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presented by

Kristine J. Lang

has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Science

Brian D. Nielsen
Major professor

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EFFICACY OF EQUI-Si™ ON INCREASING PLASMA SILICON CONCENTRATIONS AND ALTERING BONE AND COLLAGEN METABOLISM IN HORSES

By

Kristine Josephine Lang

A THESIS

Submitted to
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ABSTRACT

EFFICACY OF EQUI-Si™ ON INCREASING PLASMA SILICON CONCENTRATIONS AND ALTERING BONE AND COLLAGEN METABOLISM IN HORSES

By

Kristine Josephine Lang

Twelve Arabian mare/foal units (Exp. 1) and 20 Arabian and Quarter Horse yearlings (Exp. 2) were used to determine if EQUI-Si™ supplementation would increase plasma and milk silicon (Si) concentrations and alter systemic markers of bone metabolism. Horses were pair-matched and randomly assigned to two groups, 2% EQUI-Si™ supplemented (S) or control (C). Samples were taken on d 0, 15, 30 and 45. Plasma and milk Si concentrations and milk calcium (Ca) and phosphorus (P) concentrations were determined. Serum was analyzed for osteocalcin (OC), carboxy-terminal pyridinoline cross-linked telopeptide region of type I collagen (ICTP), and pyridinoline and deoxypyridinoline (PYD). All S horses had higher plasma Si concentrations than C (Exp. 1 & 2), and S mares' milk (Exp. 1) had higher Si concentrations on d 45 and higher Ca and P concentrations on d 15. There were no statistical differences in OC, ICTP or PYD in mares or foals (Exp. 1). In Exp. 2, S yearlings had lower ICTP concentrations on d 45. Results of these two studies suggest that EQUI-Si™ increases plasma and milk Si concentrations, and may alter bone and collagen biochemical serum markers in horses.

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Bone-related injuries and diseases involving bone loss plague the equine industry. Methods for enhancing bone formation are continually being investigated in hopes of increasing skeletal integrity and durability. Increased bone metabolism can occur for a variety of reasons: growth/development, bone damage, hormonal stimulation (endogenous factors), and exogenous factors (diet). Increasing exercise, for example, can alter bone metabolism and physiology. However, excessive exercise can be detrimental to cartilage tissue. Therefore, nutritionally supplemental means (exogenous stimulator) of enhancing bone formation appears to be an alternative way to maximize bone strength. We invested the role of providing pharmacological concentrations of silicon as a supplemental method of increasing bone formation.

Silicon (Si) is the second most abundant element of the Earth's crust (Carlisle, 1972), and is present in everything from the air that we breathe to the sand that we walk on. It is even found in many foods that we eat every day. Not all Si is available for absorption in the gut. Sodium zeolite A is a hydrated aluminosilicate compound that breaks down into monosilicic acid in the gut, and serves as an absorbable Si source. Sodium zeolite A compounds have been supplemented in a variety of animal species for reasons that range from increasing eggshell thickness (Roland, 1988) to decreasing bone-

related injuries in horses (Nielsen et al., 1993). EQUI-Si™, which is a sodium zeolite A compound, is the source of Si chosen for the current study.

Silicon has been reported to have a role in bone physiology and metabolism, by increasing osteoblastic activity (Brady et al., 1991; Keeting et al., 1992) and decreasing osteoclastic activity *in vitro* (Schütze et al., 1995). However, limited research has been done studying the effects of supplemental Si on bone metabolism and physiology *in vivo*. While increased bone metabolism in rats supplemented with a Si source has been reported (Seaborn and Nielsen, 1994), it is not clear if increased bone metabolism due to Si has a positive or negative effect on bone histomorphometry. No studies have investigated the role of supplemental Si in altering bone metabolism in horses. Nielsen et al. (1993) found that supplemental sodium zeolite A decreased the incidence of bone-related injuries in young racing Quarter Horses; however, no determination of bone turnover was attempted in that study.

Based on the limited information about supplemental Si and bone physiology and metabolism *in vitro* and *in vivo*, supplemental Si in the horse warrants investigation. Horses are continually straining (loading) their skeletal system when performing the intense activities that comprise the current sport horse industry, and advancements in increased bone formation may prove to be advantageous in increasing the longevity of the horse as well as in decreasing bone-related injuries. If supplemental Si can enhance bone formation, advancements may be made in increasing the durability of sport horses.

Therefore, our objectives were to determine if a supplemental Si source (EQUI-Si™) could: 1) increase plasma Si concentrations in mares and yearlings, 2) increase milk Si concentrations in mares, thereby increasing plasma Si concentrations in foals, and 3) improve bone and collagen metabolism in horses, as evident by changes in bone and collagen markers.

CHAPTER 1

REVIEW OF LITERATURE

Physiology of Bone

Bone is a unique and dynamic connective tissue that is characterized by distinct cells and mineralization of an organic matrix. This rigid tissue provides support for the body, protection of organs, marrow, the animal with the ability to move, and regulation of fluids and ions in the body. Bone exists in two main forms: cortical and trabecular bone. Cortical bone is a solid mass of bone, whereas trabecular is more mesh-like, or woven like a net. Because trabecular bone has a greater surface area than cortical bone, mineral is drawn from trabecular bone during short term calcium and phosphorus deficiencies in order to maintain equilibrium within the body. Trabecular, or cancellous bone, uses the arrangement of the trabeculae to help reduce stress applied to the bone from loading. The adult human skeleton consists of approximately 80% cortical bone. Trabecular bone is located on the ends of long bones, in vertebrae, and envelopes the marrow (As reviewed by Jee, 1988; Wasserman, 1984; Hays and Swenson, 1984).

Long bones are made up of the diaphysis, or shaft, and the epiphysis, or end of the bone. The diaphysis and epiphysis are connected by the metaphysis. In young animals, the epiphysis and metaphysis are separated by a thick cartilaginous tissue known as the growth plate. The long bone elongates via the growth plate, as new bone is formed at the

base of the plate where blood vessels infiltrate the cartilage matrix. In a mature animal, the growth plate is replaced by trabecular bone, and the bone will cease to grow in length (As reviewed by Jee, 1988; Wasserman, 1984).

Both cortical and trabecular bone are made up of lamellae, which consist of circular collagen fibers and mineralization. Within this layer of collagen, lacunae, or cavities, are connected by a network of channels known as canaliculi. These cavities and channels comprise the communication system for bone. One concentric unit of lamellae, and its associated lacunae and canaliculi, is known as an osteon or Haversian system. Osteoblasts, or bone forming cells, become entrapped in the lacunae and canaliculi and secrete intracellular bone matrix, known as osteoid. Osteoblasts will completely surround themselves with osteoid, mature into osteocytes, and reside in the lacunae until death (As reviewed by Jee, 1988; Lawrence and Fowler, 1997).

Bone is made up of an organic matrix and inorganic salts. The organic matrix is largely composed of collagen, mucopolysaccharides and glycoprotein. Type I collagen makes up about 90% of the organic extracellular matrix. Within the matrix, amorphous ground substance is released to house the collagen fibers in a glue-type fashion, and also accumulates the inorganic salts, or minerals. The mineral in bone is most commonly found as crystalline hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. These highly organized crystals are about 30 to 50 Angstroms wide, can be as long as 600 Angstroms, and make up about two-thirds of the dry weight of the bone matrix. Apatite provides the skeleton with

rigidity, and is also a calcium (Ca) and phosphorus (P) reservoir. Mature bone (net weight) is approximately 30% organic material (mostly collagen), 45% mineral, and 25% water (As reviewed by Jee, 1988; Lawrence and Fowler, 1997; Pritchard, 1972; Hays and Swenson, 1984, Wasserman, 1984).

Lawrence et al. (1994) reported that the mineralization process of equine bone was almost complete (76%) by 12 months of age, and that the bone mineral content of the equine third metacarpal did not differ by sex. Calcium and P have an important relationship in bone formation. Dietary Ca and P are absorbed in the small intestine, and the absorption rate depends on the form of the nutrient, the Ca:P ratio, and dietary concentrations of these and related nutrients. Additionally, vitamin D, manganese, magnesium, fluoride, copper, potassium, sodium, chloride and silicon (Si), may have a role in bone formation and metabolism by altering the structure of the organic and/or inorganic components of bone (As reviewed by Hays and Swenson, 1984; Wasserman, 1984).

Bone metabolism is regulated by a wide variety of hormones, cytokines and other factors. Metabolism is also regulated by the activity of five different types of bone cells, including: osteoprogenitor, bone lining cells, osteoblasts, osteocytes, and osteoclasts. Osteoprogenitor cells consist of preosteoblasts, which will undergo mitosis and differentiation to become osteoblasts. Bone lining cells are flat, inactive, and/or resting cells that cover the surface of bone. These bone lining cells also serve as ion barriers

between the canaliculi and interstitial fluids. The osteoblast, or bone forming cell, originates from osteogenic (undifferentiated mesenchymal stem cells) lineage and secretes minerals, amorphous ground substance, osteocalcin, osteonectin, water and type I collagen into the matrix. Osteoblasts have rough endoplasmic reticulum and golgi, and these two structures are largely responsible for producing type I collagen proteins. Osteocytes are mature bone cells that have many long cytoplasmic branches projecting from the main body. Finally, osteoclasts are the multinucleated, highly motile cells that are responsible for resorption, and are derived from preosteoclasts in the bone marrow. Active osteoclast cells are usually found in resorption pits, or Howship's lacunae. The junction of osteoclast cell and bone forms a ruffled border, which forms an impermeable microenvironment, and enhances resorption. Hormones, growth factors, ion concentrations, and nutrition regulate all five bone cells. Mesenchymal stem cells can also differentiate into bone marrow cells, which can then differentiate into blood-forming cells (As reviewed by Jee, 1988; Nijweide et al., 1986; Marks and Popoff, 1988; Pritchard, 1972; Wasserman, 1984; Lawrence and Fowler, 1997).

Bone Growth and Development

There are two forms of bone growth – endochondral and intramembranous ossification. The compound (bone or cartilage) that provides the starting material for bone formation characterizes each of these forms of osteogenesis. Endochondral ossification indicates growth occurring in the cartilage, which is made up of

chondrocytes, and occurs in long bones at the growth plate. The growth plate is composed of four zones. The first zone, known as the resting zone, is near the epiphysis of the bone, and is made up of hyaline cartilage formed by chondrocytes. Although the exact function of this zone is not clear, chondrocytes are likely recruited into the maturation process during growth. Under this layer is the proliferative zone, which consists of cells that multiply and synthesize extracellular matrix. The hypertrophic zone begins when chondrocytes differentiate and hypertrophy. Finally, in the zone of calcification, terminally differentiated chondrocytes, near the metaphysis, begin to lay down mineral into the matrix (As reviewed by Jee, 1988). The chondrocytes in this region ultimately undergo apoptosis (Hatori et al., 1995). Endothelial and phagocytic cells initiate vascularization in the empty lacunae (Jacobson et al., 1997). At the chondro-osseous junction, where calcification and chondrocyte cell death have begun, the marrow begins to form the primary spongiosa. In this region, osteoblasts in the surrounding matrix of the woven bone continue to release osteoid. Additionally, the marrow carries osteoclasts and resorbs the bone. This synergistic effect of formation and resorption helps to shape the long bone (As reviewed by Vaughan, 1980).

Intramembranous growth occurs on bone surfaces or 'in membrane', such as under the periosteum of long and flat bones. Mesenchymal stem cells congregate in the area of new bone formation and differentiate into osteoblasts. The osteoblasts can then secrete matrix, which will become calcified. This increases the amount of bone in the

area. Some osteoblasts become surrounded by osteoid and will become osteocytes. Coupled with the bone formation process is osteoclastic activity on the innermost surface of the cortical bone. In this manner, the marrow cavity grows larger at a proportional rate to the outer portion of the cortical bone. This results in an increased diameter of the diaphysis, without altering the width of the cortical bone (As reviewed by Wasserman, 1984).

After initial formation of bone, the tissue remains very dynamic. During long bone formation, certain areas need to be shaped as the bone continues to grow. Therefore, in modeling, formation and resorption are separate, and may occur at different sites. If the two processes are not coupled, a drift of the bone can occur, resulting in an eccentric shaft. During growth, modeling occurs continuously, and there is an ultimate net gain of bone via this process (As reviewed by Jee, 1988; Lawrence and Fowler, 1997; Wasserman, 1984). Modeling and remodeling increases bone strength and stiffness by adding bone to areas where deformation will be the greatest, the outside of the bone, known as the periosteum (Kimmel, 1993).

Bone formed by modeling during the growth phase tends to be structurally inferior to the bone that is formed during remodeling. Additionally, adult bone tends to change over time, become damaged by microfractures as a result of loading, or reduce in quality as it ages. For these reasons, remodeling is an important mechanism utilized by the body to replace and repair bone. Remodeling is a different process than modeling,

whereby formation and resorption events are coupled and isolated to the same location. Remodeling occurs to replace the Haversian systems upon their demise and to respond to altered stresses and strains placed on the bone (As reviewed by Jee, 1988; Lawrence and Fowler, 1997; Wasserman, 1984; Lanyon, 1994). There are three types of strain: tensile, compressive and shear. The cells that make up bone undergo these strains with normal use (El Haj and Thomas, 1994). Therefore, loaded bones must adapt by continually altering its shape and architecture (Lanyon, 1994). Remodeling may strengthen bone by replacing damaged areas caused by strain, without altering bone mass (Kimmel, 1993). Remodeling can also occur during times of disuse, and acts to decrease the total amount of bone present, thereby decreasing the strength of bone.

Remodeling stages include: quiescence, activation, resorption, reversal, formation, and return to quiescence. The bone surface is normally in the quiescent stage, and the cells are inactive. Upon activation, osteoclasts are recruited from the bone marrow, and migrate to the site of activation, where they remove mineral and matrix in the resorption period. The reversal phase is characterized by the repression of osteoclastic activity, and deposition of cement into the resorption cavity. Finally, new bone formation begins with matrix formation and sequential mineralization. Once this cycle has completed, the surface of the bone will return to quiescence (As reviewed by Parfitt, 1984). The site of resorption and formation, where the remodeling occurs, is referred to as the bone remodeling unit. Furthermore, this turnover cycle usually is

completed in about four months. Trabecular bone remodeling occurs on the surface, while cortical bone remodeling occurs by osteoclasts tunneling through bone, with new osteoblastic apposition occuring during formation from the cement line inwards, until a new Haversian canal is established (As reviewed by Jee, 1988).

Physiology of Collagen

Collagens are a family of proteins found in most tissues, including bone and cartilage. Collagen provides connective tissue with strength, and makes up a major portion of bone and a minor portion of cartilage composition. This supramolecular aggregate is associated with the extracellular matrix, and is stabilized by the triple helix that is characteristic of collagen (As reviewed by Wasserman, 1984; Lawrence and Fowler, 1997; Marks and Popoff, 1988; van der Rest and Garrone, 1991).

Collagen molecules have a repeating pattern of glycine-proline-hydroxyproline-glycine-x-y amino acids, where x and y denote any other amino acid. Hydroxyproline and hydroxylysine are two amino acids found predominately in connective tissue, and are prevalent in collagen. For steric reasons, glycine is the only amino acid that can be in the center of the superhelix, or collagen molecule.

There are many types of collagen, which are found in various connective tissues throughout the body. Type I collagen is the most abundant form of the molecule, and is found in bone, tendon and skin and many other tissues. This molecule has an $[\alpha\text{-}1(I)]_2$ α-2(I) conformation. Type II collagen is largely found in cartilage and between vertebrae,

and has an $[\alpha\text{-}1(II)]_3$ structure. Type I and II collagens are examples of fibril-forming collagens. Other collagen types, such as basement membrane, microfibrillar, anchoring fibril, hexagonal network-forming, fibril-associated with interrupted triple helices, transmembrane, and multiplexin collagens, form different structures. These collagens have various α subunits , that help differentiate each type of collagen (As reviewed by Lawrence and Fowler, 1997; van der Rest and Garrone, 1991; von der Mark, 1999).

The interfibrillar space between type I collagen fibrils is the site of bone mineralization. Minerals associate in a rigid, crystalline structure known as hydroxyapatite. Type I collagen is the most prevalent type (90%) of collagen in bone. Additionally, collagen comprises about a third of the dry weight of bone organic matrix (As reviewed by Pritchard, 1972; Wasserman, 1984; Lawrence and Fowler, 1997).

Collagen Synthesis

Collagen is synthesized intracellularly as preprocollagen, followed by hydroxylation of proline and glycosylation of hydroxylysine, to yield procollagen. The ends are cleaved, and procollagen is expelled from the cell (As reviewed by Wasserman, 1984). Extracellularly, the procollagen molecules undergo hydrolysis to form tropocollagen, which is the basis for matrix collagen fibrils (As reviewed by Lawrence and Fowler, 1997; Wasserman, 1984). The polypeptide chains of tropocollagen wind in a

left-handed fashion, and have a 30° rise, formed when every third amino acid residue is turned into the center (van der Rest and Garrone, 1991).

Fibril forming types of collagen molecules, like those in bone, are arranged parallel to each other, with spaces strategically placed between fibers, once the molecules have been cleaved at the N- and C-telopeptides to form collagen fibrils extracellularly. The ends of the fibers can then become crosslinked. Crosslinkage between collagen molecules at hydroxylysine and lysine sites provide collagen with its stabilization, and form the striations in the collagen fibrils. Collagen, in conjunction with its crosslinking, yield a scaffold for minerals to bind in the matrix (As reviewed by Wasserman, 1984; van der Rest and Garrone, 1991, Lawrence and Fowler, 1997, Marks and Popoff, 1988).

Pyridinoline (PYD), is a 3-hydroxypyridinium derivative consisting of three amino acids and three carboxyl groups (Fujimoto and Moriguchi, 1978). As crosslinks originally develop from lysine and hydroxylysine residues, lysyl oxidase converts these amino acid residues to an aldehyde, through oxidative deamination, and the residues may then mature into trifunctional, non-reducible cross-links (As reviewed by Last et al., 1990). An analog crosslink, deoxypyridinoline (DPD), is formed in the similar manner as PYD, but involves reaction with a lysine residue from the helix rather than hydroxylysine (Ogawa et al., 1982; reviewed by Robins, 1999). Eyre et al. (1988) found that hydroxypyridinium residues persist in human bone through adulthood, thereby establishing them as final crosslinks (Eyre et al., 1988).

Biochemical Markers of Bone and Collagen

Equine research has developed a need for sensitive, non-invasive techniques to measure bone metabolism. Fluid samples can be taken and analyzed for proteins or enzymes that indicate activity or bone turnover. Serum and urinary assay kits are commercially available to measure specific molecules of this type. Osteocalcin (OC) is one such marker. The function of OC is unclear (Price, 1982; Lian and Gundberg, 1988), however several characteristics are known about the protein. Osteocalcin is a non-collagenous protein synthesized largely by the osteoblast, contains three amino acids of gamma carboxyglutamic acid, and is therefore known as bone Gla protein (Fraher, 1993). Osteocalcin is vitamin K-dependent (Price, 1982), binds Ca, and may be involved in the control of mineralization (Fraher, 1993; Lepage et al., 1991). The Gla residues specific to OC are formed after translation of the protein occurs by a carboxylase enzyme system. In the presence of Ca, these Gla residues undergo conformational changes that allow OC to bind to hydroxyapatite, and then accumulate in bone matrix (Lian and Gundberg, 1988). Furthermore, OC has a high affinity for hydroxyapatite (Price, 1982). The protein exists in a 1:1 ratio with collagen in bone, and is proportional to hydroxyapatite (Lian and Gundberg, 1988). It is believed that OC may regulate the size of the hydroxyapatite crystal (As reviewed by Gundberg and Nishimoto, 1999). Osteocalcin is cleaved through proteolysis, and is cleared from circulation by the kidneys (Gomez et al., 1994). Serum concentrations of OC is probably a product of diffusion into the

circulatory system prior to binding to the hydroxyapatite (Price, 1982). Osteocalcin is mostly formed by osteoblast activity, and thus is highly correlated with bone formation (Lian and Gundberg, 1988; Kannus et al., 1996). Therefore, serum OC may be used as a marker of bone synthesis.

When animals are exposed to natural light, OC undergoes a circadian rhythm (Lepage et al., 1991). Osteocalcin concentrations in normal female Standardbred horses were found to be consistent during the day from 0700 to 1900. These concentrations then fell in the early evening hours (1900 to 2000), and increased at night until a peak was reported at 0500 the next morning (Lepage et al., 1991). Lepage et al. (1991) therefore concluded that blood samples should be taken during the daylight hours, since this period experienced the least fluctuation in OC concentrations. However, in a study where horses were exposed to only florescent lighting, a circadian rhythm was not observed (Hope et al., 1993).

Carboxy-terminal pyridinoline crosslinked telopeptides of type I collagen (ICTP) are a 9kD portion of type I collagen that is released during resorption of bone (Hassager et al., 1994), and can be quantitated in serum and urine (Risteli et al., 1993). Once type I collagen is broken down into ICTP, it cannot be used again for synthesis of new collagen in bone (Hassager et al., 1994), and is cleared from the body's circulation through the kidneys (Tahtela and Tholix, 1996; Risteli et al., 1993). Concentrations of ICTP decrease with age of the horse, up until about four years of age, after which there were no

significant differences (Price et al., 1995). A diurnal cycle has been reported with ICTP concentrations (Risteli et al., 1993).

Finally, PYD and DPD are crosslinking amino acids residues of collagen released from bone matrix during turnover. An enzyme linked immunoassay (ELISA) exists for PYD and DPD crosslinks in serum and urine. Since PYD is found in cartilage and bone, PYD and DPD can be used as biochemical markers to analyze metabolic bone or cartilage diseases. Increased urinary PYD was seen in patients with osteoarthritis and rheumatoid arthritis (Robins et al., 1986).

Milk

Milk contains carbohydrate, lipid, minerals, protein, and water. Casein is the largest portion of proteins in milk. Immunoglobins in colostrum are synthesized from amino acids in the blood. Lactose is the primary carbohydrate of milk, and is formed from blood glucose. Triglycerides make up most of the lipids in milk (As reviewed by Jacobson and McGilliard, 1984).

Peak lactation in the mare is found at 90 d postpartum (Ashcraft and Tyznik, 1976). Mares' milk has been reported to contain 1.6% fat, 2.4% protein, 6.1% lactose, and .5% ash (As reviewed by Jacobson and McGilliard, 1984). Gibbs et al. (1982) studied 14 mature Quarter Horse mares to determine milk yield and formulate a lactation curve; they reported that average milk yield was 10.9 kg/d (2.1% of body weight). Parity significantly influenced milk yield, as multiparous mares averaged 11.7 kg/d. Daily milk

yield significantly declined with time, with percent solids being highest 10 d after parturition and declining thereafter (Gibbs et al., 1982). From a second study with five mares in midlactation, milk composition was reported to be 10.5% total solids, being 1.29% fat, 1.93% protein, 6.91% sugar, and .42% ash on a whole-milk basis (Oftedal et al., 1983). Additionally, this study measured milk intake of foals using D_2O kinetics, and reported that daily milk intake averaged 16 kg at 11 d, 15 kg at 25 d and 17.6 kg at 39 d.

The mineral content of mare's milk has been the topic of few studies. Since minerals are so vital in the development of the young animal for various physiological functions, including bone mineralization, further studies are required to completely characterize the mineral content of mare milk. Utilizing five Thoroughbred mares, Schryver et al. (1986) analyzed milk for Ca, P, Mg, K, Na, Cu and Zn. They reported that the ash content of the milk was highest in the first week and decreased 26% during the first four-wk period, 16% the next four-wk period, 16% the next eight-wk period, and finally 50% in the next 16-wk period (Schryver et al., 1986).

Foals born of Ca deficient mares had significantly smaller cannon diameters (Glade, 1993). Additionally, Glade (1993) found that it took 16 wks longer for the Ca deficient mares to finally return to baseline in sound velocity and bone breaking strength of both the right and left cannon bones when compared to the Ca sufficient mares, indicating a decrease in mineral content of the long bone for a longer period of time in Ca deficient mares. The study concluded that pregnancy and lactation affects the skeletal

system and mineral homeostasis, and that adequate Ca intake is necessary during gestation and lactation to prevent skeletal mineral loss (Glade, 1993).

Trace minerals have been defined in human milk. In a study that followed six women through the first five mon of lactation, the following trace elements were found in large quantities in order of highest to lowest concentrations: zinc, Si, copper, iron, barium, and strontium. Over time, zinc, iron, and copper concentrations decreased, however strontium, barium and Si did not change as lactation advanced (Anderson, 1993). However, more research needs to be done to further understand the role of these trace minerals in milk.

Silicon

Silicon is the second most abundant element in the earth's crust. Silicon is a member of Group IV on the periodic table, and is an alkali and alkaline earth cation. Because Si can not form double bonds due to a large radius, all silicates are rigid structures (Van Soest et al., 1983). Several decades ago, most research involving Si centered around toxicity of silica and fibrous silicates, especially in their role with silicosis. However, in the 1970's, elemental Si was found to be important for normal growth and development in chicks, when specialized highly purified diets were fed (Carlisle, 1972). This element has been studied in relation to its role in the skeletal system in animals (Carlisle, 1972, 1978).

Herbivores consume large amounts of Si through the grazing process, which passes through mostly unabsorbed. However, a portion of the dietary Si is absorbed and utilized in the body or excreted. Silicon is normally excreted without side effects, although uroliths may form and block urinary passages, which may cause death (Hays and Swenson, 1984). As a result of its presence in many foodstuffs and unknown requirement status, Si deficiencies are unknown under normal dietary conditions (Van Soest et al., 1983). Additionally, in the case of systemic hypersilicemia, a decrease in the activity of rat hepatic and renal antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase was reported with .1% Si and .2% Si aqueous solutions (Najda et al., 1994). These data may be important when conducting studies using excessive dietary Si concentrations.

Silicon is naturally found in the environment as silica, SiO_2. Grains and grain fractions are very high in Si content, including whole-grain barley and oats, rice bran and flour (Pennington, 1991). When silica becomes hydrated, orthosilicic acid, $Si(OH)_4$, is formed. Once Si derivatives are converted to silicic acid in the body, Si is absorbed through the intestine and excreted via urine (Benke and Osborn, 1979; Carlisle, 1982; Van Soest et al., 1983). The concentrations of Si in blood are increased when silicates were fed to rats (Carlisle, 1982) and horses (Frey et al., 1992; Nielsen et al., 1993). In animal tissue, Si is found in the highest concentration in skin and connective tissue.

Silicon is also found in measurable quantities in milk. In the horse, Si composes .0244% of milk ash, which equates to 65 μg Si/ml of milk (Schwarz, 1978).

Because Si is so ubiquitous in the environment, and laboratory contamination may occur, special laboratory precaution must be taken to minimize contamination (Carlisle, 1972). Graphite furnace atomic absorption spectrometry is utilized to measure Si in biological samples of plasma and urine. Glass pipettes, syringes or vacuum tubes should be used, since glass contains Si. Plastic labware should be used instead (Gitelman and Alderman, 1990).

Sodium zeolite A (EZA) is a sodium aluminosilicate clay substance. Zeolites are hydrated, crystalline aluminosilicates of alkali and alkaline earth cations (Mumpton and Fishman, 1977), and have a three-dimensional framework of interconnected cavities 11.4 Å in diameter, with pore openings that are 4.2 Å in diameter (Roland et al., 1985). Furthermore, zeolites can reversibly gain or lose water, and change the bound cation easily, without altering the structure significantly. The framework consists of SiO_4^{-4} tetrahedra, and maintains an overall neutral charge. When trivalent aluminum replaces the Si in a tetrahedral, an overall negative charge is yielded. Finally, to counteract this negative charge, a cation is bound to the structure to return neutrality to the compound. These cations commonly are Na^+, Ca^{++} and K^+ (Mumpton and Fishman, 1977; Van Soest et al., 1983). This neutralizing cation provides the silicate with a high ion exchange capacity, especially for Ca^{2++}. Sodium aluminosilicate's Ca^{2++} exchange

capacity is more than 7.0 meq/g and may be beneficial in capacities ranging from bone metabolism (Seaborn and Nielsen, 1994) and mineralization (Carlisle, 1974) to egg shell quality (Roland et al., 1985). "Ion exchange capacity is basically a function of the degree of substitution of aluminum for silicon in the framework structures; the greater the substitution, the greater the charge deficiency, and the greater the number of alkali or alkaline earth cations required for electrical neutrality" (Mumpton and Fishman, 1977).

Sodium zeolite A breaks down to monosilicic acid in the gut, and when fed to horses caused a dose-related increase in plasma Si concentrations (Frey et al., 1992). Nielsen et al. (1993) found that nine h post-feeding, a high Si diet (2.8% EZA of total diet) yielded significantly higher plasma Si concentrations in horses for 30, 90, 120 and 180 d of treatment. In hens intubated with EZA, serum Si and Al concentrations increased significantly over the control. However, the aluminum concentration was only 1/100 of the Si concentration (Rabon, Jr. et al., 1995). Additionally, Rabon, Jr. et al. (1995) reported that .75% dietary EZA and 200 IU/kg cholecalciferol resulted in a two-fold increase in serum Si concentrations compared to100 IU/kg dose of cholecalciferol and .75% Si.

In vitro, silicic acid increased Transforming Growth Factor – Beta (TGF-β), which is produced in bone and platelet cells (Keeting et al., 1992). This may be important in fracture and wound healing, as an increase in TGF-β causes an increase in

cell proliferation (Lind et al., 1995). If Si supplementation can activate TGF-β *in vivo*, an enhanced repair mechanism may result.

Sodium zeolite A increases egg specific gravity as the amount of EZA is increased in the diet (Miles et al., 1986). When synthetic EZA was compared to naturally occurring zeolites in the diet of 360 Hyline W36 hens, 3% natural zeolite addition did not influence egg specific gravity, whereas EZA improved egg specific gravity (Roland, 1988). Improved specific gravity will result in reduced breakage during handling and shipping. Roland et al. (1985) determined that zeolite A improved Dekalb Xl and Hyline W36 hens' ability to use Ca in eggshell formation and improved egg specific gravity.

In a study on White Leghorn cockerels that were fed a Si supplement of sodium metasilicate, the chicks grew more rapidly and obtained a higher weight after 23 d as compared to the control chicks on a specially formulated silicon deficient diet (Carlisle, 1972). Carlisle (1972) also found that Si deficient chicks had absent wattles and much smaller combs, and their leg bones were shorter, of smaller circumference, and had a thinner cortex.

Silicon may play a role in the calcification process. Because Si is found in metaphyseal blood vessels and in Si-rich sites of bone, it is felt that as the blood vessels invade the metaphysis, the calcification process is activated, and Si may be involved in these sequences. Furthermore, Si may be coupled to Ca and aid in mineralization (Carlisle, 1974). When the Ca content of the diet is low, and high amounts of Si are

supplemented, a higher percentage of ash is recovered in a shorter period of time from chick tibia bones. Therefore, Si may increase the rate of bone mineralization when Ca levels are low (Carlisle, 1974).

Sodium zeolite A may have a role in cartilage metabolism. For example, Si supplementation in chicks increased glycosaminoglycan levels in cartilage (Carlisle, 1976). This may be important in maintaining cartilage health, as glycosaminoglycans draw water into cartilage allowing for its stiff and compressive characteristics (Nixon, 1991). Furthermore, Si deficiencies caused a decrease in articular cartilage of chicks, whereas Si supplementation increased total connective tissue (Carlisle, 1976).

Silicon's role in connective tissue seems to largely be related to collagen. Chicks fed a Si-deficient diet had thinner legs and smaller combs when compared to their size. These tissues are normally composed of a high collagen content. Also there was less hexosamine content in articular cartilage (Carlisle, 1976). Furthermore, a study done on embryonic skull bones grown in an organ culture (Carlisle and Alpenfels, 1978), showed Si supplemented systems had a marked increase in collagen content of the bones over the non-supplemented groups after 12 d.

Silicon has been shown to influence bone in numerous studies. For example, in a study where broilers had one wing immobilized and were fed sodium flouride or sodium silicate, a decrease in humeri strength was seen as a result of the immobilization in the sodium flouride group. However, the broilers fed sodium silicate showed no effects of

immobilization the last two wks of the experiment. However, sodium fluoride produced an increase in bone breaking strength over the control, where those treated with sodium silicate did not. The authors concluded that the results in bone breaking strength and bone loss in this study still leave questions to be answered (Merkley and Miller, 1983).

In vitro and *in vivo* results show that Si might improve in bone formation. Carlisle and Alpenfels (1978) found that Si supplemented bones in culture increased 23% (dry weight) over non-supplemented bones. This weight increase was due to an increase in collagen content. Silicon supplemented bones showed an 88% increase in collagen content over 12 d compared to the non-supplemented bones, which only increased 45%. Additionally, Si supplemented bone cultures had an increase in Ca content of 14%, while non-supplemented cultures remained at base level (Carlisle and Alpenfels, 1978). Furthermore, zeolite A (assessed by ^{3}H-thymidine uptake) increased proliferation of human osteoblast-like cells *in vitro* when administered at doses of .1 to 10 μg/mL. A 269% increase was seen with the 10 μg/mL dose over the control. Similarly, 100 μg/ml of zeolite A increased cellular alkaline phosphatase activity 310% (Brady et al., 1991). The authors suggest that zeolite A may have an anabolic effect on osteoblast-like cells, and may be beneficial in diseases involving bone loss, such as osteoporosis (Brady et al., 1991). It is important, however, to realize that zeolite A will break down into silicic acid in the gut, and therefore, *in vitro* studies on the whole zeolite A compound may not have a direct application to *in vivo* systems.

Bone metabolism was increased when weanling Sprague-Dawley rats were fed normal physiological amounts of Si (35 μg Si as sodium metasilicate/g diet) as evident by increased alkaline and acid phosphatase activity in homogenized femurs. In the Si deficient rats (.6 μg Si/g diet), the enzyme activities were lower (Seaborn and Nielsen, 1994). Additionally, when these rats were supplemented with a bone implant that was harvested from either Si deficient or Si supplemented rats, those that had the Si supplemented implant showed higher enzymatic activity than those rats with the deficient implant. The researchers concluded that while the mechanism for the effect of physiological amount of Si on bone turnover is not fully understood, they feel it might involve a mediator that is transported throughout the body, as evidenced by increased enzymatic activity due to the supplemented implant (Seaborn and Nielsen, 1994).

In the horse, EZA produced a dose related increase in plasma Si levels in both weanling and yearling Quarter Horses (Frey et al., 1992; Nielsen et al., 1993). Frey et al. (1992) found that horses supplemented with 2% EZA experienced a trend to gain optical bone density initially, but there was no difference between treatments at the end of the 168 d study in any of the EZA supplemented groups. Therefore, if EZA does affect optical bone density, it is likely that it occurs early on, and is short lived. Furthermore, Nielsen et al. (1993) found that Quarter Horses in race training that were fed EZA ran farther and completed more strides before experiencing bone-related injuries when compared to non-supplemented horses. A positive correlation ($R^2 = .54$) between plasma

Si concentrations and distance traveled before the horses experienced a bone-related injury was also observed (Nielsen et al., 1993). However, no attempt at determining bone metabolism as indicated by biochemical markers was made in this study, which should be examined in horses.

Rationale for Experiments

High performance sport horses may experience bone-related injuries at any point, and bone failure will halt a horse's athletic career. These injuries impact the welfare of the horse, as well as the economics of the industry, because of increased expenses to treat the injury, lose of use, and extensive therapy. Research has been conducted to examine ways of alleviating the risk of bone-related injuries, or treating them once they have occurred. Horsemen employ a variety of techniques to increase bone density and strength, in hopes of decreasing injury rates. Management, exercise and nutrition have served as means for enhancing a horse's skeletal durability. Based on the literature cited involving the relationship between supplemental Si and bone, I hypothesize that an absorbable form of Si would increase plasma Si concentrations and alter bone turnover in horses. We designed two experiments to test this hypothesis in broodmares, their foals, and yearling horses. Our overall objectives were to determine if EQUI-Si™, a sodium zeolite A compound, could: 1) increase plasma Si concentrations in the above horses, 2) increase milk Si concentrations in mares, thereby increasing plasma Si concentrations in foals, and 3) alter systemic bone and collagen metabolism in the above horses.

CHAPTER 2

EXPERIMENTAL PROCEDURE: EXPERIMENT 1

Assignment of Treatments and Management of Animals

Twelve Arabian broodmares were pair-matched by expected foaling date and assigned to one of two treatments groups of six horses each. All mares belonged to the Michigan State University Horse Teaching and Research Center. Sixteen mares were originally required for this study, however four mares were removed prior to being assigned to a treatment group due to dystocia or other foaling problems that would jeopardize the health of the foal or mare. These animals were not replaced due to a lack of pregnant mares. Prior to the study, the mares were housed on pasture, and were not brought into stalls until about a week before foaling. Mares were monitored at night, and assisted in foaling by students and staff at the Horse Teaching and Research Center.

Once foaling occurred, the horses were pair-matched by actual foaling date and placed into a randomly assigned predetermined treatment group. The treated group (S) received approximately 2% of the diet of EQUI-Si™ as fed (.22 kg/day). The control group (C) received 0% EQUI-Si™ in their diet. Mares were fed to maintain body condition, with a 56% roughage (by weight) and 44% total concentrate diet, which met

Table 1. Calculated daily feed composition for mares on an as fed basis

Nutrient	Alfalfa Hay	Grass Hay and Forage	Corn	Oats	Protein Pellet	Molasses
DE	3.76 Mcal	10.2 Mcal	5.07 Mcal	8.91 Mcal	.41 Mcal	.73 Mcal
CP	310 g	690 g	140 g	370 g	260 g	20.7 g
Ca	22.8 g	13.6 g	.75 g	2.50 g	8.78 g	2.37 g
P	4.37 g	13.6 g	4.05 g	10.6 g	6.50 g	.322 g
Mg	5.82 g	4.91 g	1.65 g	4.38 g	4.55 g	1.01 g
Cu	29.3 mg	119 mg	5.55 mg	18.7 mg	26 mg	17 mg

Table 2. Calculated total daily nutrients for mares on an as fed basis

Nutrient	Nutrient Content	Calculated Total Daily Intake
DE	2.24 Mcal/kg	29.1 Mcal
CP	13.7%	1790 g
Ca	.39%	50.8 g
P	.30%	49.4 g
Mg	.17%	22.3 g
Cu	14.6 mg/kg	216 mg

alfalfa mix. The concentrate consisted of 54.5% oats, 26.1% corn, 11.4% protein pellets (Kent Feeds Inc., Muscatine, IA), 4.0% wet molasses and 4.0% EQUI-Si™ if treated. The mares were individually fed 5.7 kg of concentrate and 7.3 kg of hay daily divided into two equal feedings at 0700 and 1600. All concentrate was consumed at each meal, and when hay was not consumed during the morning meal, additional hay was provided, and all hay was eaten overnight. The horses were maintained on their respective diets for 45 d post-foaling. Water and mineral blocks were provided to the horses at all times.

Mares and foals were housed in stalls that were 3.1 m x 3.1 m or larger from 1600 until 0800 the next morning. Beginning d 2 post-foaling, mares and foals were turned out

Mares and foals were housed in stalls that were 3.1 m x 3.1 m or larger from 1600 until 0800 the next morning. Beginning d 2 post-foaling, mares and foals were turned out during the daytime into paddocks with an average stocking rate of three pairs per paddock. Paddocks were approximately 2384.5 m^2, with brome, orchard, timothy and Kentucky blue forage. Mares and foals received health care routine for Michigan, including deworming and farrier work prior to study.

Growth Measurements

Foal weight was determined using a portable scale (Tru-Test, Inc., San Antonio, TX), and mare weight was estimated using a weight tape placed in the heart girth region at the wither on d 0 and d 45. Height at the withers and hip for all animals was found using a leveled measuring stick at the point of the withers and the tallest portion of the croup, respectively.

Sample Collection

Blood samples were collected nine h post-feeding (1600) from the mares and foals by jugular venipuncture on d 0, 15, 30 and 45. Day 0 represented the first 24 h after parturition. Two 10-mL Vacutainer® tubes with no coagulant and one 7-ml tube with K_2EDTA (Becton Dickinson and Company, Franklin Lakes, NJ), and either 22 g x 2.5 cm disposable needles for the foals or 18 g x 3.8 cm disposable needles for the mares (Becton Dickinson and Company, Franklin Lakes, NJ) were used to collect blood. Finally, approximately 10 mL of colostrum or milk samples were collected on d 0, 15, 30

and 45 from the mares. The udder was milked into acid washed plastic narrow-mouth 250-mL collection bottles (Nalgene Company, Rochester, NY).

The blood samples were centrifuged for 10 min for serum and for 20 min for plasma at 754 x g for both. After centrifugation, the serum samples were aliquotted into plastic 1.5-mL microcentrifuge tubes (DOT Scientific, Inc., Burton, MI) using disposable glass Pasteur pipettes (VWR Scientific, West Chester, PA). The plasma samples were withdrawn using acid washed plastic Samco brand transfer pipettes (Corning Samco Corporation, San Fernando, CA), and transferred into plastic 1.5-mL microcentrifuge tubes in a plastic-lined box to avoid dust contamination. This box was only open to the front, and this opening had a sheet of plastic wrap to serve as a curtain to prevent dust contamination. Milk and colostrum were handled and stored in a similar manner as plasma. Serum, plasma and milk samples were then stored at –20°C until analysis.

Minimizing Silicon Contamination and Special Cleaning of Labware

Glass vacuum collection tubes with one needle were used to reduce trauma to the foal during sample collections. Various collection methods were used on two mares in this study, to collect plasma for Si analysis. These results are discussed in appendix E.

Two to three d prior to collection, all plastic equipment, including the milk collection bottles and the Samco transfer pipettes, was specially washed using a 10% nitric acid wash, using double distilled deionized water (dd H_2O) to dilute the nitric acid (HNO_3, EM Science, Gibbstown, NJ). The equipment was then rinsed three times with

dd H_2O, and stored in a fume hood to allow for drying and to minimize Si contamination from dust. The plasticware was shielded from dust when not in use.

Biochemical Marker Analysis

Osteocalcin was determined using an enzyme-linked immunosorbent assay (ELISA) (NovoCalcin®, Metra Biosystems, Inc., Mountain View, CA) following manufacturer's instructions. Serum was diluted at 1:4 for mares and 1:15 for foals. Dilutions were determined by testing samples at dilutions of 1:2, 1:4, and 1:6 for mares, and 1:10, 1:15, and 1:20 for foals. These values were compared to the standard curve provided by Metra Biosystems. When the samples fell along the linear portion of the curve (from .4 to 1.5 optical density - OD), the corresponding dilution was chosen to run the OC assay for all samples. Standards and controls were reconstituted with .5 mL of 1X wash buffer. Twenty-five µL of standard, control, or sample was added to the osteocalcin-coated strips. Samples were tested in duplicate. Anti-osteocalcin (125 µL) was also added to each well. A two-h incubation at room temperature followed, and then the wells were washed with 300 µL of 1X wash buffer three times, with three min between each washing. Reconstituted enzyme conjugate (150 µL) was added to the wells and incubated for one h at room temperature. After incubation, the wells were then washed in triplicate with 300 µL each time, and three min between each wash. Finally, a working substrate was added (150 µL) to each well and incubated at room temperature

until the A standard read 1.5 OD, at which point 50 µL of stop solution was added, and the plate was read at 405 nm optical density on a Spectra Max 340 plate reader (Molecular Devices Corp., Sunnyvale, CA).

Serum PYD was quantified using an ELISA (Serum PYD®, Metra Biosystems, Inc., Mountain View, CA) according to manufacturer's directions, but modified for dilutions. Dilutions of 1:8 for d 0 and 1:5 for d 15, 30 and 45 for the foal were required to fit the standard curve. Mare samples were not diluted. Standard was prepared by diluting the 120 nmol/L PYD standard 1:10 with assay buffer (12 nmol/L), and then making a 1:2 dilution series for standards 6, 3, 1.5, and .75 nmol/L, with the zero standard being only assay buffer. Controls were diluted 1:10 with assay buffer. Care was taken to avoid sample exposure to light, since PYD is light sensitive. Samples were centrifuged in 30k MWCO Spinfilter tubes at 3000 x g for 30 min. After equilibrated to room temperature, coated strips were filled with 50 µL of Reagent 1, 25 µL of standard, control or filtered sample, and 75 µL of cold PYD antibody. Samples were tested in duplicate. The plate was then gently mixed on a plate mixer for one min and incubated in the refrigerator (2-8°C) in a box overnight (21.5 h). The wells were then emptied and washed with 250 µL of 1X wash buffer three times with three min between washes. Enzyme conjugate (150 µL) was then added to all wells, and the plates were incubated for one h at room temperature in a box. Another triplicate wash with 250 µL of 1X

buffer in three min intervals followed, and then 150 µL of working substrate solution was added to each well. A final incubation of 40 min at room temperature in a box followed, and then 100 µL of stop solution was added to each well. The plates were read at 405 nm optical density on a Spectra Max 340 plate reader (Molecular Devices Corp., Sunnyvale, CA).

A radioimmunoassay (RIA) was used to measure serum ICTP (ICTP, ^{125}I RIA Kit®, Diasorin, Stillwater, MN), and was performed according to manufacturer's directions. All samples, standards, controls , nonspecific binding tubes, and total count tubes were run in duplicate. The tubes contained the following: nonspecific binding tubes – 100 µL sample and 200 µL dd H_2O; standards (0 and A-F) – 100 µL standard and 200 µL ICTP Antiserum; controls and samples – 100 µL serum and 200 µL ICTP antiserum. Tracer (200 µL of ^{125}I ICTP) was added to all tubes, including the total count tubes. Tubes were mixed and incubated for two h at 37°C. Next, 500 µL of separation reagent was added to all tubes except the total count tubes. Tubes were vortexed and incubated for 30 min at 20-25°C. After incubation, tubes were centrifuged for 30 min at 2000 x g at 4°C, and immediately decanted for the supernatant. Tubes were then read using the 1290 GammaTrac Gamma Counting System (Tm Analytic, Elk Grove Village, IL).

Silicon Analysis

Plasma and milk samples were analyzed by Trace Elements Laboratory, LHSC (University Campus, 339 Windermere Road, London, Ontario N6A 5A5, Canada) for Si concentrations using electrothermal atomic absorption spectrometry as adapted from Leung and Edmond (1997).

Calcium and Phosphorus Analysis

Approximately 2.5 g of milk was weighed into a 250-mL, acid-washed, conical beaker, and ashed with 15 mL of nitric acid (Trace Element Grade, Fisher Scientific, St. Louis, MO) and 3 mL of perchloric acid (Trace Element Grade, Fisher Scientific, St. Louis, MO). Samples were diluted with dd H_20, and stored in polystyrene test tubes (Sarstedt Inc., Newton, NC).

Calcium concentrations were determined using flame atomic absorption spectrometry (Unicam 989 AA Spectrophotometer, Thermo Jarrell Ash Solutions, Franklin, MA) following a 1:250 dilution with lanthanum chloride (1% La). Inorganic standards (Fisher Scientific, St. Louis, MO) were prepared similarly to samples, and a standard reference material (Peach Leaves Standard, National Institute of Standards and Technology, Gaithersburg, MD) was analyzed as per the samples to insure accuracy of the instrument. Each sample was run in duplicate.

Phosphorus concentrations of milk samples were determined using spectrometry. Phosphorus standards (Sigma-Aldrich Chemical Co., St. Louis, MO) and samples were

diluted by a factor of 75 using dd H_2O. For the assay, 2.5 mL of molybdate-sulfuric solution and .25 mL of elon were added to all standards, blanks and sample tubes. After the incubation at room temperature for 45 min, standards were read at 700 nm on a DU 7400 Spectrophotometer (Beckman Instruments, Palo Alto, CA) using the sipper assembly to establish a standard curve. A standard reference material (Peach Leaves Standard, National Institute of Standards and Technology, Gaithersburg, MD) was analyzed as per the samples to insure accuracy of the assay. Each sample was run in duplicate, with each day being run twice, for a total of four concentrations, which were pooled to report average milk P concentrations.

Statistical Analysis

Treatment effects on Si, Ca, P, OC, ICTP, PYD and growth measurements were analyzed as a two-factor ANOVA for repeated measures using the Proc Mixed procedure of SAS (6.12) (SAS, 1997). The model included treatment, day and the interaction between treatment and day. Least Square Means (LSMeans) and Standard Error of the Mean (SEM) were determined. Correlations between plasma Si concentrations (both mare and foal) and milk Si concentrations were made using the Proc Corr procedure of SAS (6.12) (SAS, 1997). The correlations were made by day, and were reported as r^2 with P-values. For all analyses, a P-value of less than .05 was considered significant, and trends were noted at a P-value of less than or equal to .1.

Milk Ca concentrations were different at d 0 between treatment groups, and therefore each day was standardized by subtracting the day from 0, allowing each horse to serve as its own control. Proc Mixed was then utilized to report the change from d 0, and LSMeans and SEM were reported. P-values of less than .05 were still considered significant, and trends were noted at a P-value of less than or equal to .1.

RESULTS AND DISCUSSION: EXPERIMENT 1

EQUI-Si™ and Plasma Silicon Concentrations of Mares

Our first objective was to determine if EQUI-Si™ supplementation would increase plasma Si concentrations in Arabian mares. Plasma Si concentrations in the S mares increased from d 0 to d 15 ($P = .04$), to d 30 ($P = .001$) and to d 45 ($P = .05$) (Table 3). Additionally, there was a trend for plasma Si concentrations to be higher at d 30 than d 15 in S mares ($P = .07$). Sodium zeolite A supplementation in weanling Quarter Horses (Frey et al., 1992) and yearling Quarter Horses (Nielsen et al., 1993) resulted in higher plasma Si concentrations in treated animals when compared to control horses. In the current study, no change in plasma Si concentrations was seen in C mares (Table 3). On d 30, the S mares had higher Si concentrations than C mares ($P = .01$), and on d 45, S mares tended to have higher plasma Si concentrations than C mares ($P = .10$). These results illustrate that supplemental EQUI-Si™, at 2% of the diet, increases plasma Si concentrations in S mares when compared to C animals after 30 d of supplementation.

Table 3. Means of plasma Si concentrations (μg/L) in mares

	Day				
Treatment	0	15	30	45	SEM
Control (C) n=6	1339	1398	1335^{x}	1263	108
Si Treated (S) n=6	1201^{a}	1488^{b}	1739^{by}	1515^{b}	108

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

^{xy}treatments with different superscripts within columns differ ($P \leq .01$)

We concluded that EQUI-Si™ increased plasma Si concentrations in S mares (d 30) when compared to C mares in this study.

EQUI-Si™ and Milk Silicon, Calcium and Phosphorus Concentrations

We did not know whether supplemental Si would increase milk Si concentrations in horses, or if so, how long this process would take. Therefore, the next objective of this study was to determine if supplemental Si would increase the Si content in mares' milk. No studies to date have addressed this issue. In one study of mineral supplementation in 10 mares fed a high Cu and Zn diet, no differences were seen in milk Cu levels or mare and foal serum Cu levels between treatment groups (Baucus et al., 1989). In the current study, milk Si concentrations increased from d 0 to d 45 in the S mares (P = .001) (Table 4). Furthermore, on d 45, S mares' milk had higher Si concentrations then C mares' milk (P = .002). This would indicate that Si was transferred into mammary tissue, and subsequently being passed through to the milk. Therefore, supplemental Si does increase milk Si concentrations. In the current study, Si concentrations in the milk of control

Table 4. Means of milk Si concentrations (μg/L)

	Day				
Treatment	0	15	30	45	SEM
Control (C) n=6	224	460	393	265^{x}	128
Si Treated (S) n=6	242^{a}	468^{a}	547^{ab}	896^{by}	128

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

^{xy}treatments with different superscripts within columns differ (P ≤ .01)

mares were not statistically different by day (Table 4). However, there was a numerical increase in milk Si concentrations from d 0 on d 15 and 30. Due to high variability, this difference was not significant. I hypothesize that supplemental Si is necessary to change milk Si concentrations, and with higher experimental units, this numerical increase in the control group would not be present. For example, in human milk, Anderson (1993) also found no changes in Si concentrations over time in women who were not supplemented with Si. Therefore, unless horses are provided with an increased amount of Si during lactation, Si concentrations in milk will remain unchanged.

Adler et al. (1986) found that when male rats were injected with ^{31}Si, Si was quickly cleared from plasma and deposited in tissues. Labeled Si was found in highest concentrations in the kidney, followed by moderate deposition in the liver, lungs, bone, skin and spleen. Finally, low deposition of ^{31}Si was detected in skeletal and cardiac muscle and testes, and no deposition of labeled Si was found in the brain. Because all rats in this study were male, no deposition of ^{31}Si in mammary tissue was analyzed (Adler et al., 1986). In the current experiment, I hypothesized that Si was circulated through the body and transferred to mammary tissue, thereby increasing milk Si concentrations. The S mares may have passed this Si on to the foal via milk to allow the foal to deposit Si in tissues or excrete the excess. In mares, the mineral concentration of milk is highest during early lactation (first 4 to 6 weeks), and then decreases over time (Schryver et al., 1987). Based on previously cited literature, as the mineral content in milk decreases,

minerals may be utilized in other tissues, such as bone and collagen, rather than the mammary system.

To further determine the influence of EQUI-Si™ of milk mineral concentrations, Ca and P levels were determined for all milk samples. Milk Ca concentrations were different between S and C groups on d 0. This may be due to limited numbers of experimental units in this study, however, randomization was not of concern, as mares were assigned treatment groups prior to lactation. Calcium concentrations on d 15, 30 and 45 were standardized by subtracting each day from d 0, to allow each mare to serve as her own control. Milk Ca concentrations were then reported as mean differences from d 0. Actual data for milk Ca is reported in Appendix A Table 1A. Treated mares (S) had higher Ca concentrations on d 15 (P = .002), and tended to be higher on d 30 (P = .08), relative to d 0 (Table 5) than C mares. These differences in milk Ca concentrations between S and C mares may be a result of a potential Ca-Si interaction. Additionally, Si has been found to increase the bone mineral content in tibias of chicks fed a low Ca diet

Table 5. Means of differences from d 0 for milk Ca concentrations (mg/g)

	Day			
Treatment	15	30	45	SEM
Control (C) n=6	.27^{x}	.14	.24	.08
Si Treated (S) n=6	.66^{ay}	.35^{b}	.36^{b}	.08

^{ab}days with different superscripts within rows differ ($P \leq .01$)

^{xy}treatments with different superscripts within columns differ ($P \leq .01$)

(Carlisle, 1974), suggesting that Si and Ca may compete or interact to alter bone mineralization. This difference between S and C groups for milk Ca may also indicate that S mares absorbed more Ca from the gut, allowing for increased milk Ca output. In post-partum mares, milk Ca concentrations were found to increase for the first two d after foaling (Rook et al., 1997), and were highest in mares' milk the first wk of lactation, and then began to decrease as lactation progressed (Schryver et al., 1986). Milk Ca decreased 20, 30, 27, 22 and 12%, respectively, from the first wk through wk six of lactation (Schryver et al., 1986).

The role of EQUI-Si™ on milk P concentrations was also investigated. On d 15, S mares' milk contained higher P concentrations than C mares' milk (P = .05) (Table 6). This could perhaps be due to increased P absorption from the gut, or increased efficiency of P by the mare. This may also suggest a potential Si-P interaction, however, more research is needed to examine this potential interaction. Milk P levels were found to increase through d 2 of lactation (Rook et al., 1997) and were highest for the first wk, followed by a decline in P concentrations over time (Schryver et al., 1986). Milk P levels

Table 6. Means of P concentrations (mg/g) in milk

	Day				
Treatment	0	15	30	45	SEM
Control (C) n=6	.97^{a}	.68^{bx}	.61^{c}	.62^{bc}	.03
Si Treated (S) n=6	.92^{a}	.76^{by}	.61^{c}	.59^{c}	.03

^{abc}days with different superscripts within rows differ (P ≤ .05)

^{xy}treatments with different superscripts within columns differ (P ≤ .05)

decreased to the same degree as milk Ca levels from the first wk of lactation through wk six (Schryver et al., 1986).

EQUI-Si™ and Plasma Silicon Concentrations of Foals

Since Si was increased in milk concentrations from the S treated mares, the next objective was to determine if an increase in foal plasma Si concentrations could be observed. Plasma Si concentrations increased from d 0 to d 45 in S foals (P = .02) (Table 7). By d 45, S foals had higher plasma Si concentrations than the C group (P = .004). Plasma Si concentrations in S foals did not show a significant increase over C foals until d 45, which was the last day of the study. Coupled with these data are the results of the milk Si concentrations, where a treatment effect between S and C was not seen until d 45 (Table 4). There was a strong positive correlation between milk Si levels and foal plasma Si concentrations on d 45 (r^2 = .834) (P = .0007). No change in plasma Si concentrations was seen in C foals (Table 7). This is in agreement with the results of the plasma Si concentrations of control mares in the current study. Therefore, supplemental Si fed to mares increased Si concentrations in plasma of nursing foals.

Table 7. Means of plasma Si concentrations (μg/L) in foals

	Day				
Treatment	0	15	30	45	SEM
Control (C) n=6	1406	1239	1366	1146^{x}	166
Si Treated (S) n=6	1337^{a}	1463^{a}	1658^{ab}	1887^{by}	166

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

^{xy}treatments with different superscripts within columns differ (P ≤ .01)

Foal plasma Si concentrations were not different between treatment groups until d 45. It is possible that plasma Si concentrations in the S foals were not increased until d 45 because, intake from milk of S treated mares was not great enough to elicit an increase in the plasma Si concentration in the S treated foals. A longer study would be required to determine if elevated plasma Si concentrations could be maintained over time in the S foals.

EQUI-Si™ and Bone Metabolism of Mares and Foals

Silicon supplementation has been found to have effects on bone metabolism *in vitro*, however limited research has been done with Si supplementation *in vivo* and its role in bone metabolism. Researchers have reported that Si enhanced osteoblastic activity *in vitro* (Brady et al., 1991; Keeting et al., 1992), and inhibited osteoclastic activity *in vitro* (Schütze et al., 1995). Additionally, the researchers reported that bone metabolism was enhanced in rats when supplemented with sodium metasilicate, as evidenced by increased alkaline and acid phosphatase activity (Seaborn and Nielsen, 1994). However alkaline phosphatase activity is an indicator for a variety of metabolic activities, and therefore may not be an accurate marker of bone formation *in vivo*. Still, these studies indicate that Si might have a significant role in bone metabolism, and therefore our final objective of the current study was to determine if supplemental Si altered bone metabolism in lactating mares and foals. Osteocalcin in S mares tended to increase from d 0 to d 30 (P = .08) (Table 8), and to d 45 (P = .07). Treated mares tended

Table 8. Means of OC concentrations (ng/mL) in mares

	Day				
Treatment	0	15	30	45	SEM
Control (C) n=6	14.9	13.3	11.1^{x}	10.8^{x}	2.9
Si Treated (S) n=6	10.7^{a}	16.4^{ab}	18.0^{by}	18.4^{by}	2.9

[ab]days with different superscripts within rows tend to differ ($P \leq .1$)

[xy]treatments with different superscripts within columns tend to differ ($P \leq .1$)

to have higher OC levels than C mares on d 30 (P = .1) and d 45 (P = .07). This trend suggests that EQUI-Si™ may have a role in accelerating bone formation by enhancing osteoblastic activity, however higher numbers of animals, more replicates, or a longer study may be necessary to determine a statistically significant effect.

In women, bone mineral density and markers of bone formation, including OC, increased after termination of lactation (Tropeano et al., 1992). The effect of lactation remained unclear in the Tropeano et al. (1992) study. However, the increase in bone mineral density after lactation had stopped indicated that lactation yielded a negative effect on alteration of bone mineral density in postpartum women (Tropeano et al., 1992). Further research should be conducted to investigate the influence of lactation on bone mineral density, and if supplemental Si may be utilized to prevent bone mineral loss during lactation.

The rate at which Si effects OC in mares when given orally has not been established. In a study where six Arabians were orally dosed with dexamethasone at .2 mg/kg of body weight, OC concentrations were lower at two h through 72 h, when

compared to pretreated values (Geor et al., 1995). Dexamethasone is a glucocorticoid that decreases OC concentrations in humans and laboratory animals, and oral supplementation produced a rapid change in OC values in the Geor et al. (1995) study. It has not been established how quickly oral supplementation of Si effects OC in mares. In the current experiment, the study length or animal variability may have prevented treatment effects on OC in S mares.

We then analyzed the effects of supplemental Si on bone turnover in newborn foals. All foals, regardless of their dam's treatment, had increased OC over the 45 d study ($P = .0008$), by increasing from d 0 to d 15 and remaining high through the rest of the study (Table 9). This increase in OC for both S and C foals is seen immediately after birth and is evident by d 15, as the foals begin to apply load, or strain, on their skeletal system. Loading on bone has been shown to increase bone turnover, and yields greater bone mineral density and strength (Woo et al., 1981; Raub et al., 1989). This rapid rate of bone turnover will occur in young animals until they have reached skeletal maturity. Osteocalcin has been shown to decrease with age in humans (Kruse and Kracht, 1986),

Table 9. Means of OC concentrations (ng/mL) in foals

	Day			
Treatment	0	15	30	45
Control (C)	$69.4^{au} \pm 12.9$	$112.0^{bv} \pm 11.9$	$108.2^{bu} \pm 12.8$	$116.9^{bu} \pm 12.9$
Si Treated (S)	$61.4^{au} \pm 12.9$	$122.7^{bv} \pm 11.9$	$111.5^{bv} \pm 11.9$	$98.1^{bv} \pm 11.9$

^{ab}days with different superscripts within rows differ ($P \leq .01$)

$^{u}n = 5$; $^{v}n = 6$

dogs (Allen et al., 1998) and horses (Lepage et al., 1990, 1992), once the skeleton has reached maturity. Over time, OC values will decrease due to a slowdown in metabolic bone growth after skeletal maturation has peaked.

Osteocalcin is not affected by gender in horses (Lepage et al., 1992) or dogs (Allen et al., 1998). Therefore, the gender of the foals was not of concern in the current study. Concentrations of OC in this study were similar to that reported by Black et al. (1997) for one wk old Standardbred foals.

To further analyze the role of supplemental Si in bone metabolism, ICTP was studied. Postpartum ICTP concentrations for both C and S mares were constant through d 15, but decreased by d 30 and 45 (Table 10). There were no differences due to treatment in ICTP concentrations. This decrease may result from bone resorption required to mobilize Ca for milk production.

Table 10. Means of ICTP concentrations (μg/mL) in mares

	Day				
Treatment	0	15	30	45	SEM
Control (C) (n=6)	17.0^{a*}	17.4^{a}	10.6^{bu*}	9.0^{b}	1.7
Si Treated (S) (n=6)	15.0^{a}	15.7^{a*}	11.0^{b}	9.2^{b}	1.7

^{ab}days with different superscripts within rows differ ($P \leq .01$)
*SEM = 1.8; n = 5
^{u}n = 4

In a study involving postpartum dairy cows, ICTP concentrations increased from d 1 after calving through d 4, and peaked at d 5. This peak was followed by a decrease in ICTP concentrations from d 5 to d 14 postpartum (Liesegang et al., 1998). The results of

ICTP in the current study follow a similar pattern as reported by Lisegang et al. (1998), although the mares continued resorption through d 15. It was hypothesized that the resorption seen in the mares was caused by the need to utilize the Ca pool in bone to meet the increased Ca demands during early lactation. In a study analyzing plasma and milk concentrations of various minerals prior to foaling, as mares approached parturition, milk Ca concentrations increased, while serum Ca concentrations remained constant (Rook et al., 1997). Although Ca absorption rates were not discussed in this paper, this may explain the need for increased bone resorption from d 0 through d 15 in mares, thereby utilizing Ca from the resorbed bone to meet this increased Ca demand. However, over time this need for high Ca may be lessened or the mares are more efficient, and therefore bone resorption is diminished, which corresponds to the decrease from d 0 to d 30 and d 45 in ICTP concentrations in the current study. It has been established that milk Ca concentrations decrease after the first week of lactation in horses (Schryver et al., 1986).

To determine the role of supplemental Si in bone metabolism of foals, ICTP concentrations were measured to indicate bone degradation. The concentrations of ICTP did not change over time in foals from either C or S mares (Table 11). A longer experiment is necessary to determine if Si would alter bone resorption when supplemented for an extended period of time. However, overall bone turnover in this study indicates net bone formation, with an increase in OC and no change in ICTP concentrations over time for all foals. Brady et al. (1991), Keeting et al. (1992) (*in vitro)*

Table 11. Means of ICTP concentrations (μg/mL) in foals

	Day				
Treatment	0	15	30	45	SEM
Control (C) n=6	34.5	35.7	32.8	29.9	2.6
Si Treated (S) n=6	33.2	36.4	34.9	37.3*	2.6

*n = 5; SEM = 2.8

and Seaborn and Nielsen (1994) (in rats) indicate that silicon supplementation increased osteoblastic activity. Additionally, Schütze et al. (1995) indicated a decrease in *in vitro* osteoclastic activity (bone resorption) with zeolite A supplementation.

Previous reports of ICTP measurements for the young growing foal (first 45 d of life) had not been reported in the literature. The skeletal system is actively turning over in order to form bone during the rapid growth period of young animals (Parfitt, 1984). The variability observed in OC and ICTP in foals in the current study may be too great to determine a treatment effect. A follow-up study with a larger number of foals should be performed. A negative correlation was found between ICTP and age ($r = -.76$) ($P < .0001$), in a study analyzing alterations in bone metabolism with age in horses (Price et al., 1995). Price et al. (1995) found that as horses increased in age, ICTP decreased, especially through the first four years of growth. This relationship between ICTP and age was also found with dogs (Allen et al., 1998). Although age does affect ICTP levels, gender did not influence ICTP concentrations in dogs (Allen et al., 1998), and therefore gender of the foals was not of concern in the present experiment.

To further test the role of Si in skeletal metabolism of horses, PYD and DPD crosslinks may be analyzed to indicate collagen degradation (Black et al., 1999). Control mares had an increase in PYD from d 0 to d 15 (P = .04) (Table 12) and tended to increase from d 0 to d 30 (P = .09). The S group increased in PYD from d 0 to d 15 (P = .05) and decreased from d 15 to d 30 (P = .05). On d 30, C mares showed a trend for higher PYD levels than S treated mares (P = .08) (Table 12).

Table 12. Means of PYD concentrations (ng/mL) in mares

	Day				
Treatment	0	15	30	45	SEM
Control (C) (n=6)	1.2^{a}	2.0^{b}	2.0^{abx}	1.5^{ab}	.3
Si Treated (S) (n=6)	1.1^{a}	1.9^{b}	1.2^{ay}	1.0^{a}	.3

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

^{xy}treatments with different superscripts within columns differ ($P \leq .1$)

An increased urinary pyridinoline/creatinine ratio was found in cows at six d postpartum until 14 d to 16 d (Kaidi et al., 1991). On d 20, the ratios were normal for these cows. The increased excretion of PYD crosslinks correlated with the decrease in the size of the uterus during uterine involution, which took four weeks to complete (Kaidi et al., 1991). Additionally, Kaidi et al. (1991) found that PYD:DPD ratios were highest at five d postpartum, the peak involution period. Since the collagen content of the uterus increases during gestation, it is possible that the postpartum increase in PYD concentrations through d 30 in C mares and d 15 in S mares in the current study was due to uterine involution. The collagen in the uterus is being degraded as the uterus returns to

normal size, which releases PYD crosslinks. However, the period for involution for the mares in the current study appears to be longer than that necessary for the uterus to return to normal size in dairy cows.

Analysis of PYD in this study also included DPD concentrations. Deoxypyridinoline is bone specific, as it is most prevalently found in type I collagen (James et al., 1996). Therefore, increased PYD concentrations in this study may have been associated with increased bone resorption, which would be similar to the increase in resorption observed with ICTP values in the mares through the first 15 d. Additionally, Liesegang et al. (1998) found an increase in DPD levels in lactating dairy cows at d 2 through d 9 followed by a decrease from d 9 to d 14. The mares in this study showed elevated PYD concentrations through d 30 in C mares and d 15 in S mares. Supplemental Si may decrease bone resorption, as an *in vitro* experiment using purified avian osteoclasts treated with zeolite A demonstrated a three-fold decrease in osteoclastic pits at 24 h after treatment (Schütze et al., 1995). This would indicate that Si may have an inhibitory effect on bone resorption. Furthermore, in the current study, S mares may have had less of a need for bone resorption at that point in lactation, perhaps due to enhanced mineral utilization within bone. EQUI-Si™ supplementation may have reduced the need for a high Ca concentration this far into lactation, by utilizing available Ca more efficiently, and help reduce the need for bone degradation.

Finally, we determined the effects of EQUI-Si™ supplementation on collagen turnover in foals. In all foals, PYD concentrations decreased from d 0 to d 15 and remained low throughout the rest of the study (Table 13). No differences in PYD levels were seen between foal treatment groups. It is possible that supplemental Si does not affect collagen crosslink release in growing foals. Silicon may have alternative mechanisms in collagen, such as increasing the collagen content. However, it is likely that because it took 45 d for plasma Si levels to be higher in S treated foals (Table 6), a lack of treatment effect for PYD concentrations in foals may result.

Table 13. Means of PYD concentrations (ng/mL) in foals

	Day				
Treatment	0	15	30	45	SEM
Control (C) (n=6)	16.4[a]	9.2[b]	6.2[b]	6.6[b]	1.7
Si Treated (S) (n=6)	20.7[a]	7.0[b]	6.1[b]	6.7[b]	1.7

[ab]days with different superscripts within rows differ ($P \leq .01$)

A decrease in PYD concentrations for all foals was observed for the 45 d of this experiment, and therefore a decrease in collagen degradation was seen over time. This will allow for net bone deposition since OC levels are increasing. As horses mature skeletally, concentrations of PYD and DPD decrease with time (Black et al., 1997, 1999), and indicate a logical slowdown in growth as the horse matures. Black et al. (1999) found that PYD and DPD concentrations were higher in weanling horses than in adults. This relative slowdown in bone and collagen metabolism corresponds to that seen for other biochemical markers (Lepage et al., 1990, 1992; Price et al., 1995).

EQUI-Si™ Supplementation and Foal Growth

Throughout the current study all foals grew taller at the withers and hip and increased in weight over time (P = .0001) (Table 14). No differences in growth indicators existed between groups. Therefore, EQUI-Si™ treatment did not cause foals to grow more rapidly, alleviating any increased risk for growth-related diseases. The supplementation of feed additives could lead to developmental orthopedic diseases if foals grow more rapidly than their non-supplemented counterparts. The foals in the current study seemed to grow at normal rates, as growth measurements in other young horses follow the same patterns for wither and hip height growth (Black et al., 1997).

Table 14. Means of growth measurements for foals

Growth Measurement	Control (C)	Si Treated (S)	SEM (n=6 per group)
Wither Height			
D 0 Wither Height (cm)	94.2[a]	91.6[a]	1.3
D 45 Wither Height (cm)	109.5[b]	107.8[b]	1.3
Gain	15.3	16.2	
Hip Height			
D 0 Hip Height (cm)	95.8[a]	93.8[a]	1.5
D 45 Hip Height (cm)	114.3[b]	112.2[b]	1.5
Gain	18.5	18.4	
Weight			
D 0 Weight (kg)	43[a]	42[a]	2.2
D 45 Weight (kg)	93[b]	93[b]	2.2
Gain	50	51	

[ab]days with different superscripts per measurement within columns differ (P = .0001)

CHAPTER 3

EXPERIMENTAL PROCEDURE: EXPERIMENT 2

Assignment of Treatments and Management of Animals

Ten Arabian and 10 Quarter Horse yearlings were randomly assigned to one of two treatment groups of 10 horses each. All Arabians were maintained at the Michigan State University Horse Teaching and Research Center, and all Quarter Horses were maintained at the Michigan State University Merillat Equine Center. Prior to the study, the Arabian yearlings were housed on pasture continually, and were therefore brought into stalls for a three d acclimatization period. During this period, the Arabian yearlings were stalled at night, fed individually, and turned out during the day. However, prior to the study, the Quarter Horse yearlings were already housed in stalls, and were being longe-trained daily, and therefore did not need an acclimatization period.

The treated group (S) received approximately 2% of the diet of EQUI-Si™ as fed (.13 kg/day for Arabians and .16 kg/day for Quarter Horse yearlings). The control group (C) received 0% EQUI-Si™ in their diet. Horses were fed to maintain body condition. The Arabian diet was approximately 60% roughage (by weight) and 40% total concentrate, and met NRC (1989) requirements for long yearlings (Table 15 and 16). The hay was a grass and alfalfa mix. The concentrate consisted of 61.2% oats, 29.5% corn, 5.0% dairy protein pellets (Kent Feeds Inc., Muscatine, IA), 4.3% dry molasses,

and their respective EQUI-Si™ treatment. The Arabians were individually fed 5.6 kg of concentrate and 7.3 kg of hay daily divided into two equal feedings at 0700 and 1600. The Quarter Horse diet was approximately 60 % roughage (by weight) and 40 % total concentrate, and met NRC (1989) requirements for long yearlings (Table 117 and 18). The hay was an alfalfa and grass mix. The concentrate consisted of 1.8 kg of Strategy™ (Purina Feeds, St. Louis, MO), .45 kg of Equine Energy (Buckeye Nutrition, Dalton, Ohio), .15 kg of molasses, and their respective EQUI-Si™ treatment. The horses were individually fed, with each daily ration divided into two equal feedings at 0730 and 1530. The molasses enhanced palatability, and therefore feed refusal did not occur. All horses

Table 15. Calculated daily feed composition table for Arabian yearlings on an as fed basis

Nutrient	Alfalfa Hay	Grass Hay & Forage	Oats	Corn	Protein Pellet	Molasses
DE	3.43 Mcal	9.31 Mcal	9.72 Mcal	5.53 Mcal	.17 Mcal	.73 Mcal
CP	282 g	627 g	402 g	149 g	108 g	20.7 g
Ca	20.6 g	12.5 g	2.73 g	.82 g	3.65 g	2.37 g
P	3.98 g	12.5 g	11.6 g	4.43 g	2.7 g	.32 g
Mg	5.31 g	4.48 g	4.77 g	1.80 g	1.89 g	1.01 g
Cu	26.7 mg	109 mg	20.5 mg	6.05 mg	10.8 mg	17.0 mg

Table 16. Calculated daily nutrient table for Arabian yearlings on an as fed basis

Nutrient	Nutrient Content	Calculated Total Daily Intake
DE	2.35 Mcal/kg	28.9 Mcal
CP	12.9%	1590 g
Ca	.35%	43 g
P	.29%	36 g
Mg	.16%	19 g
Cu	15.45 mg/kg	190 mg

Table 17. Calculated daily feed composition table for Quarter Horse yearlings on an as fed basis

Nutrient	Alfalfa Hay	Grass Hay & Forage	Equine Athlete	Stategy	Molasses
DE	3.43 Mcal	9.31 Mcal	.572 Mcal	1.24 Mcal	.73 Mcal
CP	282 g	627 g	54 g	255 g	20.7 g
Ca	20.6 g	12.5 g	3.83 g	13.7 g	2.37 g
P	3.98 g	12.5 g	1.8 g	10.9 g	.32 g
Mg	5.31 g	4.48 g	-	-	1.01 g
Cu	26.7 mg	109 mg	9.09 mg	100 mg	17.0 mg

Table 18. Calculated daily nutrient table for Quarter Horse yearlings on an as fed basis

Nutrient	Nutrient Content	Calculated Total Daily Intake
DE	1.72 Mcal/kg	15.3 Mcal
CP	12.8%	1240 g
Ca	.55%	53 g
P	.32%	30 g
Mg	.11%	10 g
Cu	27 mg/kg	262 mg

were maintained on their respective diets for 45 d. Water and mineral blocks were provided for the horses at all times.

The Arabian yearlings were housed in stalls that were 3.1 m x 3.1 m or larger from 1600 until 0800 the next morning. They were turned out during the daytime into paddocks with an average stocking rate of 3.3 per paddock. Paddocks were approximately 2385 m^2, with brome, orchard, timothy and Kentucky blue grass forage. The Arabians were dewormed at the start of the study using a commercially available anthelmintic, but were not dewormed for the duration of the study, and received farrier work prior to commencing the study. The Quarter Horse yearlings were housed in stalls

that were 3.7 m x 3.7 m. They were exercised daily with the following exercise protocol: wk 1 – 30 min on mechanical walker at walk; wk 2 – 30 to 45 min at trot on mechanical walker; wk 3 and 4 – 10 circles at the trot and 10 circles at the lope in each direction on the longe line; wk 5 and 6 – 30 min total, with approximately 10 circles at trot and 20 circles at the lope in both directions on the longe line. The Quarter Horses were on Strongid® C (Pfizer Animal Health) Daily Dewormer prior to the start of the study, but were not dewormed for the duration of the study, and had their feet trimmed prior to the start of the study.

Growth Measurements

Weight was determined using a weight tape placed in the heart girth region at the withers. Wither and hip height for all animals was determined using a leveled measuring stick at the point of the withers and the tallest point of the croup, respectively. Hoof measurements were made by using a measuring tape, from the coronet to the bottom of the toe, to measure toe length, and from the coronet to the bottom of each heel bulb, for the right and left heel measurements. Results of hoof measurements can be found in Appendix D Tables 1 through 3. All measurements were taken on d 0 and d 45.

Sample Collection

Blood was collected as reported in experiment one, following protocol for collection time, days, and method. In addition, approximately 10 mL of blood was withdrawn using 18 g disposable sterile needles into a syringe (Luer-Lok ™, Becton

Dickinson and Company, Franklin Lakes, NJ) that was specially cleaned in a 10% nitric acid wash. The blood was then immediately transferred from the syringe into a polyurethane, Si-free collection tube (Falcon®, Becton Dickinson and Company, Franklin Lakes, NJ) with 115 μL of K_2EDTA in the bottom. The needle was punctured through parafilm covering the collection tube to prevent contamination, and the blood was mixed gently. This blood was used to obtain plasma for Si analysis. In the laboratory, the parafilm was removed from the polyurethane tubes within a special plastic-lined box, and the tubes were capped for centrifugation.

Sample centrifugation was performed as reported in experiment 1. After centrifugation, the serum samples were aliquotted into plastic 1.5-mL microcentrifuge tubes (DOT Scientific, Inc., Burton, MI) using disposable glass Pasteur pipettes (VWR Scientific, West Chester, PA). The plasma samples were withdrawn using acid washed plastic Samco brand transfer pipettes (Corning Samco Corporation, San Fernando, CA), and transferred into plastic 1.5-mL microcentrifuge tubes (DOT Scientific, Inc., Burton, MI) in a plastic-lined box to avoid dust contamination. Serum and plasma were then stored at –20°C until analysis.

Fecal samples were collected on d 45 by taking one fresh fecal ball out of each horse's stall. Fecal samples were then refrigerated until parasite analysis. Fecal samples were analyzed for parasites by the Animal Health Diagnostic Laboratory (College of

Veterinary Medicine, Michigan State University, PO Box 30076, Lansing, MI 48909). Samples were first qualitatively analyzed, and if eggs were present, then quantitative analysis was completed. The McMaster test was utilized for parasitic analysis. Results of the parasite analysis can be found in Appendix D Tables 4 and 5.

Minimizing Silicon Contamination and Special Cleaning of Labware

The procedure used in experiment 1 was also followed in this experiment to prevent Si contamination. The plasma samples from all yearlings were handled in the same manner as the plasma samples taken with the vacuum tubes in experiment 1.

Biochemical Marker Analysis

Serum was analyzed for OC, ICTP and PYD as reported in experiment 1.

Silicon Analysis

Plasma Si was again analyzed by Trace Elements Laboratory, London Health Sciences Centre (refer to experiment 1).

Statistical Analysis

Data were summarized as LSMeans and SEM. Treatment effects on Si, OC, ICTP, PYD and growth measurements were analyzed as a two-factor ANOVA for repeated measures using the Proc Mixed procedure of SAS (6.12) (SAS, 1997). The class included horse, day, treatment and farm, and the model included treatment, day, farm, time by treatment, treatment by farm, time by farm, and day by treatment by farm interactions. If the day by treatment by farm interaction was greater than $P \geq .3$, then data

were pooled from both farms and summarized as LSMeans and SEM. However, if this interaction was less than $P < .3$, as was the case with plasma Si levels, then a Student's t-test was applied to examine differences between the farms by day. If d 0 was different for the two farms, then farms were pooled and d 15, d 30 and d 45 values were standardized by subtracting them from d 0, allowing each horse to serve as its own control. Once pooled, the differences from d 0 were reported as LSMeans and SEM for each treatment group. For all analyses, a P-value of less than .05 was considered significant, and trends were studied at a P-value of less than or equal to .1.

EQUI-Si™ Supplementation and Plasma Silicon Concentrations

Supplemental Si has been shown to increase plasma Si concentrations in horses (Frey et al., 1992; Nielsen et al., 1993). Therefore, out first objective was to determine if Equi-Si™ supplementation would increase plasma Si concentrations in Arabian and Quarter Horse yearlings. Plasma Si concentrations were found to be different on d 0 at the two farms ($P < .0001$), and therefore, all plasma Si concentrations for all yearlings were standardized by subtracting them from d 0. This allowed each horse to serve as its own control, and then all yearlings were pooled to determine treatment effects. Plasma Si levels showed a greater difference from d 0 on d 15, 30 and 45 ($P = .0001$ for all days) in S yearlings when compared to the C yearlings (Table 19). Equi-Si™ supplementation increased plasma Si concentrations in S yearlings, which indicates that Equi-Si™ is an absorbable form of Si.

Table 19. Means of differences from day 0 for plasma Si concentrations (μg/L) in yearlings

	Day			
Treatment	15	30	45	SEM
Control (C) (n=6)	-70.9^{ax}	84.0^{bx}	0.1^{ax}	49.7
Si Treated (S) (n=6)	404.8^{ay}	509.9^{by}	326.9^{cy}	49.7

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

^{xy}treatments with different superscripts within columns differ ($P \leq .01$)

Interestingly, the Arabian and Quarter Horse yearlings in this study had different plasma Si values on d 0. This difference on d 0 may simply have been due to variable environmental or management factors. Silicon is very abundant in the environment, and is naturally found in grains and grain fractions (Pennington, 1991), and in soil and grass (Hays and Swenson, 1984). The Quarter Horses had a higher basal plasma Si concentration (353 μg/L ± 29) than the Arabians (176 μg/L ± 56) ($P < .0001$) on d 0. The Arabians were on pasture prior to the study, and were turned out daily during the experiment. Conversely, the Quarter Horses were housed in stalls prior to and during the study. Therefore, differences in management may have led to different basal plasma Si concentrations.

EQUI-Si™ Supplementation and Bone and Collagen Metabolism

Since plasma Si concentrations were elevated in S yearlings, our second objective was to determine if increased plasma Si concentrations would alter bone and collagen metabolism. Osteocalcin concentrations tended to decrease from d 0 to d 15 ($P = .07$) and tended to increase from d 15 to d 30 ($P = .06$) and increased from d 15 to d 45 ($P = .04$) in C yearlings (Table 20). These results were surprising that C increased in OC concentration on d 45 when compared to d 15. This increase could be due to high variability, and would likely not be seen with larger numbers of animals. The S group had no change in OC concentrations over time, and there were no differences in serum OC between treatment groups.

Table 20. Means of OC concentrations (ng/mL) in yearlings

	Day			
Treatment	0	15	30	45
Control (C) (n=10)	$77.7^{ab} \pm 22.9$	$57.6^{au} \pm 23.0$	$79.8^{abu} \pm 23.2$	$90.9^{bu} \pm 23.4$
Si Treated (S) (n=10)	$56.7^{a} \pm 22.9$	$43.6^{a} \pm 22.9$	$45.1^{au} \pm 23.1$	$48.9^{a} \pm 23.8$

[ab]days with different superscripts within rows differ ($P \leq .05$)

[u]n = 9

Markers for bone formation and resorption were increased when weanling Sprague-Dawley rats were fed normal physiological amounts of Si (Seaborn and Nielsen, 1994). An increase in alkaline and acid phosphatase activity was seen in homogenized femurs of rats, however Seaborn and Nielsen (1994) concluded that the mechanism for the altered metabolism is still unclear. Additionally, *in vitro*, zeolite A increased proliferation of human osteoblast-like cells, thereby enhancing osteoblastic activity (Brady et al., 1991). Keeting et al. (1992) found that zeolite A increased Transforming Growth Factor - ß production, human osteoblast-like cell proliferation and cellular alkaline phosphatase activity *in vitro*. Furthermore, an increase in OC was seen with zeolite A treatment *in vitro* (Keeting et al., 1992). However, *in vitro* studies using zeolite A may not be a true indicator of how this molecule would behave *in vivo*. In the current study, I hypothesized that Si was not deposited in bone for a long enough period of time to alter osteoblastic activity. This increase in activity may occur by increased cell proliferation or differentiation, upregulated protein sythesis or other potential

mechanisms. Alternatively, it is possible that Si has a role in bone and collagen structure other than altering bone metabolism, or may have no influence on bone.

Next we examined if EQUI-Si™ would alter bone degradation by measuring ICTP concentrations. In the current study, ICTP was lower in S yearlings when compared to C on d 45 (P = .04) (Table 21). This may indicate that EQUI-Si™ has a role in decreasing bone resorption. Although resorption is a crucial component of bone remodeling, these treated animals appear to be resorbing bone, but to a lesser degree than C yearlings. Therefore, since OC remains unchanged in S yearlings, net bone formation should be higher in S. Net bone formation may be seen in S, regardless of the increase seen for OC levels in C from d 15 to d 45.

Table 21. Means of ICTP concentrations (μg/mL) in yearlings

	Day				
Treatment	0	15	30	45	SEM
Control (C) (n=10)	16.7	16.2	17.1	16.9^{x}	.6
Si Treated (S) (n=10)	16.5^{ab}	16.6^{ab}	16.4^{au}	15.2^{by}	.6

^{ab}days with different superscripts within rows differ (P ≤ .05)
^{xy}treatments with different superscripts within columns differ (P ≤ .05)
^{u}n = 9

Supplemental Si might affect a mediator in bone that activates osteoclastic activity. Once this has begun, the osteoclastic enzymes may activate osteoblastic enzymes, by employing the extensions of the osteoclast cell that reach into the matrix and sending messages to the osteoblast to stimulate activity. Enzymatic activities in the

osteoblast and osteoclast alter each other's activity (Mohan et al., 1984). Chambers (1982) found that as osteoclastic activity increases, osteoblasts are activated and begin to increase in osteoblastic activity. Therefore, supplemental Si may first act on bone resorption through altering osteoclastic activity, and this in turn may activate osteoblastic action. Altering both osteoblastic and osteoclastic activity will alter total bone metabolism. However, in the current study it took 45 d to alter bone resorption in the yearlings, and there was not enough time to determine if this decreased resorption would stimulate bone formation. A longer study would be required to determine if OC release would be initiated after ICTP release has progressed. In a study demonstrating the role of supplemental Si in bone resorption, rats were implanted with mineralized or demineralized bone, taken from other Si supplemented or Si deficient rats (Seaborn and Nielsen, 1994). Seaborn and Nielsen (1994) found an interaction between Si supplementation and the implant bone source for both mineralized and demineralized bone implants in the implanted rats. Less acid phosphatase activity was found in the implanted bone portion of Si supplemented rat tibias, when compared to the implanted bone portion from Si deficient rat tibias, indicating decreased bone resorption in the Si supplemented rats (Seaborn and Nielsen, 1994). In Seaborn and Nielsen's (1994) study, bone formation was not affected by the implant, as evidenced by no change in alkaline phosphatase activity.

Since OC concentrations remained unchanged in S yearling horses in the current study, and ICTP levels decreased in the S group, net bone formation may result. This could potentially reduce the incidence of bone-related injuries later on during exercise, as there would be a greater amount of bone. In one study, a trend was observed in weanling Quarter Horses treated with sodium zeolite A to have a greater percent change in radiographic bone aluminum equivalence (RBAE) on the lateral side of the third metacarpal ($P < .1$), when compared to the non-supplemented control (Frey et al., 1992). Frey et al. (1992) concluded that this trend for a change in RBAE may have been caused by an interaction of Si and other minerals of the hydroxyapatite during early bone calcification. Carlisle (1970) found this type of Si-Ca interaction in young mice and rats, as Si was concentrated in calcification sites early during skeletal growth, but as the bone mineral reached maturity and Ca levels increased, Si levels were only present at minimal levels. A second study supporting that Si supplementation may potentially decrease the incidence of bone-related injuries was done on young Quarter Horses in race training (Nielsen et al., 1993). Nielsen et al. (1993) found that horses supplemented with sodium zeolite A had faster race times. It was felt that the faster horses in the non-supplemented group sustained more injuries, and hence, were removed from the study, thereby reducing the average speed of the non-supplemented group. Nielsen et al. (1993) also found that supplemented horses ran a greater amount of strides before experiencing a bone-related injury, when compared to non-supplemented horses and lower-dose supplemented.

Therefore, Si may make intensely exercised horses less susceptible to bone-related failure.

To further test Si's role in bone and collagen metabolism, measurements of serum PYD concentrations were made. Silicon has increased the collagen content of bone *in vitro* (Carlisle and Alpenfels, 1978), and Si deficient chicks had malformed skulls and long bones, and smaller or absent combs, which all normally contain a high collagen content. Therefore, we also studied the role of EQUI-Si™ supplementation in collagen metabolism. No differences in PYD concentrations were seen by day in the C or S treated group (Table 22). Furthermore, there were no differences in PYD concentrations between treatment groups on any day. This lack of treatment effect may be explained by one of several potential reasons. First, Si may have been deposited in collagen, but did not have time to alter the turnover of the collagen molecules. This may be possible, even though an increase in Si was seen between S and C on d 15 (Table 19), because it is unknown how long it would take to detect differences between groups for PYD, and 45 d may not have been long enough to determine differences. Secondly, the increased Si levels may have been deposited into other tissues first, and therefore, never accumulated

Table 22. Means of PYD concentrations (ng/mL) in yearlings

	Day				
Treatment	0	15	30	45	SEM
Control (C) (n=10)	4.8	4.2	4.1[u]	4.1	.3
Si Treated (S) (n=10)	4.7	4.1	4.4	4.0	.3

[u]n=9

in collagen. Collagen might not have a need for Si at this point in development, and therefore was not incorporated into the collagen tissue. In growing chicks, when the Si content of the diet was deficient, collagenous tissues were smaller and abnormal; however, supplemental Si did not increase collagen content in the normal chicks (Carlisle, 1972).

EQUI-Si™ Supplementation and Yearling Growth

All yearlings in the current study grew taller at the withers and hip, and increased in weight over time ($P \leq .002$) (Table 23). No differences existed between treatment groups for the three growth measurements. This is important, as S treatment did not alter growth as compared to C yearlings, illustrating that EQUI-Si™ does not increase the risk of growth-related diseases. Yearlings are still growing animals, and similar growth measurements are supported by measurements taken in other yearling horses (Wall et al., 1998).

Table 23. Means of growth measurements in yearlings

Growth Measurement	Control (C)	Si Treated (S)	SEM (n=10 per group)
Wither Height			
D 0 Wither Height (cm)	137.8[a]	139.3[a]	1.5
D 45 Wither Height (cm)	140.0[b]	141.2[b]	1.5
Gain	2.2	1.9	
Hip Height			
D 0 Hip Height (cm)	142.5[a]	145.0[a]	1.4
D 45 Hip Height (cm)	143.1[b]	146.0[b]	1.4
Gain	.6	1.0	
Weight			
D 0 Weight (kg)	332[a]	341[a]	9
D 45 Weight (kg)	352[b]	360[b]	9
Gain	20	19	

[ab]days with different superscripts per measurement within columns differ (P = .002)

CHAPTER 4

CONCLUSIONS AND IMPLICATIONS

Enhancing net bone formation may provide a more superior skeleton. Modeling and remodeling is increased through manipulation of hormonal stimulators, microfracture repair, or exogenous factors, such as nutritional supplements. Therefore, supplementation with certain feed additives may have the potential to alleviate bone-related failure or diseases involving bone loss.

In the present studies, EQUI-Si™, a Si containing feed additive, was fed to horses of various production levels in hopes of altering bone turnover. Silicon has been shown in the literature to increase metabolism of cells *in vitro* (Brady et al., 1991; Keeting et al., 1992; Schütze et al., 1995), and to stimulate bone formation and resorption in rats (Seaborn and Nielsen, 1994). The current studies were designed to examine potential alterations of bone metabolism due to Si supplementation in the horse. Biochemical markers were utilized to determine the effect of EQUI-Si™ in achieving altered bone turnover. While, these markers are indices of systemic trends of bone turnover, they do not provide evidence as to the quality and/or quantity of the altered bone. Terminal studies using animal models are required to fully understand the role of supplemental Si *in vivo*.

In the current study, EQUI-Si™ increased plasma Si concentrations in mares and yearlings, and increased milk Si concentrations, thereby increasing plasma Si concentrations in foals. These increases in plasma Si concentrations illustrate EQUI-Si™'s bioavailability, as it was actively absorbed into blood. The bioavailabilities of Si sources differ, but EQUI-Si™ appears to be efficacious in increasing plasma Si concentrations of horses of various ages. Furthermore, this plasma Si is transported throughout the body, as evident by the increased milk Si concentrations of S mares.

EQUI-Si™ increased milk Ca and P concentrations in S mares on d 15. Increased Ca and P concentrations in milk can be utilized by the foal for bone mineral, if foals are consuming adequate milk. However, milk yield was not determined in this study. The increase in Ca and P may suggest that EQUI-Si™ mobilized Ca and P from bone during lactation. A second potential reason for the increased Ca and P concentrations in milk on d 15 may be that EQUI-Si™ increases absorption of these minerals from the gut, thereby allowing for more output in the milk. Dietary absorption was not examined in these studies. Further experiments examining the role of EQUI-Si™ in lactating mares are required to determine supplemental Si's influence on lactation and bone turnover.

Bone turnover appeared to be altered in mares and yearling horses supplemented with EQUI-Si™. The trends seen for increased osteoblastic activity and decreased bone and collagen degradation in S mares may indicate net bone formation. Therefore,

stronger bone may result, allowing the mares to be exercised or rebred with little adverse effects on the skeletal system. The decrease in bone degradation seen in S yearlings may also indicate net bone formation, as OC was not changed in the S group. A higher degree of bone formation may equate with stronger bone, allowing for less bone-related injuries and increasing the longevity of horses. The lack of treatment effect in foals was because it took 45 d for plasma Si concentrations to be increased in foals, and this marked the end of the study. A longer study may produce different results than those found in the current study. Furthermore, normal bone turnover is very rapid during growth, and may be masking treatment effects in the bone turnover markers in foals, because of high individual variability.

Silicon may have an alternate mechanism in bone and collagen physiology and metabolism. One alternative may be that Si changes the hydroxyapatite crystal in size, structure or content. When other minerals (besides Ca and P) incorporate into hydroxyapatite, its structural and functional characteristics may be altered (Hays and Swenson, 1984; Wasserman, 1984). This may impact the strength and integrity of bone. A second alternative is that Si may alter mineralization by activating or inhibiting the calcification process. Carlisle (1970, 1974) proposes that Si may influence the mineralization process, but the exact mechanism for this has not been established. Silicon may alter the collagen crosslinks themselves, by increasing their number or structure. Additionally, Si may alter the glycosaminoglycan content of connective tissue,

by increasing the number or structure of the molecules. Carlisle (1976) found that Si supplementation in chicks increased the glycosaminoglycan levels in cartilage.

Additional research studying the histology of bone and collagen is necessary to understand the complete role of Si in these tissues. Silicon may be altering the bone mineral density, strength and porosity. Furthermore, the degree of Si deposition in bone and collagen tissue at various stages of growth and maturation still needs to be determined. Further research is required to examine these alternative mechanisms of Si in bone and collagen.

Currently, Si requirements for the horse are not included in the NRC (1989). Continued research may provide evidence for such recommendations. While Si is naturally found in grains and dirt (Pennington, 1991), not all of this Si is available. Bioavailable supplements, such as EQUI-Si™, may prove to be beneficial in enhancing skeletal health, and may aid in alleviating bone-related injuries or diseases involving bone loss. While many factors govern bone metabolism, supplemental Si may provide a nutritional means of enhancing skeletal health. More research in the area of Si and bone is needed to answer questions regarding potential biochemical mechanisms and other roles for Si in bone physiology.

APPENDIX A

APPENDIX A

Non-normalized milk Ca concentrations data and ANOVA table (Project 1)

Appendix Table 1A. Non-normalized means of milk Ca concentrations (mg/g) in Arabian mares

	Day				
Treatment	0	15	30	45	SEM
Control (C) (n=6)	1.13^{ax}	1.40^{bx}	1.28^{ab}	1.37^{b}	.06
Si Treated (S) (n=6)	$.928^{ay}$	1.59^{by}	1.28^{c}	1.29^{c}	.06

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

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

Appendix Table 1B. Proc mixed table for non-normalized milk Ca concentrations (mg/g) for Arabian mares

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	.19	.6753
Day	3	30	31.54	.0001
Day*Treatment	3	30	5.68	.0033

Non-normalized plasma Si concentrations for yearlings (Project 2)

Appendix Table 3A. Non-normalized means of plasma Si concentrations (µg/L) in yearlings

	Day				
Treatment	0	15	30	45	SEM
Control (C) (n=10)	249.7^{ab}	178.8^{ax}	333.7^{bx}	249.8^{ax}	32.7
Si Treated (S) (n=10)	278.8^{a}	683.6^{by}	788.7^{cy}	605.7^{by}	32.7

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

^{xy}treatments with different superscripts within columns differ ($P \leq .01$)

Appendix Table 4A. T-test for D 0 for plasma Si concentrations for yearlings

Breed	Mean Plasma Si concentration	Std Dev	Std Error
Arabians (n=10)	175.8	28.7	9.08
Quarter Horses (n=10)	352.7	55.7	17.6

APPENDIX B

APPENDIX B

Proc Mixed Tables

Appendix Table 1B. Proc mixed table for plasma Si concentrations (μg/L) for Arabian mares

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	2.90	.1192
Day	3	30	2.35	.0925
Day*Treatment	3	30	2.29	.0987

Appendix Table 2B. Proc mixed table for milk Si concentrations (μg/L) for Arabian mares

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	4.82	.0528
Day	3	30	2.61	.0699
Day*Treatment	3	30	2.60	.0703

Appendix Table 3B. Proc mixed table for milk Ca concentrations (mg/g) for Arabian mares

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	6.75	.0266
Day	2	20	10.30	.0008
Day*Treatment	2	20	2.75	.0877

Appendix Table 4B. Proc mixed table for milk P concentrations (mg/g) for Arabian mares

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	0.00	.9517
Day	3	29	63.90	.0001
Day*Treatment	3	29	3.47	.0287

Appendix Table 5B. Proc mixed table for plasma Si concentrations (μg/L) for Arabian foals

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	3.26	.1013
Day	3	30	0.67	.5780
Day*Treatment	3	30	2.44	.0840

Appendix Table 6B. Proc mixed table for serum OC concentrations (ng/mL) for Arabian mares

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	2.57	.1400
Day	3	30	0.22	.8827
Day*Treatment	3	30	1.77	.1732

Appendix Table 7B. Proc mixed table for serum OC concentrations (ng/mL) for Arabian foals

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	0.08	.7769
Day	3	26	8.91	.0003
Day*Treatment	3	26	0.71	.5570

Appendix Table 8B. Proc mixed table for serum ICTP concentrations (μl/L) for Arabian mares

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	0.11	.7436
Day	3	26	21.16	.0001
Day*Treatment	3	26	0.61	.6130

Appendix Table 9B. Proc mixed table for serum ICTP concentrations (μl/L) for Arabian foals

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	0.90	.3660
Day	3	29	0.56	.6468
Day*Treatment	3	29	0.97	.4199

Appendix Table 10B. Proc mixed table for serum PYD concentrations (ng/mL) for Arabian mares

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	2.45	.1486
Day	3	27	3.78	.0220
Day*Treatment	3	27	0.65	.5913

Appendix Table 11B. Proc mixed table for serum PYD concentrations (ng/mL) for Arabian foals

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	0.18	.6773
Day	3	30	23.61	.0001
Day*Treatment	3	30	1.35	.2782

Appendix Table 12B. Proc mixed table for plasma Si concentrations (μg/L) for yearlings

Source	NDF	DDF	F-value	P-Value
Treatment	1	16	128.04	.0001
Day	3	48	32.16	.0001
Farm	1	16	4.19	.0575
Day*Treatment	3	48	28.25	.0001
Treatment*Farm	1	16	10.16	.0057
Day*Farm	3	48	5.17	.0036
Day*Trt*Farm	3	48	3.48	.0229

Appendix Table 13B. Proc mixed table for normalized plasma Si concentrations (ng/mL) for yearlings

Source	NDF	DDF	F-value	P-Value
Treatment	1	18	46.48	.0001
Day	2	36	18.23	.0001
Day*Treatment	2	36	2.19	.1263

Appendix Table 14B. Proc mixed table for serum OC concentrations (ng/mL) for yearlings

Source	NDF	DDF	F-value	P-Value
Treatment	1	16	0.84	.3732
Day	3	42	2.54	.0692
Farm	1	16	1.72	.2088
Day*Treatment	3	42	0.71	.5518
Treatment*Farm	1	16	0.50	.4896
Day*Farm	3	42	1.97	.1336
Day*Trt*Farm	3	42	0.44	.7273

Appendix Table 15B. Proc mixed table for serum ICTP concentrations (µl/L) for yearlings

Source	NDF	DDF	F-value	P-Value
Treatment	1	16	0.81	.3808
Day	3	47	1.09	.3637
Farm	1	16	11.70	.0035
Day*Treatment	3	47	1.48	.2312
Treatment*Farm	1	16	0.45	.5136
Day*Farm	3	47	6.49	.0009
Day*Trt*Farm	3	47	1.28	.2936

Appendix Table 16B. Proc mixed table for serum PYD concentrations (ng/mL) for yearlings

Source	NDF	DDF	F-value	P-Value
Treatment	1	16	0.02	.8878
Day	3	46	2.23	.0970
Farm	1	16	0.21	.6566
Day*Treatment	3	46	0.23	.8727
Treatment*Farm	1	16	0.01	.9072
Day*Farm	3	46	2.53	.0685
Day*Trt*Farm	3	46	0.66	.5818

Appendix Table 17B. Proc mixed table for foal wither height (cm)

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	2.65	.1348
Day	1	10	142.05	.0001
Day*Treatment	1	10	0.09	.7683

Appendix Table 18B. Proc mixed table for foal hip height (cm)

Source	NDF	DDF	F-value	P-Value
Treatment	1	10	1.94	.1943
Day	1	10	159.06	.0001
Day*Treatment	1	10	0.00	.9734

Appendix Table 19B. Proc mixed table for yearling wither height (cm)

Source	NDF	DDF	F-value	P-Value
Treatment	1	16	0.33	.5759
Day	1	16	4.20	.0572
Farm	1	16	38.34	.0001
Day*Treatment	1	16	0.11	.7496
Treatment*Farm	1	16	3.17	.0940
Day*Farm	1	16	0.03	.8687
Day*Trt*Farm	1	16	0.04	.8514

Appendix Table 20B. Proc mixed table for yearling hip height (cm)

Source	NDF	DDF	F-value	P-Value
Treatment	1	16	0.68	.4223
Day	1	16	5.10	.0382
Farm	1	16	62.27	.0001
Day*Treatment	1	16	0.02	.8960
Treatment*Farm	1	16	2.80	.1136
Day*Farm	1	16	0.37	.5497
Day*Trt*Farm	1	16	0.01	.9375

Appendix Table 21B. Proc mixed table for yearling weight (kg)

Source	NDF	DDF	F-value	P-Value
Treatment	1	16	0.11	.7474
Day	1	16	7.46	.0148
Farm	1	16	92.85	.0001
Day*Treatment	1	16	0.13	.7228
Treatment*Farm	1	16	0.79	.3879
Day*Farm	1	16	0.00	.9994
Day*Trt*Farm	1	16	0.07	.7962

APPENDIX C

APPENDIX C

Proc Corr Tables

Appendix Table 1C. Proc corr table for D 0 milk Si concentration and foal plasma Si levels

	Milk Si Concentration	Plasma Si Concentration
Milk Si Concentration	1.0000	-0.2808
P-Value	0.0	.3766
Foal Plasma Si Concentration	-0.2808	1.0000
P-Value	.3766	0.0

Appendix Table 2C. Proc corr table for D 15 milk Si concentration and foal plasma Si levels

	Milk Si Concentration	Plasma Si Concentration
Milk Si Concentration	1.0000	0.2150
P-Value	0.0	.5022
Foal Plasma Si Concentration	0.2150	1.0000
P-Value	.5022	0.0

Appendix Table 3C. Proc corr table for D 30 milk Si concentration and foal plasma Si levels

	Milk Si Concentration	Plasma Si Concentration
Milk Si Concentration	1.0000	0.3419
P-Value	0.0	.2766
Foal Plasma Si Concentration	0.3419	1.0000
P-Value	.2766	0.0

Appendix Table 4C. Proc corr table for D 45 milk Si concentration and foal plasma Si levels

	Milk Si Concentration	Plasma Si Concentration
Milk Si Concentration	1.0000	0.8342
P-Value	0.0	.0007
Foal Plasma Si Concentration	0.8342	1.0000
P-Value	.0007	0.0

Appendix Table 5C. Proc corr table for D 0 milk Si concentration and mare plasma Si levels

	Milk Si Concentration	Plasma Si Concentration
Milk Si Concentration	1.0000	0.7313
P-Value	0.0	.0069
Mare Plasma Si Concentration	0.7313	1.0000
P-Value	.0069	0.0

Appendix Table 6C. Proc corr table for D 15 milk Si concentration and mare plasma Si levels

	Milk Si Concentration	Plasma Si Concentration
Milk Si Concentration	1.0000	0.0637
P-Value	0.0	.8441
Mare Plasma Si Concentration	0.0637	1.0000
P-Value	.8441	0.0

Appendix Table 7C. Proc corr table for D 30 milk Si concentration and mare plasma Si levels

	Milk Si Concentration	Plasma Si Concentration
Milk Si Concentration	1.0000	0.3370
P-Value	0.0	.2840
Mare Plasma Si Concentration	0.3370	1.0000
P-Value	.2840	0.0

Appendix Table 8C. Proc corr table for D 45 milk Si concentration and mare plasma Si levels

	Milk Si Concentration	Plasma Si Concentration
Milk Si Concentration	1.0000	0.0204
P-Value	0.0	.9499
Mare Plasma Si Concentration	0.0204	1.0000
P-Value	.9499	0.0

APPENDIX D

APPENDIX D

Hoof Measurement Data Tables: Experiment 2

Appendix Table 1D. Mean Toe Length (cm) for Yearlings

	Day			
	0	SEM	45	SEM
Control (C)	4.87	.3	5.47	.3
Si Treated (S)	4.78	.3	5.33	.3

Appendix Table 2D. Mean Right Heel Length (cm) for Yearlings

	Day			
	0	SEM	45	SEM
Control (C)	2.95	.22	3.63	.22
Si Treated (S)	2.77	.22	3.5	.22

Appendix Table 3D. Mean Left Heel Length (cm) for Yearlings

	Day			
	0	SEM	45	SEM
Control (C)	2.9	.22	3.62	.23
Si Treated (S)	2.83	.23	3.62	.23

Parasite Data Tables: Experiment 2

Appendix Table 4D. Mean Parasite Egg Number for Arabian Yearlings on d 45

	Mean Parasite Egg Number	SEM
Control (C)	30.0	24.24
Si Treated (S)	0	0

Appendix Table 5D. Mean Parasite Egg Number for Quarter Horse Yearlings on d 45

	Mean Parasite Egg Number	SEM
Control (C)	690	330.49
Si Treated (S)	635	294.17

APPENDIX E

APPENDIX E

Comparison of Blood Sample Collection Methods and Their Effect on Plasma Si Concentrations

Introduction

Silicon (Si) analysis using electrothermal atomic absorption spectrometry was reported by Leung and Edmond (1997). Proper sample collection appears to be critical for accurate results. Special care and handling of sampling materials is also critical. Glass pipettes, syringes or vacuum tubes should be avoided when obtaining samples, as glass will contaminate your sample. Plastic labware should be used instead (Gitelman and Alderman, 1990). Silicon is a component of glass, as glass is made of sand. Sand and dirt contain large amounts of Si.

Little is known about the extent that blood leaches Si from glass before the blood is centrifuged and stored. A potential leaching effect may be aggravated by pH differences. Additives in the collection tube, such as ethylenediamine tetracetic acid (K_2EDTA) or heparin may also chelate Si.

Because of the existing questions regarding Si collection and sampling, three methods of blood collection were originally utilized in this study. However, only glass plasma collection tubes were used in the current study to reduce stress to the foals during sample collections, as both serum and plasma were collected, and vacuum tubes made for

ease of collection. Plasma Si was analyzed by Trace Elements Laboratory, LHSC (University Campus, 339 Windermere Road, London, Ontario N6A 5A5, Canada). Because of a limited amount of funds, the three methods were analyzed for only two mares. Our objective was to determine differences in Si concentrations, as taken by plastic collection tubes containing K_2EDTA, glass tubes containing K_2EDTA and glass tubes containing heparin.

Methods

Minimizing silicon contamination:

Two to three d prior to collection, all plastic equipment, including syringes, plastic collection tubes and the transfer pipettes, was specially washed using a 10% nitric acid wash. The equipment was then rinsed three times with double distilled water (dd H_2O) and stored in a fume hood to allow for drying and to minimize Si contamination from dust. The plasticware, when taken on the farm, was kept in a cooler until needed, and replaced immediately after completion of the collection to avoid contamination.

In the laboratory, all samples were transferred to storage tubes within a plastic wrap lined box. This box was only open to the front, and this opening had a sheet of plastic wrap to serve as a curtain to prevent dust contamination. Plasma samples were only transferred with the specially cleaned plastic pipettes within the box. The Si-free storage microcentrifuge tubes were closed tightly, and then stored at -2°C.

K_2EDTA Preparation for Plastic Collection Tubes:

Fifteen g of K_2EDTA and 100 mL of dd H_20 were mixed and stored in a plastic acid washed bottle in the refrigerator. Then, K_2EDTA (115 µL) was added to the polyurethane tubes.

Sample Collection:

One 7-mL Vacutainer® tube with K_2EDTA additive and one 10-mL Vacutainer® tube with heparin additive (Becton Dickinson and Company, Franklin Lakes, NJ) were collected using the same 18 g x 3.8 cm disposable needles (Becton Dickinson and Company, Franklin Lakes, NJ) from two adult mares. Additionally, 10 mL of blood from each mare was withdrawn using 18 g disposable sterile needles into a syringe (Luer-Lok™, Becton Dickinson and Company, Franklin Lakes, NJ) that was specially cleaned in a 10% nitric acid wash. The blood was then immediately transferred from the syringe into a polyurethane Si-free collection tube (Falcon®, Becton Dickinson and Company, Franklin Lakes, NJ) with 115 µL of K_2EDTA in the bottom. The needle was punctured through parafilm covering the collection tube to prevent contamination, and the blood was mixed gently. In the laboratory, the parafilm was removed from the polyurethane tubes, and the tubes were capped for centrifugation.

The blood samples were centrifuged for 20 min at 754 x g. After centrifugation, plasma was withdrawn using specially cleaned plastic Samco brand transfer pipettes

(Corning Samco Corporation, San Fernando, CA), and transferred into plastic 1.5-mL microcentrifuge tubes (DOT Scientific, Inc., Burton, MI) in a plastic lined box to avoid dust contamination. Plasma was then stored in a –20°C freezer until analysis.

Silicon Analysis:

Plasma Si concentrations were analyzed by Trace Elements Laboratory, LHSC (University Campus, 339 Windermere Road, London, Ontario N6A 5A5, Canada). Plasma Si concentrations were determined via electrothermal atomic absorption spectrometry as adapted from Leung and Edmond (1997).

Statistical Analysis:

Plasma Si concentrations for the three methods of blood collection were analyzed using the Proc Anova procedure of SAS (6.12). The model included the type of method. Mean Si concentrations for each type of collection were compared to each other at the α = .05 level. Statistical significance was reported at a P-value of less than or equal to .05. Data were reported as mean Si concentration $\pm$ standard deviation for each method.

Results

Samples collected with the glass tubes had higher Si concentrations than the plastic collection tubes (Table 1E). Glass tubes with K_2EDTA had higher Si concentrations than tubes with heparin.

Appendix Table 1E. Mean Si concentrations (μg/L) for three blood collection methods

	Mean Si Concentration	Standard Deviation
Plastic Tubes with K_2EDTA (n=2)	203.0[a]	31.1
Glass Tubes with K_2EDTA (n=2)	1010.0[b]	66.5
Glass Tubes with Heparin (n=2)	691.5[c]	23.3

[abc]Means with different superscripts within columns are different ($P \leq .05$)

Discussion

Glass tubes yielded higher plasma Si concentrations than the plastic collection tubes. This suggests that the blood does draw Si from the glass, and contaminates the sample. Why the glass tubes containing K_2EDTA yielded higher plasma Si levels than the tubes containing heparin is unclear. Further research is required to analyze the differences seen between K_2EDTA and heparin in this study. The results of this study indicate that collection method does affect plasma Si concentrations.

Conclusions and Future Research

Blood collection using glass equipment does contaminate the sample and yields inaccurate Si analyses. Comparisons of relative Si concentrations due to treatment effects can still be determined using glass tubes, however the concentrations themselves are inaccurately elevated. It is our suggestion, that plastic tubes be utilized for plasma Si analysis.

There may be many factors contributing to the increased Si concentrations from glass collection tubes. Leaching may be accentuated by pH shifts and time of sample contact with the glass tubes. Further studies on these interactions should be conducted to

optimize collection technique. Anticoagulants may affect Si analysis as well. For example, K_2EDTA is a known chelator of some minerals in blood. Further comparison of K_2EDTA versus heparin is required. Silicon research is continually being conducted and collection methods should be solidified to allow for meaningful results.

APPENDIX F

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Osteocalcin and Carboxy-terminal Pyridinole Cross-link Telopeptide Region of Type I Collagen Measurements in Arabian Horses for the First Year of Life

Introduction

Biochemical markers are becoming an increasingly popular tool in equine research, and these markers provide valuable data on changes in various metabolic activities. In the area of equine exercise physiology, systemic markers of bone metabolism are continually used to indicate changes in turnover rates. Additionally, these markers are useful in providing evidence of alterations in the turnover rates as a result of various treatments. Young growing animals are rapidly turning over bone until they reach skeletal maturity around age four (Parfitt, 1984). This turnover is designed to optimize bone strength and integrity. However, this rapid growth may mask changes that are actually occurring as a result of research-related treatment or growth-related diseases. Therefore, having an understanding of the typical pattern of growth, as indicated by systemic markers of bone turnover, will enable scientists, veterinarians and managers to detect deviations from this general norm.

One such biochemical marker is osteocalcin (OC), which is an indicator of bone formation. Osteocalcin is predominately secreted by the osteoblast, or bone-forming cell (Lian and Gundberg, 1988; Kannus et al., 1996). It is a non-collagenous protein that is

released into circulation during new bone formation (Price, 1982). A second biochemical marker, carboxy-terminal pyridinoline cross-linked telopeptide region of type I collagen (ICTP), serves as an indicator of bone degradation. Type I collagen is the most predominant form of collagen molecule in bone (Risteli et al., 1993; Lawrence and Fowler, 1997), and ICTP is released into circulation when the collagen in the bone's extracellular matrix is degraded (Hassager et al., 1994; Risteli et al., 1993). Together, these two markers can be utilized to determine the rate of bone turnover in growing animals. Therefore, the objective of our study was to determine normal bone turnover in foals through the first two years of life by using OC and ICTP as systemic indicators of bone metabolism. With this information, a growth curve will then be formulated.

Methods

Animal Management

Twelve Arabian foals were housed with their dam, for the first 45 d of life, in stalls that were 3.1 m x 3.1 m or larger from 1600 until 0800 the next morning. During the day, foals and mares were turned out in a paddock that averaged 2384.5 m^2, with a stocking rate of three mares/foal units per paddock. Horses had ad libitum access to a mixed grass paddock (brome, orchard, timothy and Kentucky blue grasses) in the paddocks during the day. After 45 d, all mares and foals were turned out onto a mixed grass pasture that was 432.8 m x 53.6 m. Four foals were removed from the study over time due to unrelated death or other reasons.

Foals were introduced to creep feed at approximately three mon, and were weaned between three and four mon. Routine health care, including vaccinations, farrier work, and deworming, was maintained throughout the study. Creep feed was ad libitum, in a group, however the foals did not always finish the ration. Once weaned, horses were group fed, and each weanling ate approximately 1.70 kg oats, .51 kg corn and .33 kg protein pellets (Kent Feeds, Muscatine, IA) at each meal. All horses were fed this ration twice daily at 0700 and 1600. Hay was a grass and alfalfa mix, and each weanling ate approximately 5.68 kg of hay during the winter mon, and, as yearlings, ate approximately 5.0 kg during spring mon. The creep feed, weanling ration and yearling ration were approximately the same, and all horses were group fed. The daily nutrient values for these diets are summarized in Table 1F.

Appendix Table 1F. Calculated daily nutrient table on an as fed basis

Nutrient	Nutrient Content	Calculated Total Daily Intake
DE	2.05 Mcal/kg	22.07 Mcal
CP	14.26%	1540 g
Ca	.37%	40 g
P	.32%	30 g
Mg	.18%	20 g
Cu	14.23 mg/kg	178.71 mg

Blood Sample Collection

Blood samples were taken via jugular venipuncture from all foals on d 0, following parturition, d 15, 30, 45, 60 and approximately every 30 d thereafter. Two 10-mL Vacutainer® tubes with no additive (Becton Dickinson and Company, Franklin Lakes,

NJ) were filled from all animals using 22 g x 2.5 cm disposable needles (Becton Dickinson and Company, Franklin Lakes, NJ) to collect serum for biochemical marker analysis. Blood samples were then centrifuged within two h of collection for 10 min at 754 x g. After centrifugation, serum samples were removed using disposable glass Pasteur pipettes (VWR Scientific, West Chester, PA), aliquotted into plastic 1.5-mL microcentrifuge tubes (DOT Scientific, Inc., Burton, MI) and frozen at -20°C until analysis of OC and ICTP.

Osteocalcin Assay

Samples were analyzed for OC within 26 weeks of collection using an enzyme-linked immunosorbent assay (ELISA) (NovoCalcin®, Metra Biosystems, Inc., Mountain View, CA) following manufacturer's instructions. Serum was diluted at 1:15 for foals on d 0 through d 120 and 1:10 for all days thereafter. Dilutions were determined by testing samples at dilutions of 1:10, 1:15, and 1:20 for foals. These values were compared to the standard curve provided by Metra Biosystems. When the samples fell along the linear portion of the curve (from .4 to 1.5 optical density - OD), the corresponding dilution was chosen to run the OC assay for all samples. Standards and controls were reconstituted with .5 mL of 1X wash buffer. Twenty-five µL of standard, control, or sample was added to the osteocalcin-coated strips. Samples were tested in duplicate. Anti-osteocalcin (125 µL) was also added to each well. A two-h incubation at room

temperature followed, and then the wells were washed with 300 µL of 1X wash buffer three times with three min between each washing. Reconstituted enzyme conjugate (150 µL) was added to the wells, and incubated for one h at room temperature. After incubation, the wells were then washed in triplicate with 300 µL each time, and three min between each wash. Finally, a working substrate was added (150 µL) to each well and incubated at room temperature until the A standard read 1.5 OD, at which point 50 µL of stop solution was added, and the plate was read at 405 nm optical density on a Spectra Max 340 plate reader (Molecular Devices Corp., Sunnyvale, CA).

ICTP Assay

Samples were analyzed for ICTP using a radioimmunoassay (RIA) (ICTP, ^{125}I RIA Kit®, Diasorin, Stillwater, MN) according to manufacturer's directions. All samples, standards, controls, nonspecific binding tubes, and total count tubes were run in duplicate. The tubes contained the following: nonspecific binding tubes – 100 µL sample and 200 µL double 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. Tracer (200 µL of ^{125}I ICTP) was added to all tubes, including the total count tubes. Tubes were mixed and incubated for two h at 37°C. Next, 500 µL of separation reagent was added to all tubes, except the total count tubes. Tubes were vortexed and incubated for 30 min at 20-25°C. After incubation, tubes were centrifuged for 30 min at 2000 x g at

4°C, and immediately decanted for the supernatant. Tubes were then read using the 1290 GammaTrac Gamma Counting System (Tm Analytic, Elk Grove Village, IL).

Statistical Analysis

Osteocalcin and ICTP were statistically analyzed as a two-factor ANOVA for repeated measures using Proc Mixed procedure of SAS (6.12). The model included sample day. Osteocalcin and ICTP concentrations for all days were reported as LSMeans ± SEM. Statistical significance was reported at a P-value of equal to or less than .05. Trends were investigated at P-values equal to or less than .1.

Results

Results of the first 150 d for most yearlings for OC and ICTP are reported in Table 2F. Sample collections and assay analysis are in progress for the remaining animals and days. Osteocalcin appeared to increase immediately after birth, and remained higher through d 60. Osteocalcin values from d 15 through d 150 seemed

Appendix Table 2F. Mean OC and ICTP concentrations (± SEM) in horses from birth through d 150

Day	Mean OC (ng/mL)	SEM	Mean ICTP (µg/mL)	SEM
0	65.376[a]	11.5 (n=10)	33.850[a]	1.7 (n=12)
15	117.378[bc]	10.6 (n=12)	36.060[a]	1.7 (n=12)
30	110.115[bc]	11.0 (n=11)	33.823[a]	1.7 (n=12)
45	107.311[bc]	11.0 (n=11)	33.226[a]	1.7 (n=11)
60	122.135[c]	11.0 (n=11)	24.105[b]	1.9 (n=9)
90	95.534[ab]	11.1 (n=11)	23.928[b]	1.7 (n=11)
120	90.017[abc]	12.7 (n=8)	25.946[b]	2.1 (n=7)
150	102.017[bcd]	15.8 (n=5)	23.160[b]	2.8 (n=4)

[abcd]Means with different superscripts within columns differ (P ≤ .05)

variable, as evident by Table 2F. A decrease in ICTP values was seen on d 60, and ICTP remained lower through d 150.

Discussion

The increase in OC values seen after d 0 is logical, as bone begins to be loaded even on the first day of life. Loading of bone causes an increase in bone turnover and yields greater bone mineral density and strength (Woo et al., 1981; Raub et al., 1989; Bell, 1999). The decrease in ICTP concentrations was not seen until d 60. This decrease in bone resorption, coupled with increased bone formation, results in net bone formation. It is possible that it took 45 d for bone resorption to be significant in newborn foals, because until then a cell mediator was not acitvated in the osteoclasts until bone formation was increased. Studies show that enzymatic activities of bone forming and bone degrading cells alter each other's activity (Mohan et al., 1984). Chambers (1982) found that as osteoclastic activity (bone degradation) increases, osteoblasts are activated and begin to increase in osteoblastic activity (bone formation). Therefore, osteoblast and osteoclast cells appear to be capable of initiating activity for each other.

It is expected that over the short term, OC and ICTP levels will fluctuate. The results of this study will provide an indication of variability for the bone growth markers. Over the long term, the values for these markers are expected to decrease, as reduced levels were seen in horses, dogs and humans as age increased (Allen et al., 1998; Black et al., 1999; Kruse and Kracht, 1986; Lepage et al., 1990, 1992; Price et al., 1995). Once all

horses have been analyzed for OC and ICTP over 720 d more conclusive results will be published.

Conclusion and Implications

The use of biochemical markers provides indices of systemic indicators for bone metabolism. Young growing animals have high bone turnover to produce a stronger skeletal system. Therefore, the need for a growth curve for OC and ICTP has arisen. This curve can then be utilized to monitor normal skeletal growth and health, and can be used in research studies to determine treatment differences. The reported results only cover 150 d of the 720 d study. Once all data are collected, a growth curve will be established for OC and ICTP. With this information, skeletal health can be monitored, and research of young horses will be more meaningful.

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