animal to stand perfectly still for 90 sec (Jeffcott et al., 1986). The expense lies in purchasing fluid filled bandages necessary for acting as a soft tissue conductor, supplying a flat parallel surface, and the machine itself.

Dual photon absorptiometry -- Dual photon absorptiometry is very similar to single photon absorptiometry except a radiation beam at two separate energy levels are

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used instead of one. The same limitations exist with dual photon absorptiometry as in single photon absorptiometry.

Radiographic photometry -- Radiographic photometry is a popular technique because it provides precise, accurate measurements and is convenient to use. Meakim et al. (1981) measured bone mineral content of the dorso-palmar (DP) view of the equine third metacarpus using radiographic photometry. Radiographs of the DP view were measured with an aluminum stepwedge to determine radiographic bone aluminum equivalence (RBAE) expressed in mm aluminum (Al). Meakim et al. (1981) found that the RBAE measurement of the third metacarpus was highly correlated with bone mineral content ($r = .88$ for the medial side and .94 for the lateral side). This technique indicated changes in sections of the bone with greatest optical density. Using similar techniques, Nielsen and Potter (1997) looked at volumetric changes as well as changes in mineralization by determining total bone mass. This technique uses the optical area and total area of the Al stepwedge and the optical area of the third metacarpal bone. As in Meakim et al. (1981), RBAE was determined but expressed in mm^2 Al. Therefore, RBAE can be used to determine total bone mineral content of the third metacarpus.

Williams et al. (1991) compared non-invasive techniques of measuring bone mineral content of the third metacarpal bone in cattle. A correlation coefficient (r) between bone mineral content and radiographic photometry was .967 ($p < .0001$). This was a higher r than photon absorptiometry (.908, $P < .0001$) and ultrasonography (.565, $P < .0001$). Thus, it was concluded that radiographic photometry is more accurate measurement of bone mineral content then both photon absorptiometry and ultrasonography.

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Response of Bone and Cartilage to Limited Physical Activity

Bone

Numerous studies have demonstrated that depriving animals of exercise is detrimental to bone strength. Exercise allows the bone to respond and adapt to loads necessary for proper bone architecture and mass (Pratt, 1982). An increase in mechanical stress/strain causes an increase in bone mass while a decrease in loading causes bone loss (Pratt, 1982). Strain is defined as a deformation created at any point while stress is defined as internal force intensities (Hayes and Gerhart, 1985). Strain rate is directly related to velocity:

$$y = 2500v - 3067$$

where: y = strain rate in microstrain per sec
v = velocity in feet per sec

indicating that strain increases linearly with increased speeds (Pratt, 1982). In other words, the faster a horse runs the more force is placed on the leg causing an increase in strain. Frost (1987) describes strain as mechanical usage and suggests that the magnitude of load influences modeling, remodeling, and growth. A lack of exercise in racing horses will result in bone unable to tolerate high strains which leads to weaker bone potentially causing injury (Price et al., 1995). This is a major problem in racing horses due to the high degree of stress put on their legs during training while still skeletally immature (Price et al., 1995). Lameness is very common among young horses during training (18 to 24 mo of age) possibly due to their bones not reaching a sufficient mass to bear the necessary load experienced during training (El Shorafa et al., 1979; Nunamaker et al., 1990). Immature bone is more responsive to alterations in strain than mature bone (Carter, 1984).

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Housing

Knowles and Broom (1990) and Fleming et al. (1994) studied laying hens and the affects of different housing systems on bone strength. Bone strength was related to the amount of movement allowed. Both studies demonstrated that battery cages, which restrict maximal movement, yielded birds with weaker bones than those housed in percherys did, which allowed for free wing movement. Knowles and Broom (1990) specifically found that birds in battery cages had only 54% of the bone strength in the radius and tibia of birds housed in percherys. Fleming et al. (1994) found a positive correlation between bone breaking strength and bone mineral density. The increase in breaking strength was related to changes in cortical bone and not in trabecular bone. Notably, neither study discussed the effect of housing on egg production, which influences bone strength due to Ca utilization for egg formation.

While looking at the effects of mineral balance, Nielsen et al. (1998) found that Quarter Horses maintained in stalls during training showed an increase in serum osteocalcin as bone density increased. Nielsen et al. (1998) also found an initial decrease in bone mineral content at the onset of training (d 0 to d 56) and then an increase from d 56 to d 112. This initial decrease may have been due to the onset of training or a response to stall confinement. This confinement probably resulted in bone mineral resorption causing a decrease in bone mineral content, indicating decreased strength of the third metacarpus. Maenpaa et al. (1990) found decreased serum alkaline phosphatase activity and OC concentration indicating diminished bone formation in foals moved from pasture to stalls. Hoekstra et al. (1998) also found that 18.6 mo old Arabian horses removed from pasture and placed in stalls, with only 1 h/d of walking on a mechanical

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walker, showed a decrease in bone mineral content by d 28 of the study. Stalled horses also showed increased deoxypyridinoline at d 28 indicating an increase in bone resorption and a decrease in serum OC at d 14 indicating decreased osteoblastic activity. Stalling horses and limiting their access to exercise negatively affected normal bone development compared to horses maintained on pasture.

Exercise

Using photon-absorption densitometry, Rubin and Lanyon (1984) found that the ulnae of roosters not subjected to loading showed a decrease in bone mineral content to 88% of its original postoperative state. To determine the amount of loading necessary to maintain or increase bone strength in the rooster ulnae, Rubin and Lanyon (1984) found that it took four cycles of loading per day to maintain bone mineral content. With 36 cycles of loading per day on the ulnae, bone formation increased with the maximum bone mineral content being reached at d 28 and stabilizing thereafter. Woo et al. (1981) showed that exercised swine had a 35% increase in maximum load strength of the femur over those swine not undergoing exercise. Woo et al. (1981) also determined that though the mechanical properties of bone remained unchanged following prolonged exercise, the quantity (cortical thickness and volume) of bone increased.

Raub et al. (1989) noticed an increase in medial bone mineral content/density of the left third metacarpus in exercised weanling horses. This increase was associated with bones having greater bone strength, hence, being able to withstand the forces associated with increased loading. McCarthy and Jeffcott (1992) studied the effects of exercise and inactivity on 12 Thoroughbred yearlings. They found that the exercised group had lower

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cortical bone porosity, higher bone mineral content, higher bone mineral density, and lower serum OC concentrations than those receiving no exercise. The decrease in porosity indicates a decrease in bone remodeling causing an increase in bone density. These changes demonstrated the ability of cortical bone to adapt to exercise due to a structure more capable of resisting injury. Sherman et al. (1995) found that the third metacarpal bone of horses increased in peripheral thickness in response to exercise. This increase was concluded to be associated with an increase in strength. Pratt (1982) found the most efficient way to develop bone mass is to participate in short but vigorous activities. Prolonged vigorous activities may lead to fatigue fractures of the bone. These short activities provides bone the loading necessary for optimal growth without exceeding the delicate balance between formation and resorption. Jeffcott (1991) states that growing horses should remain on pasture 12 h/d for the normal development of bones and joints.

Immobilization

Biewener and Bertram (1994) immobilized the wings of 2-wk-old chickens and found that cortical thickness was greater in exercised animals than in the sedentary/immobilized or denervation (excised sciatic nerve) animals. Kannus et al. (1996) immobilized patellas of rats to identify OC concentrations. In the groups in which limbs were immobilized and then remobilized and exercised, OC increased indicating increased mineralization of newly formed bone during exercise. Yeh et al. (1993) found similar results in the limbs of immobilized rats. The resorption of the immobilized limb, analyzed by radioactive ^{45}Ca retention, was 21% greater than that of the control rats.

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Immobilization decreased bone modeling while exercise caused more active bone formation.

Cartilage

Similar to studies in bone, lack of exercise has been shown to have negative effects on cartilage. A decrease in PG content has been associated with decreased loading. Decreased PG content of the cartilage causes a decrease in elasticity and increased water content (Palmoski et al., 1980).

Exercise

The incidence of osteochondrosis in foals subjected to forced exercise decreases when compared to foals provided with limited exercise (Jeffcott, 1991). This demonstrates the importance of exercise on cartilage metabolism in young horses. Palmer et al. (1995a) found a significant increase of newly synthesized PG in the cartilage of exercised horses compared to non-exercised, stalled horses. The PG was found to be composed of both large and small monomers that reacted with monoclonal antibody 1C6 indicating the potential to form aggregates with HA. Palmer et al. (1995b) did a similar study but found no difference in PG content between exercised and stalled horses possibly due to the short time period of the study (6 wk). Palmer et al. (1995b) did find that the exercised group demonstrated a greater permeability constant of the cartilage matrix than the non-exercised group. This suggests that the exercised group had a greater ability for fluid movement through the articular cartilage.

Little et al. (1997) compared strenuous and moderate exercise in horses and found that strenuous exercise causes an increase in PG metabolism in regions of high contact stress. Thus, too much exercise can damage cartilage. Saamamen et al. (1994) found that dogs running 20 km/d for 15 wk had a higher water content and lower collagen content in the cartilage than did control animals. These changes in cartilage were thought to be a result of damaged collagen matrix due to over-exertion of the cartilage. Vasan (1983) also demonstrated cartilage degradation in dogs running 6 d/wk for 1 h over an 8 mo period at 9.7 to 12.9 km/h with a 20° incline. In order for cartilage to attempt repair caused by this excessive stress, an increase in synthesis activity was seen. However, this increase in PG synthesis remained less than PG loss.

Immobilization

Numerous immobilization studies have been performed to identify the effects of immobilization and increased weight bearing on both PG content and GAG content within the joints. Richardson and Clark (1993) describe the effects of immobilization to be complete erosion of the affected points of contact and soft tissue infiltration in non-contact areas. Comparing PG and GAG content in exercised, immobilized, and control rabbit joints, Tammi et al. (1983) found an increase in PG concentration in the cartilage of the exercised group. An increase in CS in the immobilized knee and an increase of KS in the weight bearing knee were observed when compared to controls. Immobilization yields PG richer in CS making aggregation more difficult but normal joint loading will counter-act this effect (Tammi et al., 1983). A decrease in GAG synthesis was observed by Eronen et al. (1978) when rabbit legs were immobilized in extension. Immobilization

caused a rapid induction of osteoarthritic characteristics in the weight bearing cartilage. By immobilizing a leg in extension, there is constant compression of the cartilage causing a disruption of chondrocyte function leading to a decrease in GAG synthesis. This decrease continues until the degradation becomes so extensive that an increase in GAG synthesis begins to compensate for the vast amount of degradation (Eronen et al., 1978) but by this time the damage may be greater than the synthesis causing a net loss of cartilage. A similar decrease of GAG in immobilized rabbit joints was observed as well as an increase in the KS/CS ratio (Videman et al., 1981). The increase KS/CS ratio differs from what is found in OA joints. Videman et al. (1981) could not explain this difference. Caterson and Lowther (1978) found that sheep with an immobilized foreleg demonstrated a decrease in PG content while the supporting leg had an increase in PG. Increased loadbearing on, and movement of, the joint stimulated metabolism of chondrocytes, causing an increase in CS and an increase resistance to deformation.

In a study of dogs, ankle cartilage was transected and then immobilized (Palmoski et al., 1980). The immobilized cartilage was 14 to 29% thinner than the contralateral cartilage. There was also a decrease in PG content and synthesis, an increase in water content, and defective PG aggregation. Haapala et al. (1996) found a 23 to 31% lower hyaluronan concentration in immobilized dogs than in the contralateral limb. This lower concentration of hyaluronan is thought to cause deleterious affects on cartilage matrix organization.

Palmoski et al. (1980) also adds decreased elasticity and compressive stiffness to the adverse effects of immobilized cartilage. While looking at horses immobilized with a cast, Richardson and Clark (1993) found a decrease in PG content of cartilage in the

immobilized joint due to the mechanical stimuli being below a level necessary to maintain normal cartilage. This lack of loading and motion causes a decrease in the movement of synovial fluid nutrients into cartilage causing a decrease in PG synthesis and content.

CHAPTER 2

MATERIALS AND METHODS: EXPERIMENT 1

Animal Management

Eighteen Quarter Horse weanlings from the MSU Merillat Equine Center were pair-matched by age and randomly assigned to three treatment groups of six horses each. Horses remained on the study for 56 d. Prior to the start of the study, weanlings were kept outside during the day and stalled each night with their dams. Horses were weaned in two separate groups consisting of nine horses each (three horses in each treatment group). The first weaning group began the study on June 9, 1998 while the second weaning group began on August 29, 1998. The paddock group (P1, n = 6) was maintained on a 62.6 m x 15.9 m dry lot with free access to exercise. The stall group (S1, n = 6) was housed in 3.7 m x 3.7 m stalls with no free access to exercise. The exercised group (X1, n = 6) was housed in 3.7 m x 3.7 m for the first 28 d of the study with no free access to exercise and followed an exercise protocol from d 28 to d 56 described later. One horse in X1, from the second weaning group, died after d 28 from respiratory problems. The data for this horse was still included in the results excluding d 42 and 56. The average ages, followed by the range of ages in parentheses, of each group were P1 - 143 d (115 d to 187 d), S1 - 143.2 d (116 d to 193 d), and X1 - 141.2 d (108 d to 154 d). Groups S1 and X1 were individually fed 1.5 kg of Strategy™ (Purina Feeds, St. Louis, MO) twice daily (0730 and 1530) and approximately 1.2 kg of alfalfa-grass mixed hay every morning at (0730) (Table 1). If hay was eaten in the morning, a second feeding of 1.2 kg was fed at night at (1530). Group P1 was group fed 3.8 kg of Strategy™ (1.3

kg/horse) twice daily (0730 and 1530) and followed the same regimen of hay as groups S1 and X1 (Table 2). Strongid® C Daily Dewormer (Pfizer, Inc., New York, NY) was fed every morning according to manufacturer recommendations. Water was available at all times.

Table 1. Calculated daily nutrient table for S1 and X1 weanlings on a dry matter basis

Nutrient	Nutrient Content	Calculated Total Daily Intake
DE	2.9 Mcal/kg	12.4 Mcal
CP	15.0%	645.0 g
Ca	0.81%	35.0 g
P	0.46%	20.4 g

Table 2. Calculated daily nutrient table for P1 weanlings on a dry matter basis

Nutrient	Nutrient Content	Calculated Total Daily Intake
DE	2.8 Mcal/kg	11.2 Mcal
CP	15.1%	595.0 g
Ca	0.81%	32.1 g
P	0.46%	18.0 g

Before longe training began, each weanling in the exercised group was introduced to longeing by walking 5 min daily, 5 d/wk on a mechanical walker (15.2 m diameter circle) in both directions. The longeing procedure began on d 28, 1 wk after introduction to the mechanical walker. Horses were longed on a 6.1 m to 9.1 m diameter circle once a day for 5 d/wk. They were longed in an indoor arena on a dirt surface. Horses were trotted 10 circles, cantered 20 circles, reversed and the circles repeated in the opposite direction. Longeing lasted for approximately 15 min daily.

The exercised group, in the first weaning group, were longed with the preceding procedure from d 28 to d 42. From d 42 to d 56 they were walked for 15 min, 5 d/wk on the mechanical walker. The stalled and exercised weanlings in the second weaning group were "halter broke" by walking on the mechanical walker for the first 14 d of the study. On d 28, the exercised group began the longeing procedure as stated above (Table 3).

Table 3. Exercise protocol for Quarter Horse weanlings

	Day			
Treatment	0-14	15-28	29-42	43-56
Exercise (W1)			L	MW
Exercise (W2)	HB		L	L
stall (W1)				
stall (W2)	HB			

L - longeing procedure
MW - walking on the mechanical walker
HB - halter broke on mechanical walker
W1 - weaning group 1
W2 - weaning group 2

Bone Mineral Content

On d 0, 28, and 56 radiographs of the left third metacarpal were taken to determine radiographic bone aluminum equivalence (RBAE) (Meakim et al., 1981). Dorsal-palmar and lateral-medial radiographs were taken at 17 mA at 71.1 cm focal film distance for .08 sec at 70 kV, using mobile x-ray equipment and medical x-ray film. The radiograph cassette was positioned against the palmar and medial points of the leg allowing the beam to be parallel to the ground centered at the midpoint of the metacarpal region. An aluminum stepwedge penetrometer was used as a reference standard for each

radiograph. Radiographs were scanned at the nutrient foramen, to determine RBAE, using the Bio-Rad GS-700 Imaging Densitometer (Bio-Rad Laboratories, Hercules, CA). Logarithmic regression was used to determine the dorsal, palmar, lateral, and medial RBAE (mm Al) using the thickness of the stepwedge and the maximum optical density readings of these cortices (Meakim et al., 1981). Total RBAE (mm^2Al) was used to determine density and volumetric changes using the total area of bone divided by the total area of the aluminum stepwedge (Figure 1). The total area under the stepwedge corresponding to the steps with thickness of 14, 17, 20, 23, and 26 mm Al and the total area of the third metacarpal bone curve were used. The area of the stepwedge was 1270 mm^2. The total RBAE of the third metacarpal bone was determined by multiplying the area of the third metacarpus (mm*Optical Density) by 1270 mm^2 and dividing by the scanned area of the stepwedge (mm*Optical Density) (Nielsen and Potter, 1997).

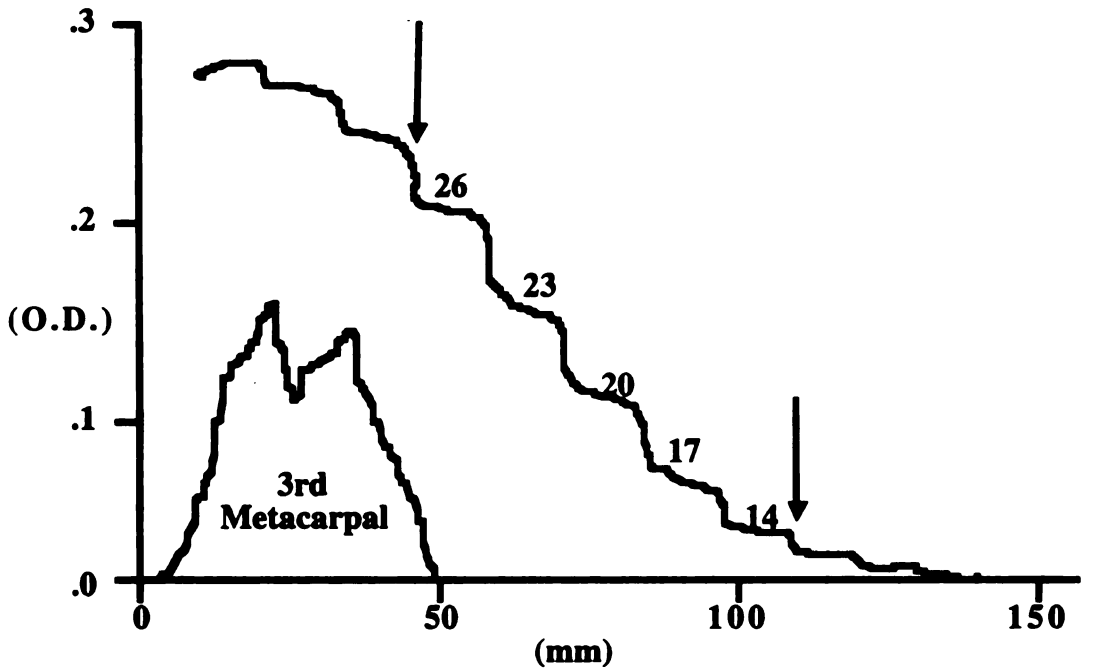

Figure 1. Scanned image of equine third metacarpal and aluminum stepwedge penetrometer (area of the stepwedge used for calculation is between the two arrows) (Nielsen and Potter, 1997)

Serum Collection

Every two wk (d 0, 14, 28, 42, 56), blood samples were taken via jugular venipuncture using a 20 gauge Vacutainer® needle and a sterile Vacutainer® tube (Becton Dickinson, Franklin Lakes, NJ) with no additives. Blood was allowed to clot for approximately 1 to 2 h and was subsequently centrifuged at 1340 g for 15 min at room temperature. Serum was collected using a disposable Pasteur pipet (VWR Scientific, West Chester, PA) and placed in 1.5 ml microcentrifuge tubes. Each sample was then stored in a -20°C freezer until analysis. Serum was analyzed for OC, ICTP, and KS. Sampling times for d 0, 14, 28, 42, and 56 for the first weaning group were 1615, 1030, 1535, 0935, and 0930 respectively. Sample collection for the second weaning group were 0930, 0930, 0945, 0930, and 0930 respectively.

Osteocalcin Analysis

Osteocalcin was quantified using Novocalcin® ELISA kits (Metra Biosystems, Inc. Mountain View, CA) according to manufacturer's instructions. Serum was diluted between 1:10 and 1:15 dependent on the sample. Dilutions were determined by running the OC assay on a few samples at dilutions of 1:2, 1:4, 1:6, and 1:8. The values were then compared to the standard curve graph in the manufacturer's instructions. When the values fell on the linear portion of the graph (between .4 and 1.5 optical density) the corresponding dilution was used. Each sample was run in duplicate. Standards and controls were reconstituted using .5 ml 1X wash buffer. Twenty-five µl of standard, sample, or control was added to each well with 125 µl of anti-OC antibody. This mix was incubated at room temperature for 2 h and then each well washed with 300 µl 1X

wash buffer three times. Enzyme conjugate (150 µl) was added to each well and incubated for 1 h at room temperature. Each well was then washed with 300 µl 1X wash buffer. The last step was the 40 min incubation of 150 µl of working substrate solution. Each plate was then read at 405 nm optical density on a Spectra Max 340 plate reader (Molecular Devices Corp., Sunnyvale, CA).

Carboxyterminal Telopeptide of Type I Collagen Analysis

Carboxyterminal telopeptide of type I collagen was quantified using ICTP ^{125}I RIA Kit® (Diasorin Corp. Stillwater, MN) according to manufacturer's instructions. Each sample, standard, control, and nonspecific binding tubes were run in duplicate. The tubes were set up as follows: nonspecific binding tubes – 100 µl sample and 200 µl distilled water, standards (0 and A-F) – 100 µl standard and 200 µl ICTP antiserum, controls and samples – 100 µl serum and 200 µl ICTP antiserum. Each tube was incubated for 2 h at 37° C with 200 µl of ^{125}ICTP. Separation reagent (500 µl) was added to each tube and incubated for 30 min at 25 ° C. Tubes were then centrifuged for 30 min at 2000 X g at 10° C. The supernatant was decanted and placed in the 1290 GammaTrac Gamma Counting System (Tm Analytic, Elk Grove Village, IL).

Keratan Sulfate Quantification

Keratan sulfate was quantified by an ELISA with an inhibition step previously described by Thonar et al. (1985) and Williams et al. (1988). The standard was diluted at 1:24 and serial diluted onto the uncoated plate. Samples were diluted to 1:4 (70 µl sample + 210 µl PBS-Tween 20:1% BSA buffer) and serial diluted to 1:32 in the uncoated plate.

Both the standard and the samples were diluted with PBS-Tween 20:1% BSA, pH 5.3. For the inhibition step, a 1:12,000 dilution of the anti-KS monoclonal antibody (ICN Pharmaceuticals Inc., Costa Mesa, CA) was made and 140 µl was added to each well. Each plate was covered and incubated, with shaking, for 1 h at room temperature. The plates were then stored at 4° C overnight. Coating buffer was removed from the coated plates with three washes of PBS-Tween 20, pH 5.3 for 5 min. Two hundred µl from each well from the inhibition step were transferred to the washed coated plates. The coated plate was covered and incubated, with shaking, for 1 h at room temperature. Ten min prior to use, the horseradish peroxidase-conjugated anti-mouse IgG antibody (second antibody) (ICN Pharmaceuticals Inc., Costa Mesa, CA) was diluted in PBS-Tween 20:1% BSA, pH 5.3, at a 1:1,000 dilution. After 1 h incubation, the inhibition mixture in the coated plates was removed and 5 min washes were performed three times. The second antibody (200 µl) was added to each well and incubated, with shaking, for 1 h at room temperature. After 1 h of incubation, the inhibition mixture in the coated plates was removed and 5 min washes were performed three times. O-phenylenediamine was added (200 µl/well) and incubated at room temperature for 15 min (Sigma Chemical, St. Louis, MO). To stop the substrate-enzyme color development, 50 µl/well of 2M H_2SO_4 was added. The plates were then read with the Spectra Max 340 plate reader (Molecular Devices Corp.).

Growth Measurement

Cannon circumference, hip height, wither height and weight were taken every 28 d. Weight was determined using a weight tape placed around the girth just below the

withers. Cannon circumference was measured around the mid-section of the third metacarpus. Hip and wither heights were measured using a measuring stick from the ground to the point of the hip or the withers.

Statistics

Differences between treatments, day of study and day*treatment interactions were determined using a two-factor ANOVA (PROC MIXED) in SAS 6.12 (1997). The blocking effect of weaning in two groups was included in our model. This was added to account for any differences that may have existed due to weaning time. The LSMEANS statement was included in the analysis to obtain treatment means, differences between means and standard errors. Data were normalized (d 0 values subtracted from other days to examine changes from d 0) when treatment differences existed at d 0. A P-value of less than .05 was considered significant while a trend was investigated at a P-value of less than .1.

RESULTS: EXPERIMENT 1

Bone Mineral Content

The medial RBAE of the paddocked weanlings (Figure 2) increased from 16.6 ± .7 mm Al on d 28 to 18.3 ± .7 mm Al on d 56 (P = .03). Stalled weanlings demonstrated a decrease in medial bone mineral content from 17.1 ± .7 mm Al on d 0 to 15.6 ± .7 mm Al on d 28 (P = .05). On d 56, P1 tended to have a greater mineral content of 18.3 ± .7 mm Al than the stalled horses at 16.4 ± .7 mm Al (P = .06) in medial RBAE. There were no differences seen in the exercised weanlings or between X1 and P1 or S1 weanlings.

There were no time or treatment differences in lateral RBAE (Figure 3).

No treatment differences were seen in palmar bone mineral content. However, the palmar RBAE of S1 (Figure 4) tended to decrease from 15.2 ± .7 mm Al on d 0 to 13.6 ± .7 mm Al on d 56 (P ≤ .1). No differences were seen in paddocked or exercised weanlings.

Due to differences on d 0, dorsal, total (LM) and total (DP) views were normalized by subtracting all values from d 0. The exercised group tended to decrease in dorsal bone mineral content from d 0 to d 28 (P = .06) but increased from d 28 to d 56 (P = .04) (Figure 5). On d 28, the paddocked group had greater dorsal mineral content than X1 (P = .02) while S1 tended to have greater mineral content than X1 on d 28 (P = .1).

Total (DP) RBAE demonstrated a tendency to decrease in X1 from d 28 to d 56 (P = .09) (Figure 6). On d 56, paddocked weanlings had greater total (DP) mineral content than exercised weanlings (P = .03). Total (DP) RBAE did not change in the stalled weanlings.

The exercised group decreased in total (LM) RBAE from d 0 to d 56 (P = .002) (Figure 7). An increase in the paddock group was seen from d 0 to d 28 (P = .04). The total (LM) RBAE of P1 was greater than that of X1 on d 28 (P = .02) and on d 56 (P = .01). Stalled weanlings tended to decrease from d 0 to d 56 ($P \leq .1$) in the total (LM) view. On d 28, the stalled group tended to be greater than the exercised group (P = .1).

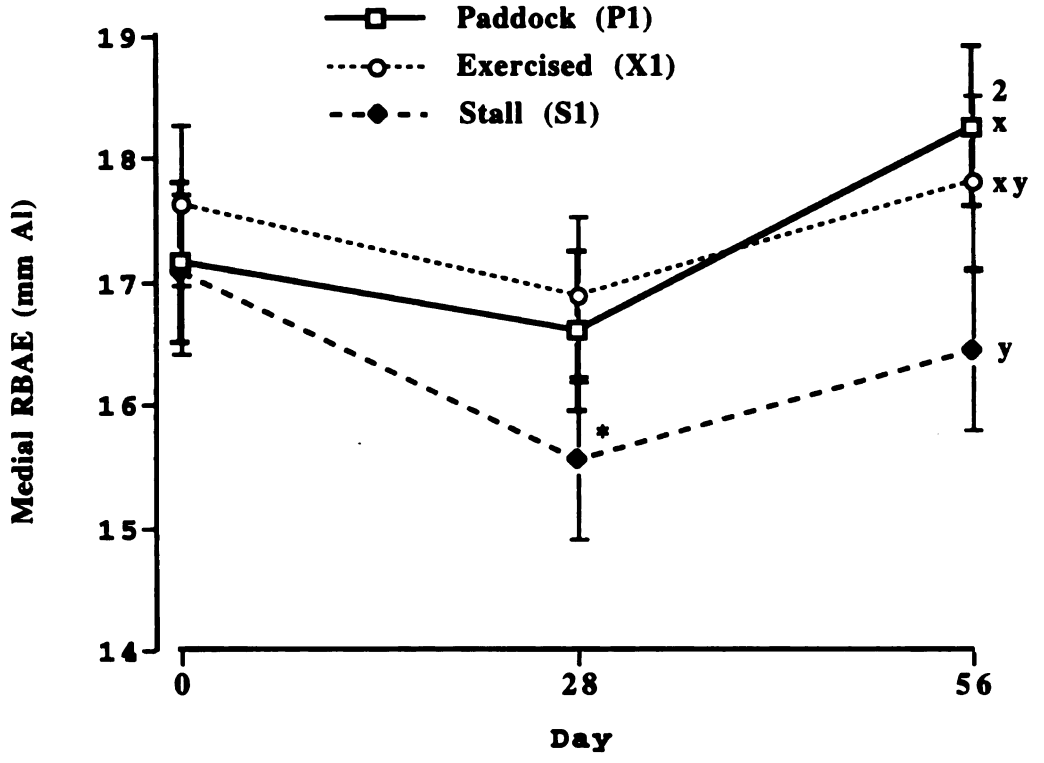

Figure 2. Medial RBAE (mm Al) versus day of project in weanling Quarter Horses
[2]differences between d 28 and d 56 ($P \leq .05$)
[*]different from d 0 ($P \leq .05$)
[x,y]treatments with different superscripts at a given day differ ($P \leq .1$)

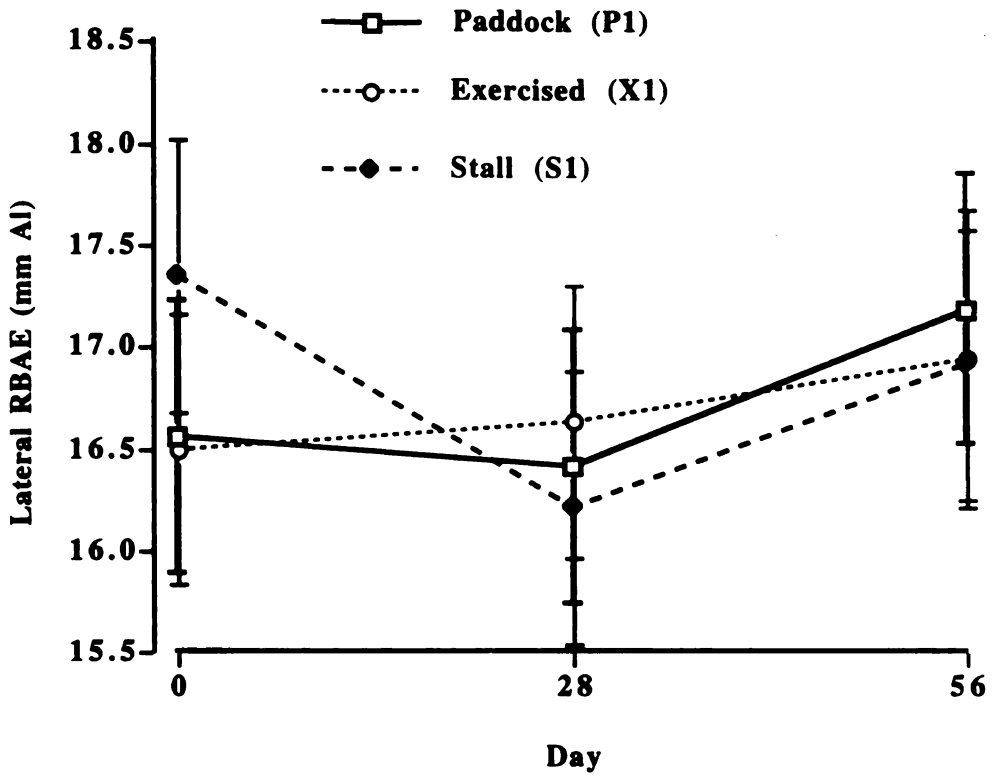

Figure 3. Lateral RBAE (mm Al) versus day of project in weanling Quarter Horses

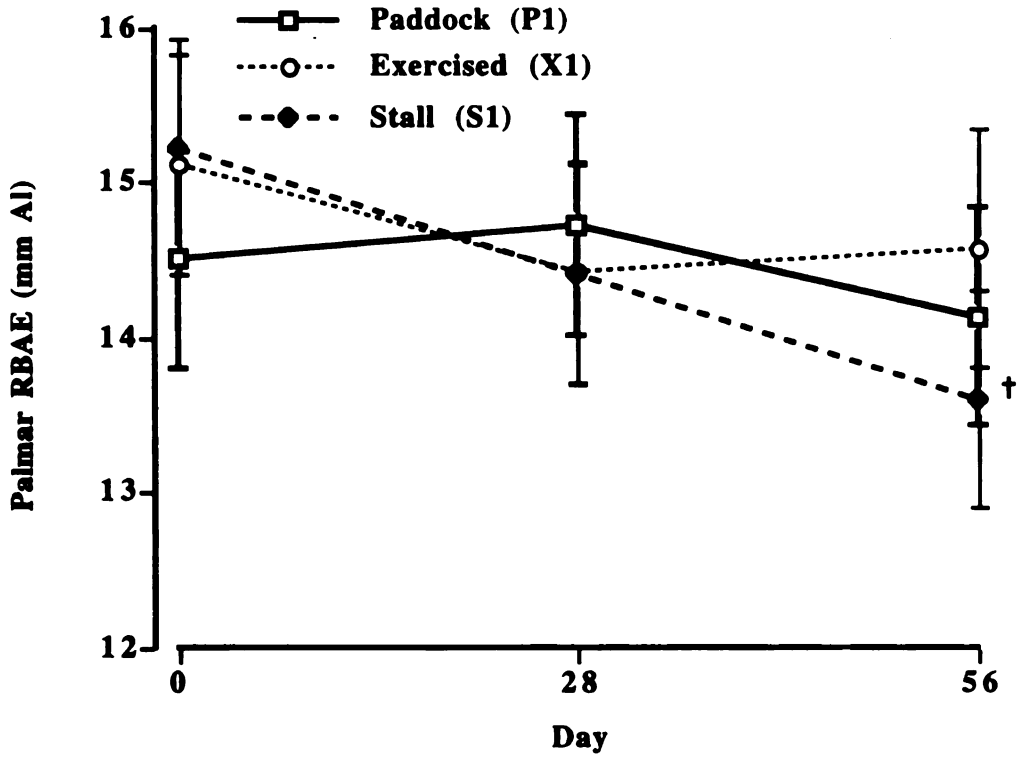

Figure 4. Palmar RBAE (mm Al) versus day of project of weanling Quarter Horses
†different from d 0 ($P \leq .1$)

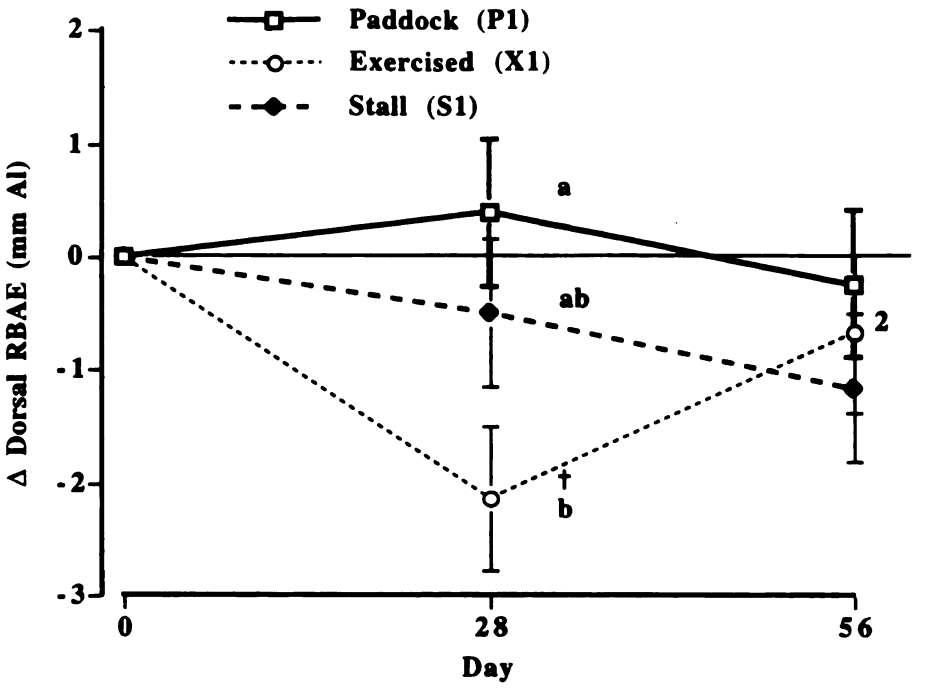

Figure 5. Change in dorsal RBAE (mm Al) versus day of project in weanling Quarter Horses
[†]different from d 0 (P ≤ .1)
[2]differences from d 28 to d 56 (P ≤ .05)
[a,b]treatments with different superscripts at a given day differ (P ≤ .05)

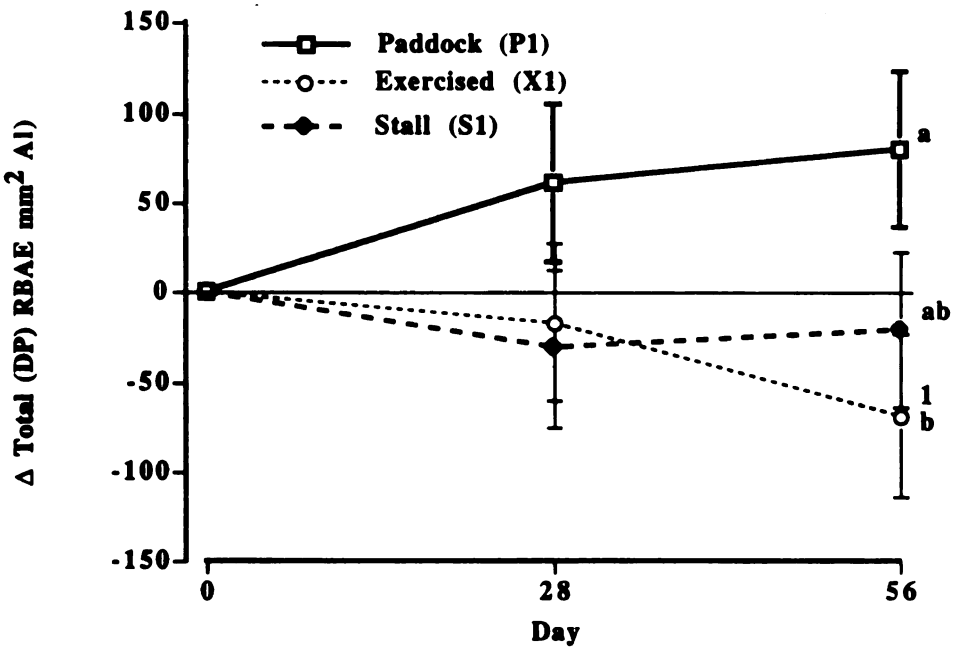

Figure 6. Change in Total (DP) RBAE (mm^2 Al) versus day of project in weanling Quarter Horses
[1]differences from d 28 to d 56 (P ≤ .1)
[a,b]treatments with different superscripts at a given day differ (P ≤ .05)

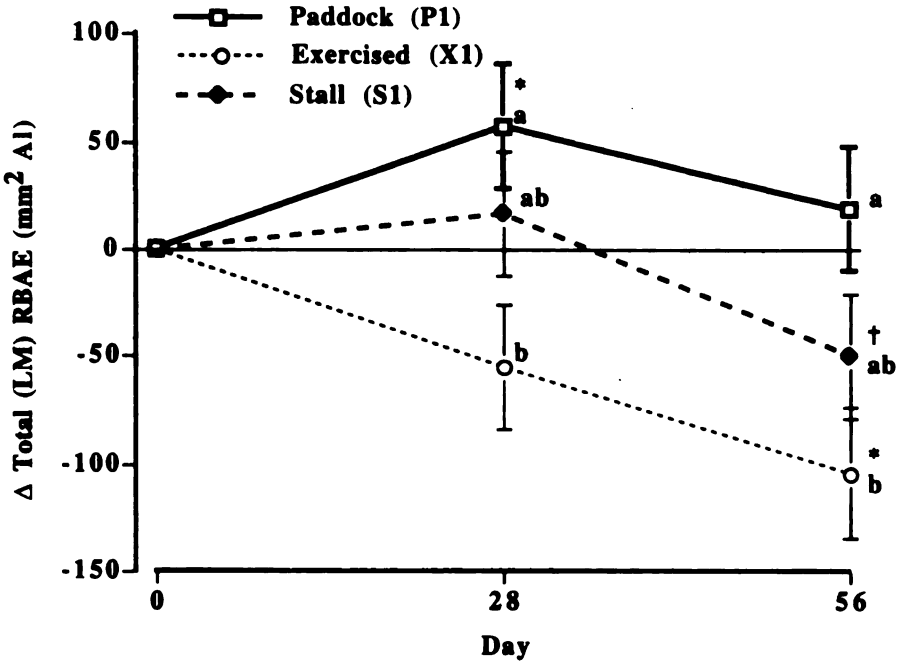

Figure 7. Change in total (LM) RBAE (mm^2 AI) versus day of project in weanling Quarter Horses
***different from d 0 ($P \leq .05$)**
†different from d 0 ($P \leq .1$)
a,b treatments with different superscripts at a given day differ ($P \leq .05$)

Osteocalcin

The serum OC of the paddocked group tended to decrease from 152.3 ng/ml on d 0 to 108.3 ng/ml on d 28 (P = .06) and continued to decrease to 101.5 ng/ml on d 42 (P = .03) (Table 4). The final P1 serum OC concentration was 98.4 ng/ml (P = .02). The serum OC concentration of the exercised weanlings decreased from 154.7 ng/ml on d 0 to 99.3 ng/ml on d 14 (P = .01) and continued to decrease to 91.9 ng/ml on d 28 (P = .007). Serum OC of the exercised group tended to increase from 91.9 ng/ml on d 28 to 134.2 ng/ml on d 56 (P = .06). Stalled weanlings had greater OC concentration than X1 on d 14

(P = .05) and tended to be greater than X1 on d 28 (P = .1). The serum OC of the stalled group also tended to be greater than the paddocked weanlings on d 42 (P = .1).

Table 4. Means table for osteocalcin (ng/ml) in Quarter Horse weanlings

	Day					
Treatment	0	14	28	42	56	SEM
Paddock	152.3^{a}	123.4^{abxy}	108.3^{ab}	101.5^{b}	98.4^{b}	16.4
Exercise	154.7^{a}	99.3^{bx}	91.9^{b}	117.1^{ab*}	134.2^{ab**}	16.4
Stall	147.6^{a}	145.8^{ay}	129.4^{a}	139.0^{a}	113.8^{a}	16.4

^{ab}days with different superscripts within rows differ ($P \leq .05$)
^{xy}treatments with different superscripts within columns differ ($P \leq .05$)
*SEM = 17.9
**SEM = 18

ICTP

Due to differences in ICTP on d 0 (Table 5), ICTP data was normalized by subtracting all values from d 0 (Table 10A). Paddocked weanlings decreased from d 0 to d 42 (P = .05) with a continued decrease of 3 ng/ml to d 56 (P = .05). The exercised weanlings demonstrated a decrease in ICTP concentration of 2.8 ng/ml from d 14 to d 28 (P = .05) and increased by 3.0 ng/ml from d 28 to d 56 (P = .05). The stalled weanlings decreased by 6.7 ng/ml from d 0 to d 28. An increase of S1 serum ICTP of 4 ng/ml was seen from d 28 to d 42 (P = .007). On d 28, paddocked weanlings had greater ICTP concentration than the stalled group (P = .02).

Table 5. Means table for ICTP (ng/ml) in Quarter Horse weanlings

	Day					
Treatment	0	14	28	42	56	SEM
Paddock	15.7^{axy}	13.1^{ab}	14.3^{abx}	12.6^{b}	12.6^{b}	1.1
Exercise	16.8^{ax}	14.9^{a}	12.1^{bxy}	13.9^{ab*}	15.1^{a*}	1.1
Stall	20.0^{ay}	14.7^{bc}	13.3^{by}	17.3^{ac}	15.7^{c}	1.1

^{abc}days with different superscripts within rows differ ($P \leq .05$)
^{xy}treatments with different superscripts within columns differ ($P \leq .05$)
*SEM = 1.2

Keratan Sulfate

All treatment groups demonstrated a decrease in KS concentration from d 0 to d 56 (Table 6). Initial KS concentrations of P1, X1, and S1 were 618.6, 548.5, and 623.8 ng/ml respectively. All treatment groups decreased to d 14 ($P \leq .02$), d 28 ($P \leq .003$), and d 42 ($P \leq .03$). The final concentrations were 473.2 ng/ml for P1 ($P \leq .001$), 476.1 ng/ml for X1 ($P = .08$), and 535.9 ng/ml for S1 ($P = .02$). On d 14, the paddocked group demonstrated greater KS concentration than the exercised group ($P = .02$). Serum KS concentration of the stalled group tended to be greater than the exercised group on d 28 ($P = .06$).

Table 6. Means table for keratan sulfate (ng/ml) in Quarter Horse weanlings

	Day					
Treatment	0	14	28	42	56	SEM
Paddock	618.6^{a}	565.2^{bx}	488.5^{cd}	524.7^{bd}	473.2^{c}	36
Exercise	548.5^{a}	440.6^{by}	435.0^{b}	464.1^{b*}	476.1^{ab**}	36
Stall	623.8^{a}	514.0^{bxy}	533.8^{b}	522.9^{b}	535.9^{b}	36

^{abcd}days with different superscripts within rows differ ($P \leq .05$)
^{xy}treatments with different superscripts within columns differ ($P \leq .05$)
*SEM = 37.1
**SEM = 37.9

Growth Measurements

All groups demonstrated an increase from d 0 to d 56 in height at the withers ($P \leq .01$). Initial wither heights were 123.5 cm for P1, 125.3 cm for X1, and 123.3 cm for S1. Final height at the withers were 128.5 cm for P1, 128.5 cm for X1, and 128.5 cm for S1. There were no treatment differences seen in wither height (Table 11A). All groups increased from d 0 to d 56 ($P \leq .05$) in hip height with no treatment differences observed. Initial and final hip heights were 129.3 cm to 133.6 cm for P1, 131.4 cm to 135.0 cm for

X1, and 130.1 cm to 135.3 cm for S1 (Table 12A). All treatment groups increased from d 0 to d 56 ($P \leq .02$) in weight with no treatment differences seen. Initial weights were 233 kg for P1, 232 kg for X1, and 221 kg for S1. Final weights for P1, X1, and S1 were 265 kg, 250 kg, and 257 kg respectively (Table 13A). Both paddocked weanlings and stalled weanlings demonstrated increases in cannon circumference from d 0 to d 56 (Table 14A). The cannon circumferences increased from 15.7 cm on d 0 to 16.4 cm on d 56 ($P = .0003$) for P1 and 15.5 cm on d 0 to 15.9 cm on d 56 ($P = .03$) for S1. The exercised group decreased from 16.0 cm on d 0 to 15.7 cm ($P = .02$) on d 28. The cannon circumference of exercised weanlings then increased from d 28 to 16.2 cm on d 56 ($P = .002$).

DISCUSSION: EXPERIMENT 1

Bone Mineral Content

As expected, the paddocked group increased in both medial and total (LM) RBAE and demonstrated greater bone mineral content than the stalled group in medial RBAE. The stalled weanlings demonstrated a decrease in medial, palmar, and total (LM) bone mineral content. The greater bone mineral content of the paddocked weanlings, demonstrated in the present study, parallel those of Hoekstra (1998) where paddocked yearlings demonstrated greater bone mineral content than the stalled horses on d 28 and d 56 in both the lateral and medial cortices. The results are also similar to Knowles and Broom (1990) and Fleming et al. (1994) that demonstrated that allowing an animal room to move (i.e. hens in perchery's or weanlings in paddocks) causes an increase in bone mineral content and, hence, in bone strength. The increase in space to move allows for free access to exercise. McCarthy and Jeffcott (1992) found that exercised yearlings demonstrated greater bone mineral content than those horses not receiving exercise. The lack of exercise received by the stalled group would account for the decreased bone mineral content.

Along with the present study, the influence of housing on behavior was examined using the stalled and paddocked treatment groups (Heleski et al., 1999). It was found that stalled weanlings spent more time lying down than paddocked weanlings ($P < .001$). The stalled animals also spent less time standing, interacting with other horses, and moving around than the paddocked weanlings. The increase in lying down and lack of movement

from the stalled horses would account for the decreases in bone mineral content observed in the present study.

The lack of changes seen in the palmar and lateral cortex of the paddocked group and in the lateral cortex of the stalled group is dependent on where the most compressive loads were experienced. Where compression occurs may relate to the conformation of the animal, the ground surface, the architecture of the bone, and other factors. During "normal everyday activities", the dorsomedial quadrant of bone receives the most concussion (Rooney, 1978). Because the dorsolateral quadrant does not receive as much concussion as the dorsomedial quadrant the risk of injury to that area increases (Rooney, 1978). As cortical bone thickens in response to training, the medial, lateral, and dorsal cortices change while the palmar cortex remains unchanged (Nielsen et al., 1997). This results in the dorsal, medial, and lateral cortices increasing in strength and size while the palmar does not. This lack of change in the palmar cortex may be due to the lack of compression the palmar cortex receives (Pratt, 1982). The palmar cortex has been shown to primarily undergo tension and not compression (Pratt, 1982).

Exercised weanlings demonstrated an initial decrease in dorsal RBAE followed by an increase. The exercised group also decreased in both total (DP) and (LM) bone mineral content. The paddocked and stalled weanlings demonstrated greater dorsal and total (LM) bone mineral content then the exercised weanlings. The sharp increase in the dorsal view, of the exercised group, corresponds with the onset of longe training, which began on d 28. Raub et al. (1989) found that weanlings worked on the mechanical walker at the trot 5 d/wk demonstrated an increase in medial bone mineral content with no change in the lateral cortex. The differences of the cortices was attributed to the medial

cortex having greater bending properties than the lateral cortex. Though an increase was seen in dorsal RBAE, the increase of bone mineral content was not great enough to detect an increase in the total bone mineral content. The initial decrease observed in dorsal RBAE, of the exercised group, may be due to the lack of exercise the weanlings were receiving for the first 28 d of the study.

Paddocked weanlings may have demonstrated greater bone mineral content than the exercised weanlings because the longeing procedure and the work on the mechanical walker did not supply enough concussion on the bone to stimulate an increase greater than that seen in the paddocked group. There seems to be no explanation for the greater bone mineral content of the stalled horses, in dorsal and total (LM) RBAE, when compared to the exercised weanlings.

When looking at the weaning groups separately, we find that in weaning group 1 (data not reported), the stalled weanlings never demonstrate greater bone mineral content than the exercised weanlings. In fact, the stalled weanlings decrease in total (LM), palmar, and medial RBAE. The stalled weanlings tended to increase in lateral RBAE from d 28 to d 56. This increase in the stalled weanlings does not correspond with any increase in activity as seen by Heleski et al. (1999) and does not seem to have an explanation. The exercised weanlings in weaning group 1, tended to decrease in total (DP) RBAE from d 28 to d 56 and increase in medial RBAE from d 28 to d 56. The exercised group also decreased from d 0 to d 28 in medial RBAE. The decrease demonstrated in the exercised group in medial RBAE may be due to being stalled from d 0 to d 28. The increase in medial RBAE is likely due to the longeing procedure counteracting the affects of stalling this group for the first month of the study. Because

the bone received little concussion during the first month of the study, due to stalling, one would expect to see a decrease or no change in the bone mineral content (Hoekstra et al., 1998; Fleming et al., 1994; Knowles and Broom, 1990). Once longe training began however, an increase in bone mineral content is expected because of the increase in concussion the bone would then be receiving (Hoekstra et al., 1998; McCarthy and Jeffcott, 1992). In the exercised group, the bone went from little concussion on d 0 to d 28 to loads associated with cantering and trotting on the longe line from d 28 to d 56. In both lateral and total (DP) RBAE, exercised weanlings demonstrated greater bone mineral content than the stalled weanlings on d 28. This response of the exercised weanlings corresponds with the start of longe training following the 1 wk of walking on the mechanical walker. Though walking does not result in much change in bone mineral content (strain is related to speed which is lacking in the walk), as weanlings are introduced to a mechanical walker they usually jump, rear, or bolt forward to escape the pressure on their halters or because they are startled. These actions may cause enough strain on the bone to induce increases in bone mineral content, which would result in greater bone mineral content than the stalled weanlings.

The total (LM) or (DP), lateral, and medial RBAE in the paddocked group in the first weaning group is always greater than the stalled or the exercised weanlings. An increase in medial RBAE from d 28 to d 56 was seen in the paddocked group. This correlates with other studies indicating that confinement rearing is detrimental to bone strength (Hoekstra et al., 1997; Knowles and Broom, 1990; Fleming, 1994). There were no differences seen in the normalized data of dorsal RBAE. This lack of change may have been due to the lack of compression the dorsal cortex was receiving. Though the

everyday activity causes compression on the dorsomedial quadrant of the bone, there may not have been enough concussion surpassing this "normal activity" to elicit an increase in the dorsal cortex of the paddocked weanlings. In racing horses, it is necessary to have compression of the dorsal cortex in order to increase bone strength of the dorsal cortex. The dorsal cortex is the area that is injured the most due to increases in strain (Nunamaker et al., 1990).

In the second weaning group, the stalled weanlings increased from d 0 to d 56 in both medial and lateral RBAE. The exercised group increased in lateral RBAE from d 0 to d 56 and tended to be greater than stalled weanlings on d 28 in medial RBAE. The paddocked group decreased from d 28 to d 56 in total (LM) RBAE but increased from d 28 to d 56 in medial RBAE. The increase demonstrated by the stalled weanlings does not have an explanation. The increase seen in the exercised group is likely due to the introduction to the mechanical walker and the longeing procedure. The decrease seen in the paddocked group may be due to the weanlings lack of activity in the paddock (Heleski et al., 1999).

Since the exercised group was treated so differently between weaning groups, this may explain why the data of the exercised group demonstrate both increases and decreases in RBAE when both weaning groups are combined. No blocking effect was seen because only six horses (three stalled and three exercised from the second weaning group) out of the 18 weanlings used in the study were treated differently. However, the weaning groups were quite different and yielded different responses in all groups although significant power in the design was not present to detect a difference by weaning groups. Due to the relative inactivity of the exercise group from d 0 to d 28, one

would expect the bone mineral content to either decrease or remain unchanged. But as the longeing procedure began on d 28 an increase in bone mineral content was expected.

Serum Analysis

Osteocalcin

Paddocked weanlings decreased in serum OC concentration. The stalled weanlings remain unchanged throughout the study and resulted in the stalled weanlings demonstrating greater OC concentration than both the exercised and paddocked weanlings. Osteocalcin decreases with age in growing horses (Hope et al., 1993; Lepage et al., 1991; Lepage et al., 1990). This decrease can be seen in the paddocked weanlings. The exercised weanlings demonstrated an initial decrease followed by an increase in serum OC. This increase may represent the increase in bone formation or bone turn-over once the longeing procedure began. Kannus et al. (1996) found similar results as in the present study. Kannus et al. (1996) immobilized the hind legs of rats for 3 wk, similar to the inactivity of the exercised group, and found a decrease in OC concentration. After 8 wk of remobilization and forced exercise, similar to the exercised group working on the longe line, the OC concentration increased to baseline. Though immobilization is more severe than the lack of activity the exercised group underwent, both the present study and Kannus et al. (1996) demonstrated similar results indicating that decreased activity followed by an increase in activity may result in an increase in OC.

Osteocalcin has been shown to fluctuate with time of day. Lepage et al. (1991) found that OC serum concentration levels remained constant from 0700 to 1900. The

serum analyzed in the present study was collected between 0700 and 1900. Hence, this should not have contributed greatly to any variation.

ICTP

All treatment groups had decreases in serum ICTP concentration from d 0 to d 42 or d 56. This corresponds with numerous studies indicating that serum ICTP concentration decreases with age in equine (Price et al., 1995a; 1995b), canine (Allen et al., 1998), and humans (Crofton et al., 1997; Zanze et al., 1997). Price et al. (1995a) reported ICTP serum concentrations of 15 female Thoroughbred horses less than 1 yr to be 13.7 to 26.7 μg/l. In the present study our ICTP serum concentrations on d 0 ranged from 15.7 ± 1.0 ng/ml to 20.0 ± 1.0 ng/ml, thus falling within the range Price at al. (1995a) reported. Price et al. (1995a) reported the ICTP serum concentrations to decrease to 7.9 to 22.8 μg/l by 1 to 2 yr of age. The final concentrations in the present study ranged from 12.6 ± 1.0 ng/ml to 15.7 ± 1.0 ng/ml, also falling within the range of Price et al. (1995a). The stalled weanlings increase in serum ICTP concentration from d 28 to d 42 is likely due to the lack of exercise received by this group. The increase in the exercised group from d 28 to d 56 may be due to the increase in bone turn-over from being longed returning serum ICTP concentrations to baseline. The increase of ICTP and OC serum concentrations represents an increase in bone turn-over due to remodeling and modeling. There is no explanation as to why differences existed on d 0, randomization should have accounted for this difference.

Keratan Sulfate

All treatment groups decreased in KS serum concentration from d 0 to d 56. This decrease parallels other studies that demonstrate decreases in KS concentration with increasing age (Todhunter et al., 1997; Okumura et al., 1997; Roughley and Lee 1994; Spector et al., 1992; Nixon, 1991; Sweet et al., 1990; Leipold et al., 1989). Okumura et al. (1997) reported KS concentrations in 15 foals to be approximately 4000 ng/ml at 3 mo of age and decreasing to 500 ng/ml at 6 mo of age. Todhunter et al. (1997) reported KS values in ten 3-mo-old foals to be approximately 300 ng/ml. The differences between these two studies may be due to Okumura et al. (1997) analyzing serum while Todhunter et al. (1997) analyzed plasma levels. The present study reports KS concentration values to range from 435.0 $\pm$ 37.5 ng/ml to 623.8 $\pm$ 37.5 ng/ml, in the range of Okumura et al. (1997). Okumura et al. (1997) found KS serum concentrations to decrease rapidly after 3 mo of age. To have an average concentration at 4 mo of age of 597.0 $\pm$ 37.5 ng/ml would seem to agree with Okumura et al. (1997) study. The paddocked group demonstrated greater KS concentrations than the exercised weanlings on d 14 while the stalled weanlings demonstrated greater KS concentrations than the exercised weanlings on d 28. These differences may be due to the paddocked and stalled groups having greater (though not significant) starting values. The paddock group demonstrated a smaller decrease to d 14 than the exercised group resulting in the paddocked group having greater serum KS concentration. Similarly, the stalled group demonstrated a smaller decrease to d 28 than the exercised group resulting in the stalled group having greater serum KS concentration.

Growth Measurements

There were no treatment differences in height at the withers, hip, or weight of the weanling horses. All groups increased which follows the normal pattern of growth. The third metacarpus circumference increased from d 0 to d 56 in the paddocked and stalled groups. The exercised group demonstrated an initial decrease from d 0 to d 28 and then an increase from d 28 to d 56. The increases in the paddocked and stalled groups may be due to normal growth resulting in larger bones. The decrease initially observed in the exercised weanlings may be due to experimental error. One would expect to see either an increase related to normal growth or no change in circumference at all due to lack of activity. The increase seen from d 28 to d 56 is related to growth of the animal and the onset of longe training which may have caused thickening of the bone and of the tendons and ligaments (Raub et al., 1989). An increase in all growth measurements was expected due to normal growth of the weanlings. The initial decrease of the cannon bone in the exercised group was unexpected but was likely due to experimental error.

CHAPTER 3

MATERIALS AND METHODS: EXPERIMENT 2

Animal Management

Eighteen Arabian weanlings from the Michigan State University Horse Teaching and Research Center were pair-matched by age and randomly assigned to three treatment groups of six horses each. Horses remained on the study for 56 d. Before the start of the study, weanlings were kept outside with their dams. Horses were weaned in three separate groups of six horses each with two horses in each treatment group. The first weaning group began the study on August 25, 1998, the second group on September 21, 1998, and the third group on October 19, 1998. The pasture group (P2, n = 6) was maintained on a 432.8 m x 53.6 m pasture allowing free access to exercise. The stall group (S2, n = 5) was housed in 3.1 m x 3.1 m stalls with no free access to exercise. The partial pasture group (PP2, n = 6) was maintained with the pasture group allowing free access to exercise for 12 h and housed in 3.1 m x 3.1 m stalls for 12 h. One horse in the S group died and resulted in a smaller treatment group. The average ages of each group, followed by the range of ages in parentheses, were P2 – 136 d (119 d to 151 d), S2 – 134 d (127 d to 143 d), and PP2 – 135 d (128 d to 149 d). Each group was individually fed .9 kg corn, .85 kg oats, and .05 kg 40% dairy protein pellets (Kent Feeds Inc., Muscatine, IA) twice daily (0700 and 1900). Stalled horses had ad libitum access to alfalfa and grass mixed hay (Tables 7 and 8). Horses on pasture had ad libitum access to a mixed grass pasture (brome, orchard, timothy and Kentucky blue grasses). Water and mineral blocks were available at all times.

Table 7. Calculated daily nutrient table for S2 weanlings on a dry matter basis

Nutrient	Nutrient Content	Calculated Total Daily Intake
DE	3.06 Mcal/kg	14.7 Mcal
CP	14.0%	670.0 g
Ca	0.27%	12.8 g
P	0.33%	15.9 g

Table 8. Calculated daily nutrient table for P2 weanlings on a dry matter basis

Nutrient	Nutrient Content	Calculated Total Daily Intake
DE	3.0 Mcal/kg	14.3 Mcal
CP	11.9%	570.0 g
Ca	0.16%	7.8 g
P	0.33%	16.0 g

Table 9. Calculated daily nutrient table for PP2 weanlings on a dry matter basis

Nutrient	Nutrient Content	Calculated Total Daily Intake
DE	3.02 Mcal/kg	14.5 Mcal
CP	12.9%	620.0 g
Ca	0.21%	10.3 g
P	0.33%	15.9 g

Bone Mineral Content

Bone mineral content was measured as reported in Experiment 1 with the exception of no lateral-medial radiographs were taken due to an insufficient number of available radiographic cassettes.

Serum Collection

Serum was collected as reported in Experiment 1. The times of sample collection for d 0, 14, 28, 42, and 56 for the first weaning group were 1700, 0730, 1430, 1300, and

1300 respectively. Sample collection for the second and third weaning groups were between 1300 and 1400.

Serum Analysis

Serum was analyzed for OC, ICTP and KS as reported in Experiment 1.

Growth Measurement

Cannon circumference, hip height, wither height and weight were taken every 28 d as described in Experiment 1.

Statistics

Data was analyzed as reported in Experiment 1. The blocking effect of weaning in three groups was included in our model. This was added to account for any differences that may have existed due to weaning time.

RESULTS: EXPERIMENT 2

Bone Mineral Content

The pastured group tended to increase from 17.4 ± .4 mm Al on d 0 to 18.4 ± .4 mm Al on d 28 in medial RBAE (P = .06) (Figure 8). The partial-pastured group demonstrated an increase in medial RBAE from 17.2 ± .4 mm Al on d 0 to 18.5 ± .4 mm Al on d 56 (P = .02). In medial RBAE, the stalled group was lower than P2 (P = .003) and PP2 (P = .05) on d 28. On d 56, S2 was lower than PP2 in medial RBAE (P = .01).

The lateral RBAE of pastured weanlings increased from 15.7 ± .4 mm Al on d 0 to 16.8 ± .4 mm Al on d 28 (P = .05) and continued to increase to 17.8 ± .4 mm Al on d 56 (P = .001) (Figure 9). The lateral RBAE of the partial-pastured group was lower than the pastured group on d 56 (P = .02). Partial-pastured weanlings tended to have greater lateral RBAE than the stalled weanlings on d 28 (P = .08). Stalled weanlings had a lower lateral RBAE than pastured weanlings on d 28 (P = .005) and d 56 (P = .007).

In total (DP) RBAE (Figure 10), pastured weanlings increased from 150 ± 20 mm^2 Al on d 0 to 208 ± 20 mm^2 Al on d 56 (P = .05) and tended to be greater than the stalled weanlings on d 28 (P = .08) (Figure 10). The partial-pastured weanlings tended to increase in total (DP) RBAE from 145 ± 20 mm^2 Al on d 0 to 196 ± 20 mm^2 Al on d 56 (P = .08) and was greater than the stalled weanlings on d 28 (P = .05). The stalled group increased from 136 ± 22 mm^2 Al on d 0 to 204 ± 22 mm^2 Al on d 56 in total (DP) RBAE (P = .04).

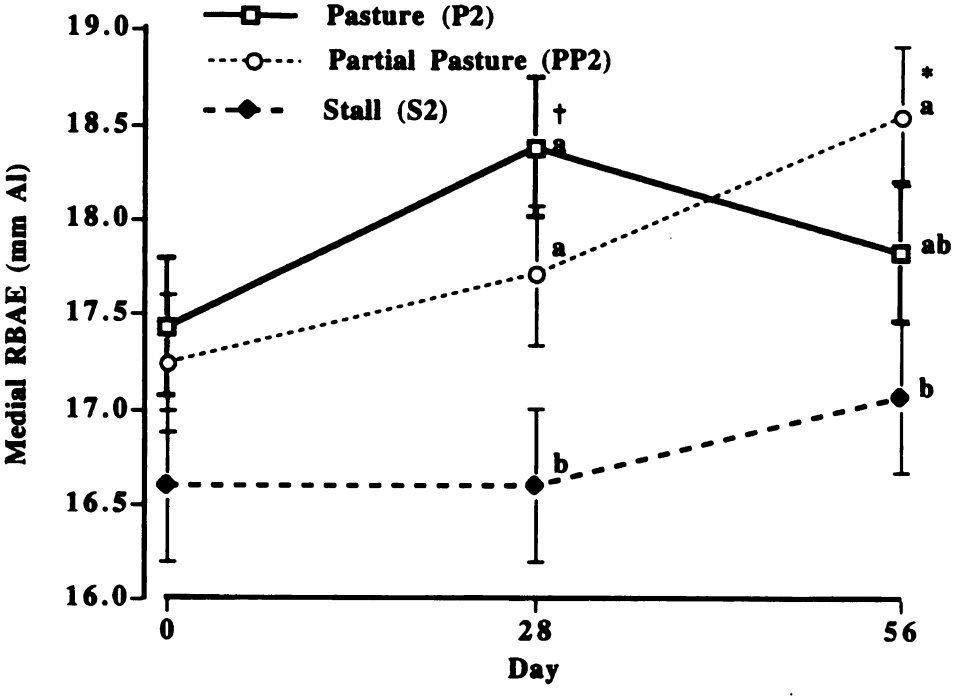

Figure 8. Medial RBAE (mm AI) versus day of project in weanling Arabians

*different from d 0 ($P \leq .05$)

†different from d 0 ($P \leq .1$)

a,b treatments with different superscripts at a given day differ ($P \leq .05$)

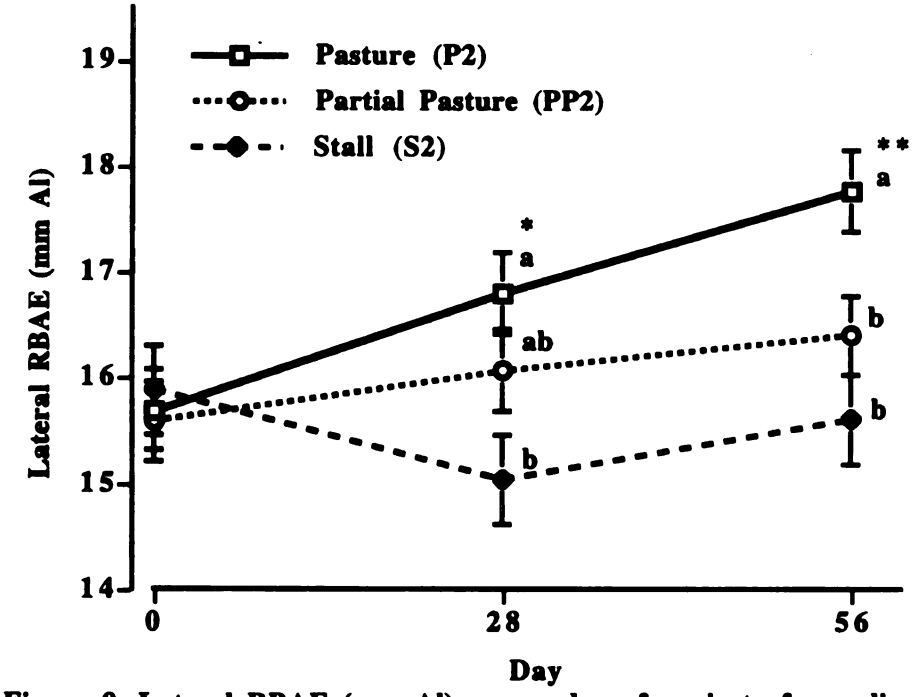

Figure 9. Lateral RBAE (mm AI) versus day of project of weanling Arabians

*different from d 0 ($P \leq .05$)

**different from d 0 ($P \leq .01$)

a,b treatments with different superscripts at a given day differ ($P \leq .05$)

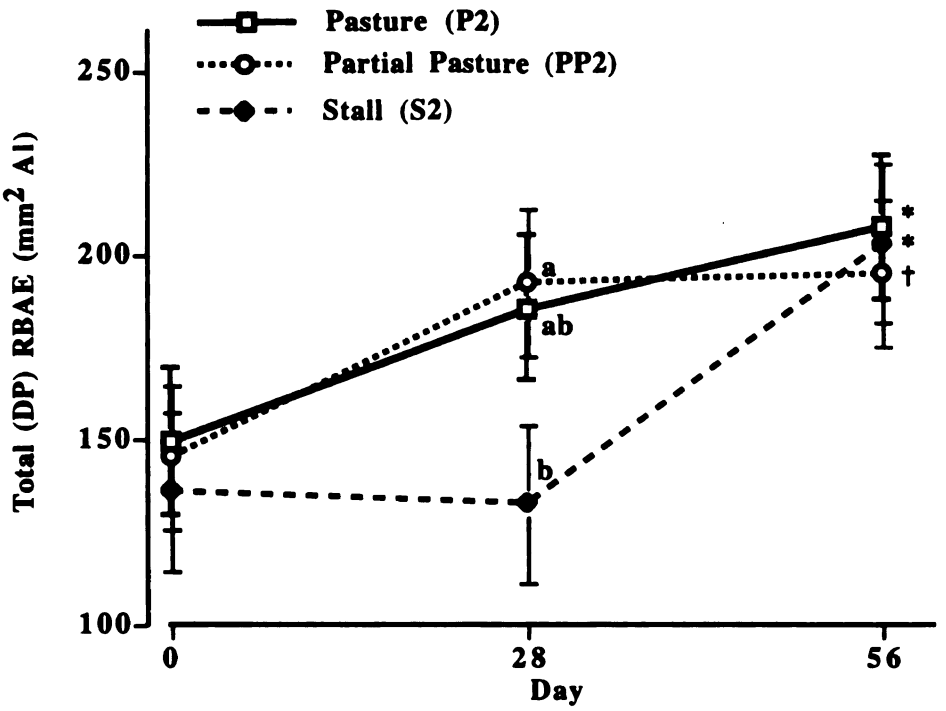

Figure 10. Total (DP) RBAE (mm^2 Al) versus day of project in weanling Arabians

*different from d 0 ($P \leq .05$)

†different from d 0 ($P \leq .1$)

a,btreaments with different superscripts at a given day differ ($P \leq .05$)

Osteocalcin

Due to differences in serum OC on d 0 (Table 10), the data were normalized (Table 18A) by subtracting all days from d 0. Pastured weanlings decreased by 46.6 ng/ml from d 0 to d 42 (P = .006) and continued to decrease to d 56 (P = .06). Partial-pastured weanlings also demonstrated a decrease of 66.0 ng/ml from d 0 to d 56 (P = .01). The stalled group tended to decrease by 34.7 ng/ml from d 0 to d 56 (P = .06). On d 42, stalled weanlings had a greater serum OC concentration than partial-pastured weanlings (P = .05).

Table 10. Means table for osteocalcin (ng/ml) in Arabian weanlings

	Day					
Treatment	0	14	28	42	56	SEM
Pasture	120.6^{ax}	114.7^{ac}	108.6^{ac}	74.1^{bx}	$88.5a^{bc}$	11.6
Partial Pasture	154.5^{ay}	113.7^{b}	106.0^{b}	89.4^{bx}	112.7^{b}	11.6
Stall	144.5^{axy}	124.1^{a}	132.5^{a}	126.4^{ay}	109.4^{a}	12.8

^{abc}days with different superscripts within rows differ ($P \leq .05$)
^{xy}treatments with different superscripts within columns differ ($P \leq .05$)

ICTP

All treatment groups decreased in serum ICTP from d 0 to d 56 with no treatment differences shown (Table 11). The pastured group demonstrated a decrease in serum ICTP from 20 ng/ml on d 0 to 17 ng/ml on d 14 (P = .006) remained low until d 28 (P = .04) and experienced a further decrease to 14 ng/ml on d 42. On d 56, the ICTP concentration of the pastured group was 16 ng/ml (P = .003). Partial-pastured weanlings had an initial ICTP concentration of 19 ng/ml and decreased to 16 ng/ml on d 14 (P = .006). Concentrations continued to decrease to 16 ng/ml on d 42 with final ICTP concentrations of 14 ng/ml (P = .003). The stalled weanlings demonstrated a similar decrease in ICTP concentrations from 19 ng/ml on d 0 to 14 ng/ml on d 42 (P = .0001) and 15 ng/ml on d 56 (P = .002).

Table 11. Means table for ICTP (ng/ml) in Arabian weanlings

	Day					
Treatment	0	14	28	42	56	SEM
Pasture	19.9^{a}	17.0^{b}	17.5^{b}	14.4^{c}	16.1^{bc}	0.9
Partial Pasture	19.1^{a}	16.4^{b}	16.0^{b}	16.3^{b}	14.8^{b}	0.8
Stall	19.0^{a}	17.1^{bd}	17.9^{ab}	14.4^{c}	15.4^{cd}	0.8

^{abcd}days with different superscripts within rows differ ($P \leq .05$)

Keratan Sulfate

There were no treatment differences in KS concentration (Table 12). The serum KS concentration of the pastured group decreased from 537 ng/ml on d 0 to 465 ng/ml on d 28 ($P = .04$), with a continued decrease to 460 ng/ml on d 42 ($P = .05$). Stalled weanlings demonstrated a decrease from 497 ng/ml on d 14 to 434 ng/ml on d 56 ($P = .05$). There was no change in the serum concentration of the partial-pastured weanlings.

Table 12. Means table for keratan sulfate (ng/ml) in Arabian weanlings

	Day					
Treatment	0	14	28	42	56	SEM
Pasture	536.6^{a}	537.2^{a}	465.2^{b}	460.4^{b}	491.1^{ab}	25.9
Partial Pasture	495.6^{a}	507.8^{a}	492.9^{a}	501.5^{a}	474.8^{a}	23.6
Stall	486.0^{ab}	497.4^{a}	490.1^{ab}	460.2^{ab}	434.2^{b}	23.6

^{ab}days with different superscripts within rows differ ($P \leq .05$)

Growth Measurements

Initial height at the withers for P2, PP2 and S2 were 119.9 ± 1.2 cm, 119.7 ± 1.2 cm and 119.2 ± 1.4 cm respectively. All groups increased from d 0 to d 56 ($P \leq .01$) with no differences between groups. Final wither heights were 125.1 ± 1.3 cm for P2, 124.9 ± 1.2 cm for PP2, and 125.0 ± 1.4 cm for S2 (Table 19A). On d 0, pastured weanlings (127.0 ± 1.1 cm) tended to be taller at the hip than stalled weanlings (123.8 ± 1.2 cm) ($P = .06$). The partial-pastured group had an initial hip height of 124.7 ± 1.1 cm. All groups increased from d 0 to d 56 ($P \leq .001$). Final heights at the hip were 131.8 ± 1.2 cm for P2, 132.0 ± 1.1 cm for PP2 and 129.5 ± 1.2 for S2 (Table 20A). All treatments increased in weight from d 0 to d 56 ($P \leq .01$) and demonstrated no differences between groups. Initial weights were 187 ± 7 kg for P2, 195 ± 7 kg for PP2, and 188 ± 8 kg for S2. Final weights for P2, PP2, and S2 were 235 ± 8 kg, 228 ± 7 kg, and 238 ± 8 kg respectively

(Table 21A). Partial-pastured weanlings were greater than stalled weanlings in cannon circumference (P = .03). Pastured weanlings tended to have greater cannon circumference than stalled weanlings ($P \leq .07$). In cannon circumference, both P2 and PP2 increased from d 0 to d 56 (P = .0001) with no change in S2. The initial and final circumference of P2 were 15.2 ± .2 cm on d 0 to 16.6 ± .2 cm on d 56. The cannon circumference for PP2 on d 0 was 15.5 ± .2 cm and 16.5 ± .2 cm on d 56. The stalled group had an initial circumference of 15.3 ± .2 cm with a final circumference of 15.6 ± .2 cm. Both P2 and PP2 had larger cannon circumferences than S2 on d 28 ($P \leq .05$) and d 56 (P = .004) (Table 22A).

DISCUSSION: EXPERIMENT 2

Bone Mineral Content

In the present study, the pastured group increased in bone mineral content in both lateral and total (DP) RBAE and tended to increase in medial RBAE. The stalled group remained lower than the pastured group in the medial and lateral views and tended to be lower in total (DP) RBAE. These data correspond with a study looking at the influence of housing in yearling horses (Hoekstra et al., 1998). Hoekstra et al. (1998) normalized the lateral and medial RBAE data due to differences on d 0 but found that the pastured horses had greater bone mineral content than the stalled horses on d 28 and d 56 in both cortices. Hoekstra et al. (1998) found no change in total RBAE.

A partial pasture group was used to begin to elicit how much time on pasture was necessary to prevent the disuse osteoporosis seen by Hoekstra et al. (1998). The present study indicates that the partial-pasture horses increased in medial and total RBAE. The stalled group had lower medial RBAE and total RBAE than the partial-pastured group and tended to be lower in lateral RBAE. This demonstrates the benefits of 12-h daily turn-out on bone strength. The results seen in the partial-pasture group are similar to the pastured group.

The data in the present study concur with studies indicating that allowing an animal room to move (i.e. placing an animal in a larger cage or area) causes an increase in bone strength. Knowles and Broom (1990) found that restricting the amount of movement in laying hens was detrimental to bone breaking strength. When the hens were housed in percherys versus battery cages or the Elson terrace system, more

movement was allowed and hence those birds housed in percherys had stronger bones. In fact, birds housed in battery cages demonstrated 54% less strength than their counterparts in percherys. A similar study by Fleming et al. (1994) found that laying hens housed in percherys or Naturel housing systems allowed more wing movement, which in turn caused an increase in bone strength.

The increases in the pastured and partial-pastured group and the differences found between both groups and the stalled group in this study are supported by numerous studies looking at the effects of exercise or immobilization on the equine third metacarpus. McCarthy and Jeffcott (1992) found an increase in bone mineral content of exercised Thoroughbred yearlings. By wk 12, exercised horses had greater bone mineral content than the non-exercised horses. This increase in the exercised horses was determined to be due to the horse exercising to its maximum potential (galloped for up to 1.2 km or until exhaustion). Both groups in the McCarthy and Jeffcott (1992) study were relatively inactive for 2 mo before the start of the study. This was suggested to be enough time for the horses to reach a steady state of bone remodeling so all horses started the study at the same baseline. In the present study, when the partial-pastured horses were brought out to the pasture every morning, it was very rare that they did not sprint off to join the other weanlings and once all the horses were together they would often run around chasing one another. This was probably enough loading on the bone to initiate increases in bone mineral content. This parallels Jeffcott's (1991) suggestion that growing horses should be pastured for 12 h/d to develop normal bones and joints. Pratt (1982) states that the most efficient way to increase bone strength is to run short vigorous sprints, hence it is likely that even less than 12-h of turn-out per day may be adequate.

The stalled group became very active in their stalls after d 28 of the study. Their activity included jumping, bucking, and rearing as well as running around in small circles. This increase in activity may explain the increase of the stalled group from d 0 to d 56 in total (DP) RBAE. This activity may have provided the third metacarpus enough strain to increase the mineral content. The increase in bone mineral content may not have been great enough to see in the lateral and medial views separately, but combined were great enough to detect in the total (DP) RBAE. In the medial and lateral cortices, the stalled group demonstrated no changes with time though remained lower than the pasture group.

The only concern as to whether 12-h of turn-out is as good as continuous turn-out was that partial-pastured weanlings demonstrated lower lateral RBAE than pastured weanlings on d 56. The partial-pastured weanlings demonstrated no increase in lateral RBAE which may account for the difference with pastured weanlings in lateral RBAE on d 56. No increase may have been seen based on the amount of compression the lateral cortex received. Each cortex will receive differing amounts of compression depending on how the animal moves and where most of the force is placed, as previously discussed in Experiment 1. The increases seen in total RBAE are consistent with other studies indicating an increase in RBAE in growing horses (Nielsen et al., 1997; Frey et al., 1992; Meakim et al., 1981). Nielsen et al. (1997) found an initial decrease in lateral and medial RBAE in 18 mo old horses entering race training. This decrease may be due to only working the animals at slower gaits in order to get them broke to ride. But, after speed was placed on theses horses, lateral and medial RBAE increased. This is consistent with the present study indicating that the lateral and medial cortices were greater in the

pastured or partial-pastured weanlings versus the stalled weanlings. This was probably due to the applied forces on the animals legs when out in the pasture running around and playing with each other.

Serum Analysis

Osteocalcin

All treatment groups decreased in OC serum concentration. But, on d 42 the stalled weanlings demonstrated a greater OC concentration than the partial-pastured weanlings. The greater concentration may be explained by the increase in activity of the stalled weanlings after d 28. The decrease with age seen in this study corresponds with other studies demonstrating the OC serum concentrations decrease with age in equine (Hope et al., 1993; Lepage et al., 1991; Lepage et al., 1990). Lepage et al. (1990) reported the OC serum concentration of eight horses, under the age of 1 yr old (4 mo to 1 yr), to be 47.3 ± 10.1 ng/ml and found that it decreased in nine horses, between 1.5 and 2.5 yr of age, to 35.7 ± 14.2 ng/ml. These values are clearly lower than the values reported in the present study of 74.1 ± 11.2 ng/ml to 154.5 ± 11.2 ng/ml. The lower concentrations reported in the present study were from horses ranging in age from 119 d to 151 d (4 mo to 5 mo) and ended with them being 179 d to 211 d old (6 mo to 7 mo). This range of ages is smaller than the range in the Lepage et al. (1990) study. This decrease in bone formation corresponds with the decrease in modeling and remodeling with increasing age (Fraher, 1993; Maenpaa et al., 1988). Osteocalcin has been shown to fluctuate with time of day. Our samples were collected around 1400 on all sample

days (with exception to the first two days of the first weaning group). Lepage et al. (1991) found that OC serum concentration levels remained constant from 0700 to 1900.

ICTP

All treatment groups decreased in ICTP serum concentration. This corresponds with numerous studies indicating that ICTP concentration decreases with age in equine (Price et al., 1995a, 1995b), canine (Allen et al., 1998), and humans (Crofton et al., 1997; Zanze et al., 1997). Price et al. (1995a) reported ICTP serum concentrations of 15 female Thoroughbred horses less than 1 yr to be 13.7 to 26.7 µg/l. In the present study our ICTP serum concentrations of 14.4 ng/ml to 19.9 ng/ml fall within the range Price at al. (1995a) reported. Price et al. (1995a) reported the ICTP serum concentrations decreased between 7.9 to 22.8 µg/l in 15 horses ranging in age from 1 to 2 yr of age.

Keratan Sulfate

Stalled and pastured weanlings decreased in KS serum concentration. This decrease parallels other studies that demonstrate decreases in KS concentration with increasing age (Todhunter et al., 1997; Okumura et al., 1997; Roughley and Lee, 1994; Spector et al., 1992; Nixon, 1991; Sweet et al., 1990; Leipold et al., 1989). Okumura et al. (1997) reported KS concentrations, in 15 foals, to be approximately 4000 ng/ml at 3 mo of age and decreasing to 500 ng/ml at 6 mo of age. The present study reports KS concentration values to range from 434.2 ± 24.4 ng/ml to 537.2 ± 27.0 ng/ml, in the range of Okumura et al. (1997). Okumura et al. (1997) found KS serum concentrations to decrease rapidly after 3 mo of age. The average concentration at 4 mo of age of 506.1 ±

25, in the present study, is in the correct range when compared to the Okumura et al. (1997) study. The failure of the partial-pastured group to decrease with age seems to have no explanation .

Growth Measurements

Height at the withers, hip, and weight increased in all treatment groups from d 0 to d 56. There was an overall treatment difference between the pastured weanlings and the stalled weanlings on d 0 in hip height. For cannon bone circumference, the pastured and partial-pastured weanlings increased from d 0 to d 56 with no change in the stalled weanlings. The pastured and partial-pastured horses also demonstrated larger cannon circumferences than the stalled horses on d 28. Sherman et al. (1995) measured the breaking strength and cortical cross-sectional area of the third metacarpal bone in 24 Thoroughbreds (2 to 4 yr of age) with various training backgrounds. Horses with the more extensive training background had greater peripheral thickening and greater area moment of inertia. By looking at the correlation between area moment of inertia and failure load, Sherman et al. (1995) found that this increase in size corresponds with an increase in strength. Woo et al. (1981) agrees with Sherman et al. (1995) that the increase in femoral cross-sectional area of the exercised swine compared to the non-exercised group is directly related to bone strength. The increase of the pastured and partial-pastured weanlings in the present study may correspond with the increase in bone mineral content seen in the radiographs and hence is associated with an increase in bone strength (Fleming et al., 1994; El Shorafa et al., 1979).

There were nutritional differences in the present study where the stalled weanlings had access to hay while the pastured weanlings had access to the pasture grasses and the partial pastured weanlings had access to both hay and pasture grasses. The nutrition tables indicated inverse Ca to P ratio but no detrimental effects of this inverted ratio were observed. It is not believed that nutrition had a part in the results observed. Hoekstra (1998) performed a similar study to the present study, but the study took place during the winter months while Experiment 2 took place from late summer to early winter. During the winter months, there was no grass in the pasture so all horses had ad libitum access to alfalfa-grass hay and were fed the same amount of concentrate (Hoekstra, 1998). The results observed in Hoekstra's (1998) study were very similar to those in the present study.

CHAPTER 4

DISCUSSION: EXPERIMENTS 1 & 2

There were no breed differences demonstrated in the present study. Numerous studies have identified the effects of exercise or lack of exercise on bone mineral content or bone strength in different breeds of horses (McCarthy and Jeffcott, 1992; Hoekstra et al., 1998; Nielsen et al., 1998). Though the methods of exercise differed in these studies the results remained the same -- increase in availability to exercise through pasturing causes an increase in bone mineral content. In the present study, the paddocked group of Experiment 1 and the pastured group of Experiment 2 both demonstrated increases in bone mineral content. The exercised group and the partial-pastured group are not able to be compared because of the vast number of differences between the two.

Though not scientifically proven, the increase in total RBAE of the stalled group of Experiment 2 is related to the amount of activity of the stalled weanlings. Though these weanlings were not exercised, they were quite active in their stalls. In Experiment 1, stalled weanlings had no significant increase in bone mineral content. Differences in housing conditions may explain why S2 were more active than S1. In Experiment 1, the stalls had walls that allowed no interaction with other horses. The walls were from the floor to the ceiling with only a small window looking outside and a small window allowing the horses to interact vocally across the barn aisle. This design does not allow for much interaction while the stalls of Experiment 2 were open on three sides allowing both vocal and physical interactions. The barn of Experiment 2 was a very busy barn with a lot of activity. Numerous classes were held in the barn and during feeding times

students would groom the weanlings. All of the activity in the barn caused the weanlings to become excited and run or jump around. The barn in Experiment 1 was not as active besides the running of the broom vacuum during the mornings and cleaning of the stalls.

Yeh et al. (1993) found that rats whose legs were immobilized demonstrated a decrease in bone formation for the first 31 d of the study and then stabilized until the end of the study on d 41. This stabilization may have occurred from the rats adapting to the decrease in load. In contrast, the exercised rats demonstrated an increase in bone formation of the cortical bone. Yeh et al. (1993) related his results to be in agreement with Frost's (1987) mechanostat theory (Figure 11). This theory is based on the mechanical usage of the bone determining its bone mass. Frost explains the mechanostat as acting like a home thermostat that activates in response to an error and turns "off" in absence of an error. The mechanostat can be growth, modeling, or remodeling. As these properties increase or decrease the mass of the bone is effected which then loops around and affects how the bone adjusts in growth, modeling, and remodeling.

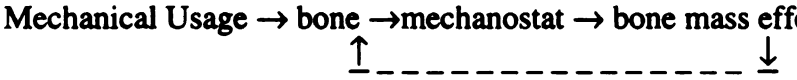

Figure 11. The Mechanostat Theory (Frost, 1987)

The present study corresponds with the mechanostat theory in that horses maintained on pasture or partial-pasture demonstrated increases in bone mineral content and hence bone strength given the correlation between bone breaking strength and the amount of structural bone present (Fleming et al., 1994). El Shorafa et al. (1979) found

a positive correlation between bone breaking strength and ash content of the third metacarpal (r^2 = .58; P< .01). The mechanical usage of the stalled weanlings has changed which caused a decrease in bone formation which decreased bone mass. Hoekstra (1998) hypothesized that once the bone has maintained a bone mass that has adapted to the optimal strain placed on it, the bone will stop increasing in bone mass and reach a steady state.

The bone and cartilage serum markers indicate changes of the entire skeleton which may have masked any specific changes that were occurring in the left third metacarpal bone and its joints. Any treatment differences that were seen may have been due to changes occurring in other parts of the body and not necessarily changes in the third metacarpus as a response to housing. During the growth phase of young horses, modeling and remodeling occur at a very high rate allowing for appositional growth and strengthening of long bones with the rate of formation exceeding the rate of resorption (Maenpaa et al., 1988; Fraher, 1993). This increased bone turn-over is occurring throughout the skeleton and is not known how it is affected by environmental changes such as stalling. Nunamaker et al. (1990) believes that any alterations to this growth phase may have a large impact on bone strength later in life. Cartilage turnover is also occurring at a faster rate in young animals than in older animals (Leipold et al., 1989). In 8 to 10 mo old dogs predisposed to OA (dogs from a genetic line with a high incidence of hip dysplasia), no change in KS or HA serum concentrations were seen (Leipold et al., 1989). That study concluded that KS and HA were poor markers of indicating predisposition to OA but it may have been due to the age of the dogs masking any changes that may have been occurring. The serum markers were found to be

inconclusive but mirror the results of a previous study indicating that bone turnover decreases with age (Price et al., 1995). This was indicated by the decrease in both OC and ICTP. The decrease in KS is also similar to a previous study that found KS decreases very rapidly between 3 to 5 mo of age in horses (Okumura et al., 1997). Synovial fluid KS is more specific for the fetlock joint and the carpal joint of the third metacarpus but is an invasive technique (Campion et al., 1991).

As we did not know how variable the serum measurements would be in the weanling horse, we can use this study to determine the relative power. As power of a test determines the probability of correctly rejecting the null hypothesis when the hypothesis is not correct, a low power study often fails to find differences between treatments when one actually exists. After completing this study and having a better indication of the variability of the serum measurements in weanlings, we found that our power ranges from a high of 91% to a low of 5%. Hence, the failure to find treatment differences, for instance in some of the serum markers, is due to a relatively low number of animals. Thus, we conclude that because of large overall changes in the entire skeletal system of the weanling during growth, the likelihood of detecting a difference due to our imposed treatments would require an unrealistic number of animals for an equine study.

CHAPTER 5

CONCLUSIONS

An increase in mineral content of the third metacarpal of the pastured and paddocked groups in both experiments suggests an increased strengthening of bone. A decrease in bone mineral content of the third metacarpal bone or a lack of change in the stalled weanlings in both experiments suggests a weakening of the third metacarpal bone or prevention of maximal bone mineral deposition, respectively (Fleming et al., 1994; El Shorafa et al., 1979). The increase in RBAE is likely due to exercise while the decrease in RBAE is most likely due to the lack of exercise and loading on the bones of the stalled horses. These studies are a practical example of Wolff's Law which states that as the function of a bone changes, the architecture of that bone must change to adapt to this new function (Woo et al., 1981).

Maintaining weanlings on pasture for 12 h/d in Experiment 2 proved to be just as effective in increasing bone mineral content in horses pastured continuously. Pasture-rearing may not be very feasible for many horse owners because of feeding and aesthetic reasons. Differences between the handling of the longeing groups in Experiment 1, prevent us from drawing conclusions about the positive or negative effects of longeing at the trot and canter or walking on the mechanical walker. However, Raub et al. (1989) found that weanlings worked on the mechanical walker at the trot 5 d/wk demonstrated an increase in medial bone mineral content. Longe training at the trot or canter or working on the mechanical walker at the trot would likely be more advantageous to bone strength than stalling with no exercise. Though the exercise received on the longe line or

mechanical walker may not be sufficient to increase bone mineral content it may be enough to prevent disuse osteoporosis and maintain bone mineral content.

Thoroughbreds do not train at racing speeds which predispose the horse to an increase risk of injury once racing begins due to the animals legs not being sufficient to withstand the increase in strain (Nunamaker et al., 1990). Maintaining a horse in stalls preventing strains necessary for racing will lead to an increase of injury once speed is placed on the leg (Nielsen et al., 1997). In order to prevent this, a training regimen that includes a slow progression of speed work may be beneficial to increase the strength of the bones so eventually they will be capable of withstanding strains associated with racing.

Photometric radiography is an accurate measure of bone mineral content which correlates with bone strength (Fleming et al., 1994; El Shorafa et al., 1979). The measurements were specific for the third metacarpal and allowed conclusions to be made regarding bone mineral content without dependency on the serum markers. We are confident that pasture- or paddock-rearing horses is beneficial for increasing bone mineral content based on similar results in previous studies. Though pasture- and paddock-rearing horses responded similarly in bone mineral content, pasture-rearing elicited more consistent responses in increases in bone mineral content. The larger pasture allows for more area to run at fast sprints while a smaller paddock may be too small to really allow the ability to pick up a fast sprint. When raising horses in a paddock or a pasture, the size must be considered. Enough room must be allowed for the horses to run at fast sprints.

We conclude that stalling weanlings without exercise prevents maximal mineral deposition in the third metacarpal. This may prove detrimental to some athletic horses when they enter training later in life. Stalling horses while "sale prepping" is a common occurrence in the horse industry. Horses are often stalled for 3 to 4 mo before a sale to improve hair coat and prevent injuries. This "prepping" likely yields horses with weaker bones that are more prone to injury once training commences. The results of the present study indicate that if "sale prepping" is necessary, 12-h daily turn-out or perhaps only a few hours outside allowing for the horse to sprint are enough to prevent decreases in bone mineral content. Exercise is particularly important during the skeletal maturation stage so that once skeletal maturation is complete, the bone is strong enough to withstand increases in strain. For example, if a horse is raised with only access to walking (no fast sprints), the bone will mature capable of withstanding the strains of standing and walking. Once speed work is introduced, the bone will not be strong enough to withstand the increase in strain associated with training and injury will occur.

The next research to be performed should be to determine how much time is necessary outside to prevent disuse osteoporosis. The present study indicates 12 h is plenty but this may not be feasible for many horse producers. Because this study determined the influence of housing on bone mass and cartilage markers, it has not been determined what exactly causes the increase in bone strength seen in the pastured/paddocked horses. Though it is thought to be growth and exercise that facilitate this increase and a lack of exercise that causes a decrease, a more controlled study would need to be done to determine why a decrease or lack of growth is seen in stalled horses.

A more controlled study would be one that allowed the stalling of horses outside so that all horses experience the same daylight, weather conditions, and feeding conditions.

Pratt (1982) states that the most efficient way to develop bone mass and hence bone strength is to participate in short vigorous activities (i.e. sprints). The combination of this study with the results indicated in the present study implies that it should only take a minimal amount of strides at a sprint to prevent disuse osteoporosis in the horse. Therefore, we have concluded that disuse osteoporosis can be prevented by 12-h daily turn-out and quite possibly less.

APPENDIX A

APPENDIX A

Means Tables

Table 1A. Means table for medial RBAE (mm Al) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	17.2	16.6	18.3	0.6
Exercise	17.6	16.9	17.8*	0.6
Stall	17.1	15.6	16.4	0.6

*SEM = 0.7

Table 2A. Means table for lateral RBAE (mm Al) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	16.6	16.4	17.2	0.7
Exercise	16.5	16.6	16.9	0.7
Stall	17.4	16.2	16.9	0.7

Table 3A. Means table for palmar RBAE (mm Al) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	14.5	14.7	14.1	0.7
Exercise	15.1	14.4	14.6*	0.7
Stall	15.2	14.4	13.6	0.7

*SEM = 0.8

Table 4A. Means table for dorsal RBAE (mm Al) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	16.1	16.5	15.9	0.6
Exercise	17.5	16.2	16.7	0.6
Stall	16.5	16.0	15.3	0.6

Table 5A. Means table for change in dorsal RBAE (mm Al) in Quarter Horse weanlings

	Day		
Treatment	28	56	SEM
Paddock	0.4	-0.2	0.7
Exercise	-2.1	-0.7	0.7
Stall	-0.5	-1.2	0.7

Table 6A. Means table for total (DP) RBAE (mm2 Al) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	168	229	255	42
Exercise	287	270	221*	42
Stall	232	220	211	42

*SEM = 45

Table 7A. Means table for change in total (DP) RBAE (mm2 Al) in Quarter Horse weanlings

	Day		
Treatment	28	56	SEM
Paddock	60.6	79.8	43.8
Exercise	-16.8	-68.7*	43.8
Stall	-31.3	-20.6	43.8

*SEM = 45.3

Table 8A. Means table for total (LM) RBAE (mm2 Al) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	84	140	99	21
Exercise	188	133	83*	21
Stall	117	104	67	21

*SEM = 23

Table 9A. Means table for change in total (LM) RBAE (mm2 Al) in Quarter Horse weanlings

	Day		
Treatment	28	56	SEM
Paddock	56.6	18.8	28.7
Exercise	-54.6	-103.9*	28.7
Stall	16.5	-49.5	28.7

*SEM = 30.7

Table 10A. Means table for change in ICTP (ng/ml) in Quarter Horse weanlings

	Day				
Treatment	14	28	42	56	SEM
Paddock	-2.6	-1.3	-3.0	-3.1	1.5
Exercise	-1.9	-4.7	-2.9*	-4.3*	1.5
Stall	-5.3	-6.6	-2.6	-1.5	1.5

*SEM = 1.6

Table 11A. Means table for height at the withers (cm) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	123.5	125.3	128.5	1.4
Exercise	125.3	125.2	128.5*	1.4
Stall	123.3	125.2	128.5	1.4

*SEM = 1.5

Table 12A. Means table for height at the hips (cm) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	129.3	132.1	133.6	1.7
Exercise	131.4	132.1	135.0*	1.7
Stall	130.1	131.4	135.3	1.7

*SEM = 1.8

Table 13A. Means table for weight (kg) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	233	236	265	14
Exercise	237	232	250	14
Stall	221	224	257	14

Table 14A. Means table for third metacarpal circumference (cm) in Quarter Horse weanlings

	Day			
Treatment	0	28	56	SEM
Paddock	15.7	15.5	16.4	0.3
Exercise	16.0	15.7	16.2	0.3
Stall	15.5	15.4	15.9	0.3

Table 15A. Means table for medial RBAE (mm Al) in Arabian weanlings

	Day			
Treatment	0	28	56	SEM
Pasture	17.4	18.4	17.8	0.4
Partial Pasture	17.2	17.7	18.5	0.4
Stall	16.4	16.6	17.1	0.4

Table 16A. Means table for lateral RBAE (mm Al) in Arabian weanlings

	Day			
Treatment	0	28	56	SEM
Pasture	15.7	16.8	17.8	0.4
Partial Pasture	15.6	16.1	16.4	0.4
Stall	15.9	15	15.6	0.4

Table 17A. Means table for total (DP) RBAE (mm2 Al) in Arabian weanlings

	Day			
Treatment	0	28	56	SEM
Pasture	149	186	208	20
Partial Pasture	145	193	196	20
Stall	136	133	204	22

Table 18A. Means table for change in OC (ng/ml) of Arabian weanlings

	Day				
Treatment	14	28	42	56	SEM
Pasture	-6.0	-12.1	-46.6	-32.1	19.2
Partial Pasture	-40.8	-48.5	-65.1	-41.7	19.2
Stall	-13.1	-5.8	-18.0	-30.1	22.1

Table 19A. Means table for height at the withers (cm) in Arabian weanlings

	Day			
Treatment	0	28	56	SEM
Pasture	119.9	122.1	125.1*	1.2
Partial Pasture	119.7	124.4	124.9	1.2
Stall	119.2	122.9	125.1	1.4

*SEM = 1.3

Table 20A. Means table for height at the hip (cm) in Arabian weanlings

	Day			
Treatment	0	28	56	SEM
Pasture	127.0	129.7	131.8*	1.1
Partial Pasture	124.7	127.5	132.0	1.1
Stall	123.8	127.1	129.5	1.2

*SEM = 1.2

Table 21A. Means table for weight (kg) in Arabian weanlings

	Day			
Treatment	0	28	56	SEM
Pasture	186	205	235*	7
Partial Pasture	195	209	228	7
Stall	188	208	238	8

*SEM = 8

Table 22A. Means table for third metacarpal circumference (cm) in Arabian weanlings

	Day			
Treatment	0	28	56	SEM
Pasture	15.2	15.9	16.6	0.2
Partial Pasture	15.5	15.9	16.5	0.2
Stall	15.3	15.2	15.6	0.2

APPENDIX B

APPENDIX B

ANOVA Tables

Table 1B. ANOVA table for medial RBAE (mm Al) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	14	1.62	0.2326
Day	2	29	4.72	0.0169
Day*Treatment	4	29	0.56	0.6917
Block	1	14	0.14	0.7098

Table 2B. ANOVA table for lateral RBAE (mm Al) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	14	0.04	0.9637
Day	2	29	0.55	0.5854
Day*Treatment	4	29	0.30	0.8752
Block	1	14	0.08	0.7768

Table 3B. ANOVA table for palmar RBAE (mm Al) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	14	0.10	0.9091
Day	2	29	1.20	0.3170
Day*Treatment	4	29	0.46	0.7612
Block	1	14	0.55	0.4695

Table 4B. ANOVA table for dorsal RBAE (mm Al) in Quarter Horse weanling

	NDF	DDF	F-Value	P-Value
Treatment	2	14	1.10	0.3596
Day	2	29	1.42	0.2588
Day*Treatment	4	29	1.06	0.3955
Block	1	14	1.68	0.2160

Table 5B. ANOVA table for total (DP) RBAE (mm2 Al) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	14	0.53	0.6002
Day	2	29	0.14	0.8731
Day*Treatment	4	29	1.11	0.3717
Block	1	14	0.18	0.6810

Table 6B. ANOVA table for total (LM) RBAE (mm2 Al) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	14	2.09	0.1611
Day	2	29	4.83	0.0154
Day*Treatment	4	29	2.86	0.0412
Block	1	14	0.52	0.4843

Table 7B. ANOVA table for Osteocalcin (ng/ml) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	1.53	0.2555
Day	4	54	3.12	0.022
Day*Treatment	8	54	1.04	0.4163
Block	1	12	0.18	0.6791

Table 8B. ANOVA table for ICTP (ng/ml) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	9.02	0.0041
Day	4	54	8.19	0.0001
Day*Treatment	8	54	2.05	0.0579
Block	1	12	38.69	0.0001

Table 9B. ANOVA table for Keratan Sulfate (ng/ml) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	1.42	0.2803
Day	4	54	14.90	0.0001
Day*Treatment	8	54	2.78	0.0118
Block	1	12	0.26	0.6224

Table 10B. ANOVA table for height at the withers (cm) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	14	0.08	0.9260
Day	2	29	25.92	0.0001
Day*Treatment	4	29	0.99	0.4307
Block	1	14	4.75	0.0468

Table 11B. ANOVA table for height at the hip (cm) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	14	0.16	0.8555
Day	2	29	10.71	0.0003
Day*Treatment	4	29	0.77	0.5529
Block	1	14	5.67	0.0320

Table 12B. ANOVA table for weight (kg) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	14	0.16	0.8500
Day	2	29	21.92	0.0001
Day*Treatment	4	29	0.80	0.5334
Block	1	14	2.38	0.1454

Table 13B. ANOVA table for third metacarpal circumference (cm) in Quarter Horse weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	14	0.42	0.6621
Day	2	29	37.24	0.0001
Day*Treatment	4	29	1.84	0.1477
Block	1	14	4.26	0.0580

Table 14B. ANOVA table for medial RBAE (mm Al) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	7.90	0.0065
Day	2	28	3.55	0.0423
Day*Treatment	4	28	1.14	0.3576
Block	2	12	2.98	0.0888

Table 15B. ANOVA table for lateral RBAE (mm Al) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	7.29	0.0085
Day	2	28	3.85	0.0332
Day*Treatment	4	28	2.55	0.0613
Block	2	12	0.35	0.7103

Table 16B. ANOVA table for total (DP) RBAE (mm2 Al) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	1.23	0.3256
Day	2	28	6.26	0.0057
Day*Treatment	4	28	0.68	0.6125
Block	2	12	0.02	0.9772

Table 17B. ANOVA table for osteocalcin (ng/ml) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	8	4.51	0.0488
Day	4	48	6.22	0.0004
Day*Treatment	8	48	1.11	0.3722
Block	2	8	3.14	0.0987

Table 18B. ANOVA table for ICTP (ng/ml) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	0.19	0.8332
Day	4	56	13.58	0.0001
Day*Treatment	8	56	1.74	0.1087
Block	2	12	0.47	0.6358

Table 19B. ANOVA table for keratan sulfate (ng/ml) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	11	0.63	0.5494
Day	4	52	1.89	0.1267
Day*Treatment	8	52	0.99	0.4571
Block	1	11	0.55	0.4757

Table 20B. ANOVA table for height at the withers (cm) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	0.13	0.8818
Day	2	25	17.40	0.0001
Day*Treatment	4	25	0.87	0.4973
Block	2	12	7.52	0.0076

Table 21B. ANOVA table for height at the hip (cm) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	1.71	0.2218
Day	2	25	36.84	0.0001
Day*Treatment	4	25	1.31	0.2929
Block	2	12	5.20	0.0236

Table 22B. ANOVA table for weight (kg) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	0.06	0.9457
Day	2	25	25.72	0.0001
Day*Treatment	4	25	0.48	0.7466
Block	2	12	0.89	0.4360

Table 23B. ANOVA table for third metacarpal circumference (cm) in Arabian weanlings

	NDF	DDF	F-Value	P-Value
Treatment	2	12	2.92	0.0928
Day	2	25	34.0	0.0001
Day*Treatment	4	25	4.70	0.0057
Block	2	12	1.37	0.2923

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