CALCIUM BALANCE AND BONE DENSITY IN IMMATURE HORSES FED A HIGH PROTEIN DIET

A Thesis

by

HOLLY SUE SPOONER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Animal Science

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Approved by:

Co-Chairs of Committee,	Gary D. Potter
	Pete G. Gibbs
Committee Member,	Michael Walker
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ABSTRACT

Calcium Balance and Bone Density in Immature Horses Fed a High Protein Diet.

(August 2005)

Holly Sue Spooner, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Gary Potter
Dr. Pete Gibbs

Studies in other species indicate high protein diets increase urinary calcium (Ca) excretion and may lead to negative calcium balance and reduced bone density. As overfeeding of protein is commonplace in the horse industry, this study was undertaken to determine the effects of excess dietary protein on growth, physiologic response, mineral balance, bone density, and bone geometry in immature horses. Sixteen 10-month-old American Quarter Horses were blocked by age and sex into two dietary treatments. The control diet was formulated to provide the NRC (1989) recommended concentration of crude protein, while the high protein diet provided 130% of NRC (1989) recommendations. All other nutrients were formulated at or slightly above NRC (1989) recommendations. Blood samples, feces, and urine were collected during the 116-day study to determine any diet effect on pH and mineral balance. Radiographs were made of the left third metacarpal (MCIII) to determine bone density via radiographic bone aluminum equivalence (RBAE), and bone geometry was determined metrically from the radiographs.

Urine pH decreased over time ($p < 0.001$), but there were no diet effects on blood pH or urine pH. Conversely, when normalized to day 0 values, fecal pH was reduced by feeding the high protein treatment ($p < 0.02$). Density of dorsal and palmar cortices increased over time ($p < 0.001$), but no differences were observed between diets. But, normalized total medial-lateral (ML) width of the MCIII was higher in the control diet ($p < 0.05$). Fecal Ca loss was greater in horses fed the high protein diet ($p < 0.005$), while Ca absorption and retention were lower for horses on the high protein treatment ($p < 0.02$). Phosphorus (P) balance was not different between diets, although feeding the high protein diet resulted in higher P intake overall ($p < 0.001$).

While excess dietary protein may decrease fecal pH, increase fecal Ca excretion, and decrease Ca absorption and retention, there was no consistent effect of the high protein diet on bone density over the course of this study. Further research is necessary to determine if feeding high-protein diets is detrimental to bone quality in the growing horse.

To my husband,

Scott Spooner.

Without your love, devotion, and assistance

this thesis would not have been possible.

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CHAPTER I

INTRODUCTION

Bone quality in the equine athlete is a primary concern in the horse industry with both economic and animal welfare implications. While initial research focused on the treatment of orthopedic injuries, recent studies have sought to identify the causes of skeletal weakness and develop methods of prevention. It is known that bone strength is a direct result of bone mineral content (BMC), specifically calcium and phosphorus. While calcium needs in young and exercising horses have been elucidated, problems with bone development persist.

Recent work in others species indicate that factors other than calcium intake can play a role in the amount of calcium retained within the body and available for use within the bone. One such factor is the amount of protein in the diet. High protein intake specifically has been associated with hypercalciuria and reduced bone density, purportedly as a result of an acidogenic effect of the diet.

In the equine industry, the feeding of protein in amounts above those recommended by the National Research Council (NRC, 1989) is commonplace. In the horse, however, forage protein is digested primarily in the large intestine,

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This thesis follows the style of the Journal of Animal Science.

which results in limited amino acid absorption and high ammonia absorption. Protein in cereal grains and especially oilseeds are digested effectively in the small intestine and provide amino acids to the tissues. When horses are fed protein in excess of the amount digestible in the small intestine, the protein is passed on to the large intestine where it too is fermented and additional ammonia absorbed.   Because of the potential for excess amino acid metabolism and acid production in the large intestine, then, it is plausible that an acidogenic situation could occur, and calcium retention and bone quality could be affected.

The purpose of this study then is to determine the effect of feeding excess dietary protein on physiological parameters including pH, calcium balance, and bone density in young horses and any interrelationships among them. This information, then, can be used within the equine industry to develop dietary protein feeding recommendations for young horses in training.

CHAPTER II

REVIEW OF LITERATURE

Bone failure in race and performance horses is an unfortunate, yet common occurrence. Necropsies performed on Thoroughbred racehorses that were fatally injured or euthanized on California racetracks over a two year period indicated that 83% of deaths were the result of a musculoskeletal injury (Johnson et al., 1994). Of these, 85% were found to be the result of fractures. Such high occurrences of fractures in these animals may be the result of skeletal immaturity, as fatal injuries were also found to occur most often in animals aged 2-4 years (Johnson et al., 1994).

Even in cases where complete bone failure does not occur, skeletal injuries are more common in young animals. Norwood (1978) reported as many as 70% of two-year-olds in race training may have stress-induced microfractures of the third metacarpal (MCIII), commonly referred to as “bucked shins” or dorsal metacarpal disease, while only 10% of racehorses three and over are affected. Selnow et al. (1991) report similar findings with up to 90% of two-year-olds affected. Furthermore, a study of the Australian race industry found shin soreness to be the most frequent injury among two-year-olds in training and racing, and was viewed as a major cause of lost training days (Bailey et al., 1997).

It is known that bone strength is strongly correlated to bone mineral content, specifically calcium and phosphorus (Currey, 1969), and a study by Lawrence et al. (1994) indicated that maximum bone mineral content in horses was not achieved until approximately 6 years of age. Similarly, Buckingham et al. (1992) reported that metacarpal bone size and ultrasound speed (an indirect measure of bone density) did not peak until at least 5 years of age. Still, economics drive race and performance horse trainers to work horses harder at a younger age, so research has recently been focused on identifying means of obtaining higher bone density earlier in life, as well as identifying factors which may adversely impact bone quality.

Wolff's law indicates that bone will adapt to the physical demands placed upon it (Norwood, 1978). This adaptation may occur in two ways, modeling and remodeling. In the young animal, a process known as modeling occurs. This coordination of resorption and formation increases bone size and may modify shape (Buckwalter et al., 1995). In both young and mature animals, remodeling may occur to help replace old or damaged bone tissue. Repetitive stresses or strains placed on a bone cause flex signals within the bone to stimulate bone remodeling units (BRUs). Within a BRU, cells known as osteoclasts work to remove bone in areas referred to as resorbtion pits. Osteoblastic cells then move into the area and replace the pit with new bone and bone mineral, often in a new shape or configuration (Frost, 1973). In humans, the remodeling process generally takes 4 months, with up to 6 months required for complete mineralization of the affected area, although many sites may be remodeling simultaneously. In young humans the rate of bone turnover may be as high as 100% annually (Buckwalter et al., 1995). Like many physiological processes, rate of bone turnover slows with age. In adult animals, greater than 75% of bone surfaces may be quiescent at any given time (Parfitt et al., 1987).

It is important to remember, however that total bone strength is the result of three main factors: stiffness/elasticity, mineral content, and geometry (Jeffcott et al., 1988). While bone must be stiff enough to endure loads placed upon it, it must also be flexible enough to withstand torsional strain. Elasticity can be correlated to mineral content, as an increase in stiffness is the result of replacement of bone water content with mineral (Loveridge, 1999). Still, there becomes an optimum point for mineral content, as too much BMC may result in an overly stiff bone that cannot flex and is more likely to fracture. In terms of geometry, bone can be thought to begin as a hollow cylinder. Its strength then is a result of both the bone's physical properties and its cross-sectional area (Buckingham and Jeffcott, 1987). Over time, however, remodeling may occur on one side of a bone more than on others as a result of the stresses placed upon it, leaving the bone more oblong with an offset medullary cavity.

It can be predicted that when young horses enter race training, new stresses placed upon the bones of the leg, particularly MCIII, initiate the remodeling process. In the horse, this strain may be tremendous. Research conducted by Nunamaker and colleagues (1990) used strain gauges placed inside bone to reveal that the strain placed upon MCIII in the galloping horse may be as high as 5,600 microstrains, compared to values of 2,000 – 3,000 microstrains in other species. This strain, then, can be expected to result in changes in both bone geometry (Sherman, 1995) and density (Nielsen et al., 1997), while also having the potential to cause micro-fractures. Because the process of bone remodeling may occur in numerous areas within the bone at any one given time, overall density of the bone may also decrease as the remodeling process begins (Nielsen et al., 1997), making it even more susceptible to injury. Unfortunately, this period of decreased bone density often corresponds to the time that high speed sprint work is introduced, setting the stage for increased risk of injury.

Unlike the muscular and cardiovascular system, race and performance horse trainers also have little ability to assess skeletal maturity, and as a result identification of skeletal weakness may not occur until lameness is present. This is primarily due to a shortage of accurate, non-invasive measures of bone strength that can be performed on large animals. Meakim et al. (1981) developed a means of using standard radiographs to compare bone density to known densities of aluminum, a technique referred to as radiographic bone aluminum equivalence (RBAE). While this technique is easy and non-invasive, the variability among horses is quite high, and thus it is best used for observations of changes in bone density over time for a specific animal. Computer assisted tomography (CAT scan) and dual energy x-ray absorptiometry (DXA) have also been used, although their use remains limited due to cost and necessity of sedation.

Evaluation of the problem of skeletal failure in immature horses from a nutrition perspective quickly identifies mineral requirements as an important consideration. Calcium, in particular, makes up the majority of the bone crystal, hydroxyapatite, which along with type I (fibrous) collagen form the structural component of the bone (Arnett, 2003). Initially, nutritional Ca requirements in mature, idle horses were identified (Hintz et al., 1986) and these values were extrapolated to produce values for horses in training recommended by the National Research Council (NRC, 1989). Currently, NRC (1989) recommendations indicate that horses at work should receive:

Ca (g/day) = 1.22*(Mcal of DE/day).

Growing horses not in training should receive:

Ca (g/day) = 0.04*Body Weight (kg) + 32*Average Daily Gain (kg/day).

However, work by Nielsen et al. (1998), indicated a need for dietary Ca above NRC requirements between 80-120 days of training when there is an enhanced rate of bone formation (1998). This was further substantiated by Stephens and associates (2004) who found maximal Ca absorption and retention at 138% and 136% of NRC recommendations, respectively.

The relationship of Ca intake to phosphorus intake has been debated as P has been shown to be inhibitory to Ca absorption (Schryver, 1978). Meacham (1981) suggests that while a Ca to P ratio of 1.1:1 to 2:1 seems to meet the requirement for proper growth, it is unlikely that the minerals are being absorbed from the intestine at this ratio. The important factor, according to Schryver (1978), is that the ratio not be less than 1:1.

Still, work in other species, including humans, indicates factors other than dietary Ca and P intake may play a role in the amount of Ca absorbed or retained within the body. Dietary protein level is one such factor. As early as 1918, it was reported that an acidic diet resulted in skeletal depletion in rabbits (Goto, 1918). Then, in 1920, studies showed that an all meat diet in humans increased urinary Ca losses (Sherman, 1920). During the 1970s and 1980s more than 30 human studies were published on the matter. Although study groups and experimental design varied, the majority reported a positive relationship between protein intake and urinary Ca (Kersetter et al., 2004). A review of 26 studies in which diet was controlled, protein intake was manipulated, and urine Ca was measured as the response criterion, found a strong relationship between protein intake and Ca excretion ($p < .001$, $r = 0.7$) (Kersetter et al., 2004).

It is important to note that over time, if Ca lost in the urine and feces exceeds the amount of Ca absorbed from the diet, a phenomenon known as negative Ca balance can occur. In this instance, Ca must be pulled from the body's skeletal stores to maintain normal blood Ca concentrations, perhaps at the expense of bone density. A study by Licata (1981) reported negative Ca balance in individuals consuming a high protein diet, as a result of a doubling of urinary Ca over the control group which received a lower level of protein.

The mechanism proposed for this increase in urinary Ca has been attributed to the metabolism of sulfur-containing amino acids such as methionine. As these amino acids are broken down, sulfuric acid is produced leading to metabolic acidosis (Sellmeyer et al., 2001). Because urinary excretion of acid alone is insufficient, other systems such as the skeleton may be used to buffer this excess dietary acid load. As base is lifted from bone to restore acid-base balance, the accompanying minerals such as Ca are also excreted through urine (Barzel, 1976). At the same time, it has been shown that bone cells themselves may also be affected by a change in pH as a result of acidosis. Bushinsky (1995) reports that osteoblasts show reduced collagen synthesis and mineralization as pH decreases, while osteoclasts were upregulated. The method for this regulation remains unknown (Arnett, 2003). Whether or not intestinal Ca absorption can be affected by dietary protein level also remains unclear. Heaney (2000) illustrated in humans that protein intake did not contribute to the variability inherently observed in Ca absorption, but Kerstetter et al. (2004) report that dietary protein levels may alter Ca absorption as a result of changes in parathyroid hormone (PTH).

Protein requirements in the horse have been studied persistently; however, within the equine industry, overfeeding of protein is commonplace. A field study examining the diets of Thoroughbreds at a Detroit racetrack found crude protein consumption to be at least 25% greater than NRC (1989) recommendations (Gallagher et al., 1992), while a central North Carolina study found more than 70% of all horses were consuming excess protein (Honore and Uhlinger, 1994). This may be due to a lack of understanding on the part of horse owners and trainers, who may believe that feeding a higher amount of protein enhances performance (Custalow, 1991). Still, little research supports that theory. A study of endurance horses found high protein diets to show no benefit and to require increased water consumption to allow for the excretion of excess N (Hintz et al., 1980). Additional studies indicate that heat production associated with higher levels of protein intake may decrease the time to fatigue in performance horses (Kronfeld, 1996), while restriction of dietary protein may help diminish the acidogenic effects of exercise (Graham-Thiers et al., 2001).

Two studies have examined the effect of excess dietary protein in young horses with conflicting results. Glade et al. (1985) reported that 6 to 8-month-old Thoroughbred foals fed 130% of NRC requirements had increased urinary excretion of Ca and P when compared with similar foals consuming 70% or 100% of their requirements. Investigation into the renal function of these animals indicated that the losses were the result of decreased reabsoption of Ca and P immediately following feeding. This decreased reabsorption was attributed to the methionine content of the diet, as glomerular filtration of sulfurous amino acids, such as methionine, has been shown to interfere with reabsorptive functions when the amino acids are oxidized and sulfuric acid is formed. Changes in bone density were not determined in that study.

Conversely, Schryver et al. (1987) investigated 24 foals beginning at 4 months of age. The foals were placed on one of three diets, formulated to NRC requirements except for protein, with protein levels of 9%, 14%, and 20%. No significant differences were found in regard to growth or Ca balance between the recommended (14%) and high protein (20%) diets. Additionally, bone turnover as measured with ^{47}Ca was not significantly different between the recommended and high protein diets. The authors suggested an adaptive response to the high protein diet; a theory observed in rats (Allen and Hall, 1978) but unsubstantiated in humans (Allen et al., 1979). It is important to note, however, that the animals on this study consumed the concentrate portion of the diet *ad lib* which may have resulted in higher than normal Ca intake. Thus, it may be possible that flooding the digestive system with Ca may counteract any negative effects of a high protein diet.

This study was designed to investigate the effects of excess dietary protein on mineral balance and bone density in immature horses.

CHAPTER III

MATERIALS AND METHODS

Management of Animals

Sixteen American Quarter Horses were obtained from the Texas A&M Horse Center herd at approximately 10 months of age. The horses were blocked by age and sex and placed into one of two treatment groups (eight horses each). Prior to initiation of the study all horses were adapted to handling, vaccinated, and dewormed per Texas A&M Horse Center protocol. Horses were housed in groups of 3-4 in large dirt paddocks with access to shelter. Throughout the course of the study, all horses received regular hoof care and deworming. The protocol for management and treatment of the study animals was approved by the Institutional Agricultural Animal Care and Use Committee.

Experimental Diets

Prior to the start of the study, all horses were subjected to a background period of at least 14 days during which time they were fed a 16% crude protein pellet (Producer's Cooperative, Bryan, Texas) and coastal bermudagrass hay at 3% of body weight in a 65:35 concentrate:hay ratio.

Two rations were formulated to meet NRC (1989) requirements except for protein, with one diet formulated to meet the NRC recommendation for dietary protein, and the other exceeding the recommendation by approximately 30% (Table 1). Horses were offered a ration of 3% of body weight daily in a 65:35

ratio of concentrate to coastal bermudagrass hay. Concentrate portions of the rations were provided by Cargill-Nutrena, and were developed using common feedstuffs found in horse rations (Table 2). Analyzed compositions of the diets on a 100% dry matter basis is found in Table 3.

Horses were fed individually at 12h intervals (0600 and 1800) throughout the study. Horses were allowed at least 2h to consume their ration, and any feed refused was weighed and recorded. Feed refusals were infrequent. Adjustments to feed intake were made after d60 to correspond with increases in body weight.

Table 1. Calculated compositions of concentrates, as fed

Nutrient	Concentrate A	Concentrate B
DE, Mcal/lb	1.4	1.45
Crude Protein, %	14.5	19.75
Crude Fiber, %	7.2	6.66
Lysine, %	0.64	1.07
Fat, %	2.9	2.62
Calcium, %	0.65	0.65
Phosphorus, %	0.48	0.56
Magnesium, %	0.2	0.23
Potassium, %	0.81	1.09
Sodium, %	0.3	0.3
Chloride, %	0.6	0.6

Table 2. Ingredient formulation of concentrate diets, %

Ingredient	Concentrate A	Concentrate B
Wheat Midds, 27-34% NDF	25.59	29.60
Milo, fine ground	25	25
Corn, coarse ground	22.34	10.33
Soybean Meal, 48% protein	6.95	19.33
Rice Hulls	7.5	5.0
Dried Distillers Milo	5.06	3.21
Corn Germ Meal	5.0	5.0
Calcium Carbonate	1.38	1.30
Salt, 90%	0.790	0.790
Potassium Ch 50	0.19	0.16
L-Lysine HCL	0.103	0.212
Trace Mineral Pre-mix	0.020	0.011

Table 3. Analyzed nutrient profiles of diets, 100% DM

Nutrient, 100% DM	Total Diet A	Concentrate A	Total Diet B	Concentrate B	Hay
Dry Matter, %	90.35	90	91	91	91
Crude Protein, %	15.36	17.02	18.46	21.78	12.28
Calcium, %	0.80	0.94	0.72	0.82	0.54
Phosphorus, %	0.46	0.61	0.50	0.66	0.19
Magnesium, %	0.26	0.26	0.28	0.29	0.26
Potassium, %	1.23	0.93	1.36	1.13	1.78
Sodium, %	0.56	0.69	0.50	0.59	0.33
Zinc, ppm	83	116	69	94	23
Iron, ppm	47	18	47	19	99
Copper, ppm	15	19	10	11	8
Manganese, ppm	130	155	112	127	83

Exercise Protocol

All horses received forced exercised with a “moving-gate” mechanical exerciser 3X weekly. Horses were walked for 10 min, strongly trotted for 15 min, and then walked for 5 min to minimize the effect of confinement on bone density.

Physical Measurements

Physical measurements were taken prior to the study (d0) and in 28-day increments (d28, d56, d84, and d112). Measurements included weight, wither height, hip height, body length, heart girth circumference, forearm circumference, gaskin circumference, and rump-fat thickness (via ultrasonic measurement). Weight was determined using a certified scale with readings in pounds and converted to mass units of kilograms. Wither height and hip height were measured using a standard height stick with a level, at the highest points of the wither and hip respectively. Body length, heart girth circumference, forearm circumference, and gaskin circumference were measured in centimeters using a vinyl measuring tape. Locations for each measurement were identified by clipping small areas of hair to ensure repeatability. Rump fat thickness was measured in two locations on the left side of the rump using ultrasound. The first location was 1 cm lateral from the midline, 3 cm caudal from the top of the rump. The second location was 1 cm lateral from the midline and 8 cm caudal from the top of the rump. The area to be ultrasounded was shaved as near the skin as possible and alcohol was used to improve transduction.

Sample Collection

Blood samples were collected via jugular venipuncture on d0, d28, d56, d88, and d116 at various times post-feeding. Samples were immediately tested for pH then centrifuged at 53000 rpm for 30 minutes, and resulting serum and plasma stored at -20ºC for later analyses.

Prior to the start of the study (d-4 to d-1), during the study (d57-d60), and at the conclusion (d112-d116), total collections of feces and urine were conducted. All horses were confined to tie stalls with rubber mats for a 4 day period to prevent coprophagy and sample contamination. Feces were allowed to fall onto the rubber mats then were immediately collected into a clean storage bucket. Manure produced during each three hour period was weighed for each horse, and a 10% aliquot was frozen at -20ºC and retained for analyses. Urine was collected via urine collection harnesses fitted to each horse, and urine volume was recorded via a graduated cylinder and a 10% sample retained in a plastic storage bottle. At the end of each collection period, urine was strained through three layers of cheesecloth and individually frozen in Nagalene tubes at -20ºC.

All horses were walked on the mechanical exerciser at least 1 hour daily during the total collection period to minimize the stress of confinement. Any feces eliminated during this time was weighed and recorded but not retained to avoid contamination of samples. Horses were successfully discouraged from urinating while on the exerciser by continual forward motion.

Hay and concentrate were sampled from each bale or bag fed during a total collection period and representative combined aliquots were retained for later analyses.

Radiographic Measurements

Radiographs of the left third metacarpal bone (MCIII) were made at the Texas A&M University Large Animal Hospital at d0, d56, and d112. A dorsal-palmar view was made with the cassette against the palmar aspect of the leg and the beam centered on the midpoint of MCIII and directed parallel to the ground in the midsaggittal plane. A lateral-medial view was made with the cassette placed medially, while the beam is centered on the midpoint of MCIII and directed parallel to the ground 90° from the midsaggittal plane. For all views the x-ray machine was set at 70kV, with an exposure time of 0.16s, and a focal length of 90 cm. An aluminum stepwedge penetrometer was attached to each radiographic cassette.

Radiographic Bone Aluminum Equivalence (RBAE)

Radiographs were scanned into a Bio-Rad GS-800 densitometer and logarithmic regression was formed using known thickness of steps on the aluminum penetrometer attached to each radiograph to determine bone density.

Bone density was expressed as RBAE. Maximum optical density values for all cortices were recorded in millimeters of aluminum (mm Al) 1 cm distal to the nutrient foramen. Total RBAE was then calculated on the dorsal-palmar radiograph using the area under the curve concept described by Nielsen et al. (1998) and reported in mm^2 Al. All radiographs were viewed by a radiologist for determination of any abnormalities.

Geometric Bone Measurements

Geometric changes in bone were identified by measurement on the radiographs 1 cm distal to the nutrient foramen. Using a bright back light, a pair of digital calipers was used to measure the width of the dorsal, palmar, medial, and lateral cortices and total bone width. Width of the medullary cavity was determined by difference. When necessary, a light box was utilized to assist in determining the edges of the bone.

Laboratory Analyses

Feed and fecal samples were dried in a 62ºC oven for a period of at least 72 hours, then ground in a Wiley mill with a 2mm screen. The ground samples were mixed thoroughly and a composite sample for each horse for each collection was stored in a sealed, mineral-free plastic container at room temperature. Feed and fecal samples were sent to the Texas A&M University Soil, Water, and Forage Testing Laboratory for analyses of Ca and P. Corn stalk (zea mays) reference material from the National Institute of Standards and Technology, Gaiterhersburg, Maryland was used as a standard for mineral analyses. Urine was sent to the Texas Veterinary Medical Diagnostic Lab (TVMDL), College Station, Texas, for analysis of Ca and P. Serum was also analyzed by TVMDL for Ca, P, and blood urea nitrogen (BUN).

Statistical Analyses

For statistical analyses, physiological differences between diets, day of study, and diet*day interactions were determined by two-factor analysis of variance using STATA statistical software (StataCorp, 2001). When necessary, means were further separated using the Bonferroni means comparison test (StataCorp, 2001). Differences were considered significant at $p < 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

Physical Measurements

Horses began the project at an average weight of 299 ± 4.86 kg and increased weight to 323 ± 5.39 kg for an average gain of 24 kg (Table 4) which is normal growth for horses this age (NRC, 1989). While wither height was not significantly different from d0, hip height increased from 54.8 ± .4 cm at d0 to 56.7 ± .3 cm at d112. Body length increased from 140.8 ± 4 cm to 149.5 ± 1 cm from d0 to d128. Rump fat at location 1 increased from 1.25 ± .1 mm at d0 to 5.19 ± 0.5 mm at d112. Similarly, rump fat at location 2 increased from 1.19 ± 0.1 mm to 4.75 ± 0.5 mm. The increases in height and weight are within normal ranges for growing horses. All other measured variables were not significantly different from d0 to d112 (Table 4, Appendix 1A, 1B).

Differences due to diet were observed for heartgirth and body length (Table 4, Appendix 1A). Heartgirth averaged 153.6 ± 0.7 cm for diet A, while diet B averaged 155.4 ± 0.7 cm. Body length averaged 142.1 ± 1.1 cm and 146.0 ± 1.1 cm for diet A and diet B, respectively. These differences are slight, and may simply be the result of errors in taking measurements, such as having the horses positioned differently, as there was no corresponding change in body weight. There were no observed diet*day interactions detected at the $p < 0.05$ level.

Table 4. Physical measurements by diet and day

Day	0	28	56	84	112	Mean
Body Weight (kg)						
Diet A	297.5	306.5	311.3	316.0	319.6	310.2
SEM	7.8	7.0	7.5	6.1	7.8	3.3
Diet B	302.8	310.6	320.4	326.1	327.6	317.5
SEM	6.2	7.0	8.0	7.2	7.7	3.4
Mean	300.2[a]	308.6[a,b]	315.8[a,b]	321.0[b]	323.6[b]	
SEM	4.9	4.8	5.4	4.8	5.4	
Wither Height (cm)						
Diet A	52.3	52.9	53.1	53.5	53.6	53.1
SEM	0.5	0.7	0.5	0.6	0.3	0.3
Diet B	52.7	53.3	53.3	53.7	53.6	53.3
SEM	0.6	0.5	0.5	0.5	0.4	0.2
Mean	52.5	53.1	53.1	53.6	53.6	
SEM	0.4	0.4	0.3	0.4	0.4	
Hip Height (cm)						
Diet A	54.1	55.3	55.2	56.1	57.1	55.7
SEM	0.6	0.6	0.6	0.5	0.5	0.3
Diet B	54.9	55.6	55.7	56.3	56.3	55.8
SEM	0.6	0.6	0.5	0.7	0.5	0.3
Mean	54.8[a]	55.5[a,b]	55.4[a,b]	56.2[a,b]	56.7[b]	
SEM	0.4	0.4	0.4	0.4	0.3	
Heartgirth (cm)						
Diet A[e]	151.3	152.1	152.8	155.6	156.4	153.6
SEM	1.3	1.3	1.5	1.3	1.4	0.7
Diet B[f]	151.8	154	155.2	157.8	158.4	155.4
SEM	1.5	1.6	1.4	1.1	1.4	0.7
Mean	151.5	153.1	154.0	156.7	157.4	
SEM	1.0	1.0	1.0	0.9	1.0	
Body Length (cm)						
Diet A[e]	136.5	138.8	143.0	144.0	148.0	142.1
SEM	1.9	1.6	1.8	2.4	2.2	1.1
Diet B[f]	145.1	142.6	145.3	145.9	151.1	146.0
SEM	6.9	1.7	1.7	1.9	1.4	1.5
Mean	140.8[a]	140.7[a]	144.1[a,b]	145.0[a,b]	149.5[b]	
SEM	3.6	1.2	1.2	1.5	1.3	
Forearm Circumference (cm)						
Diet A	51.4	51.1	51.3	50.9	50.7	51.1
SEM	0.8	0.6	1.0	0.5	0.5	0.3
Diet B	49.8	50.5	51.1	51.4	50.8	50.7
SEM	0.9	0.9	0.8	0.8	1.4	0.4
Mean	50.6	50.8	51.2	51.2	50.7	
SEM	0.6	0.5	0.6	0.4	0.7	

Table 4. Continued

Day	0	28	56	84	112	Mean
Gaskin Circumference (cm)						
Diet A	41.5	41.6	41.5	42.2	42.1	41.8
SEM	0.4	0.3	0.4	0.5	0.5	0.2
Diet B	41.1	41.6	41.9	42.3	42.1	41.8
SEM	0.5	0.5	0.5	0.5	0.7	0.2
Mean	41.3	41.6	41.7	42.2	42.1	
SEM	0.3	0.3	0.3	0.3	0.4	
Rump Fat 1 (mm)						
Diet A	1.13	1.75	2.75	3.13	5.38	2.83
SEM	0.13	0.31	0.25	0.58	0.60	0.29
Diet B	1.37	2.00	2.75	3.50	5.00	2.92
SEM	0.18	0.27	0.25	1.02	0.82	0.33
Mean	1.25^{a}	$1.88^{a,b}$	$2.75^{b,c}$	3.31^{c}	5.19^{d}	
SEM	0.11	0.20	0.17	0.57	0.49	
Rump Fat 2 (mm)						
Diet A	1.13	1.75	2.25	2.87	4.75	2.55
SEM	0.13	0.25	0.25	0.48	0.49	0.25
Diet B	1.25	1.50	2.38	3.00	4.75	2.56
SEM	0.16	0.19	0.18	0.60	0.80	0.28
Mean	1.19^{a}	1.63^{a}	$2.31^{a,b}$	2.94^{b}	4.75^{c}	
SEM	0.10	0.15	0.15	0.37	0.45	

[a,b]Row means not sharing a common superscript differ ($P < 0.05$)
[e,f]Diets not sharing a common superscript differ ($P < 0.05$)

Blood Parameters

All measured blood parameters were within normal ranges, but because serum values were highly variable between horses (Appendix 2A, 2B, 2C) and values for the diets were significantly different at d0 as a result of random assignment into treatment groups, data for each horse was normalized to that horse's baseline values (d0) for serum Ca, serum P, and blood urea nitrogen (BUN). There were no effects of diet or day on normalized serum Ca or normalized serum P (Figure 1, Figure 2). This is easily attributed to the body's tight homeostatic mechanisms for calcium. The mean change in BUN was significant for both diet and day, with d28 being significantly lower than d116, at -0.394 ± .55 mg/dl and 2.125 ± 0.70 mg/dl respectively (Figure 3). Change in BUN in diet B was greater than diet A ($p < 0.001$) (Figure 3). BUN increased from baseline in the high protein group (diet B) while decreasing in the control group (diet A). This was expected and is easily explained as prior to the start of the study all horses were consuming a concentrate formulated to contain 16% crude protein. Thus, the horses in the control group began consuming less crude protein at the initiation of the study, while protein intake in diet B increased. BUN, then, increases as a result of deamination of protein consumed above the body's needs. These findings are in agreement with Schryver and colleagues (1987) who found BUN to be directly related to the protein content of the diet.

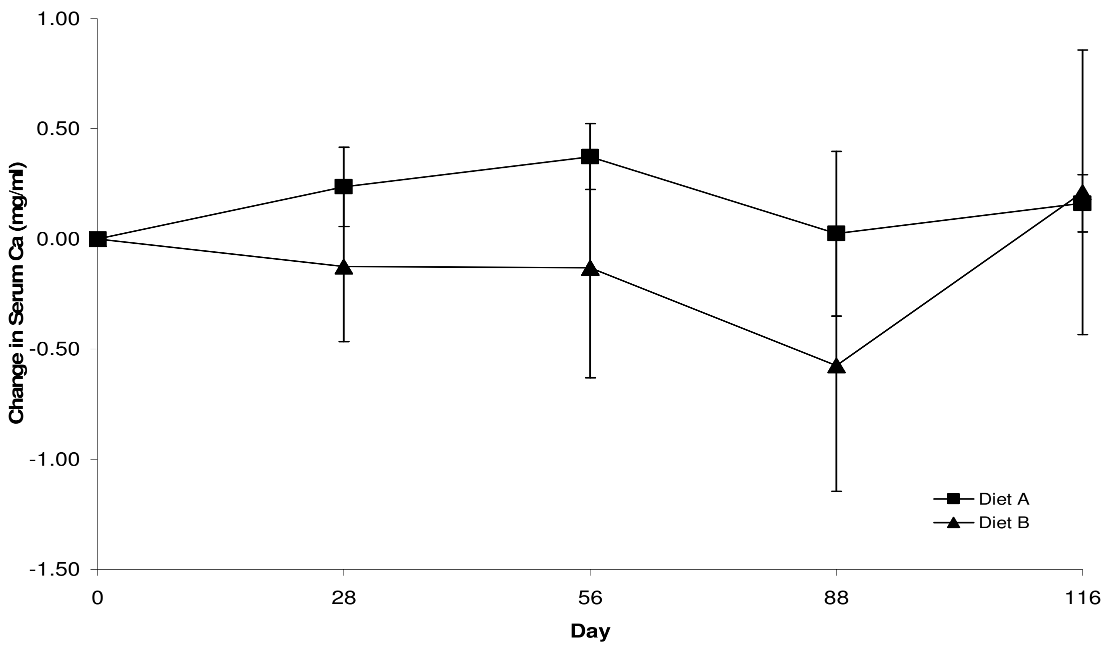

Figure 1. Normalized serum Ca by diet and day.

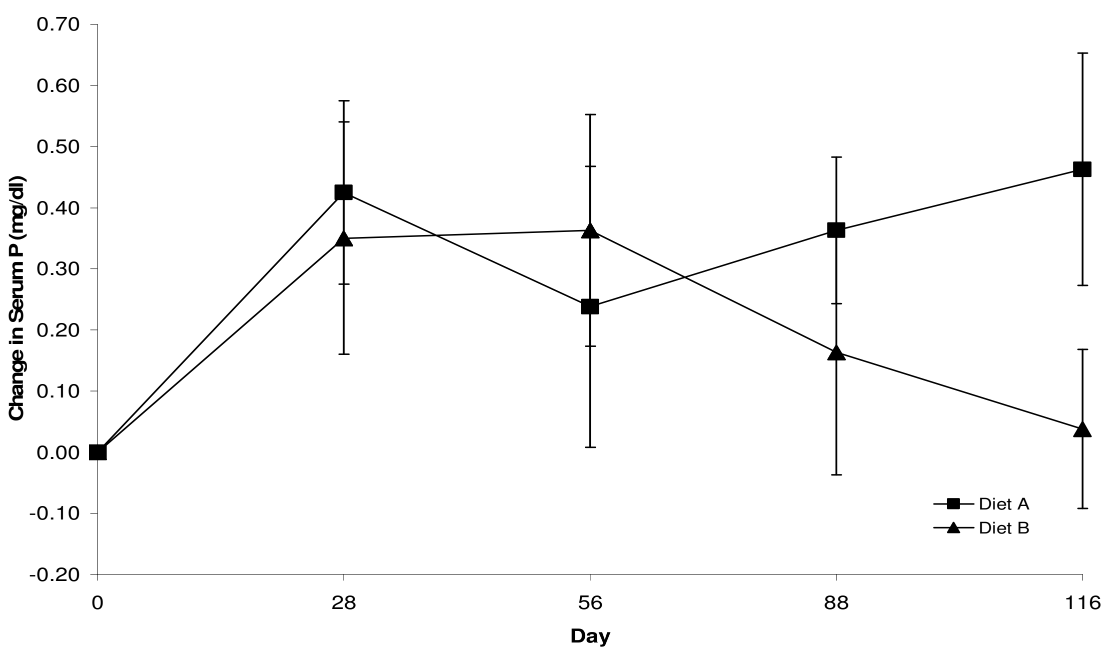

Figure 2. Normalized serum P by diet and day.

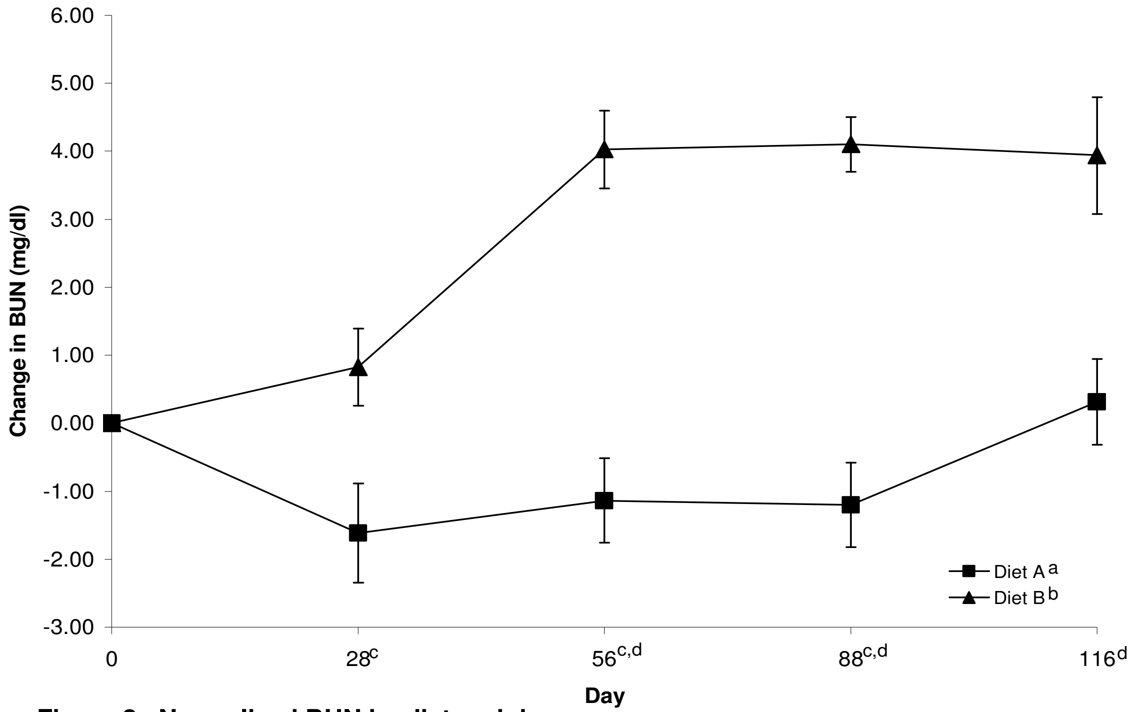

Figure 3. Normalized BUN by diet and day.

[a,b]Diets not sharing a common superscript differ (P< 0.001)
[c,d]Means across all diets for days not sharing a common superscript differ (P< 0.0001)

pH Measurements

Blood pH was found to exhibit a day effect ($p < 0.001$) (Table 5, Appendix 3A, 3B). Mean blood pH increased from d0 to d116, from 7.50 ± 0.01 to 7.58 ± 0.01 (Figure 4). Day 88 was not significantly different from d0, but at 7.49 ± 0.02 was lower than d116. Such day differences may be the result of variations in sampling time such as time post-feeding or post-exercise as sampling times were not consistent. Previous research indicates that blood pH may not be affected by protein content of the diet with the subject at rest; however, protein-restricted diets may result in lower blood pH post-exercise (Graham-Theirs et al., 2001). No diet or diet*day effect was seen on blood pH at a significance of $p < 0.05$. While this does not seem to support an acidogenic effect of the high protein diet, it may be that the body's homeostatic mechanisms are able compensate for such pH changes over the length of time of this study. It may also be possible that protein being digested in the hind-gut is increasing ammonia absorption, as evidenced by increased BUN, and thus working to increase blood pH at the same time, thereby nullifying any acidogenic effects.

Urine pH was found to decrease over time ($p < 0.001$) (Table 5, Figure 5). Mean urine pH was 7.95 ± 0.05 at d0 and decreased to 7.50 ± 0.1 at d112. This change is presumably the result of a change in dietary cation-anion difference (DCAD) from the pre-study diet to treatment diets. The DCAD is defined as the difference between positively and negatively fixed ions in the diet, primarily sodium (Na^+), potassium (K^+), chloride (Cl^-) and sulfur (S^-). As DCAD increases linearly, urine pH has been shown to rise (Baker et al., 1992). Thus, it is suspected that the diet consumed prior to the start of this study had an unusually high DCAD. This is further supported by the fact that an unrelated group of horses on an unrelated research project consuming the same pre-study diet were observed to also have high urine pH values.

No change in urine pH was observed due to dietary treatment. This may be the result of length of the study combined with abnormally high readings at d0, as urine pH was lower in diet B by d116, although not significant. Conversely, Glade et al. (1985) suggest that changes in urinary pH, while not measured in that study, would likely be in direct proportion to methionine content, as ingestion of methionine above threshold amounts is followed by renal filtration of sulfur in excess of local buffering capacity, thus lowering pH. While crude protein was higher in diet B, amino acid analysis was not performed, so there may not have been enough difference in methionine between diets to warrant a change in pH. Furthermore, like blood pH, changes in urine pH may have also been affected by increased ammonia absorption in the hind-gut, as evidenced by increased BUN in the high protein diet.

Fecal pH values were significantly different between diets at d0 (Table 5, Appendix 3A, 3B) and thus were normalized to baseline (d0) values. Normalized fecal pH was significantly lower at days 56 and 116 for horses consuming diet B ($p < 0.02$) (Figure 6). This finding is similar to that reported by Barzel and Massey (1998) who found excess dietary protein to cause an acidogenic effect. Still, it is puzzling that if this difference in pH is in fact indicative of an acidogenic state, that urine and blood pH would remain unchanged or the changes would not be discernible by methods used in this project. Again, it may be possible that the unique digestive physiology of the equine is a factor here. Digestion of protein in the hind-gut may have increased production of volatile fatty acids (VFA) and lactate as the carbon structures are broken down, which could be responsible for decreasing gut pH and ultimately fecal pH.

Table 5. Blood, urine, and fecal pH by diet and day

Day	0	28	56	88	116	Mean
Blood						
Diet A	7.51	7.56	7.54	7.44	7.59	7.53
SEM	0.02	0.03	0.03	0.04	0.02	0.14
Diet B	7.49	7.57	7.55	7.53	7.58	7.55
SEM	0.03	0.02	0.03	0.02	0.01	0.01
Mean	7.50[a]	7.57[a,b]	7.54[a,b,c,d]	7.49[a,c]	7.58[b,d]	
SEM	0.01	0.02	0.02	0.02	0.01	
Urine						
Diet A	7.92		7.73		7.59	7.76
SEM	0.07		0.11		0.16	0.07
Diet B	7.98		7.74		7.42	7.73
SEM	0.08		0.12		0.14	0.07
Mean	7.95[a]		7.73[b]		7.50[c]	
SEM	0.05		0.08		0.10	
Fecal						
Diet A[e]	6.67		6.45		6.29	6.49
SEM	0.05		0.07		0.06	0.04
Diet B[f]	6.90		6.42		6.40	6.60
SEM	0.07		0.07		0.08	0.05
Mean	6.79[a]		6.44[b]		6.35[b]	
SEM	0.03		0.04		0.05	

[a,b,c,d]Row means not carrying a common superscript differ ($P < 0.001$)
[e,f]Diets not carrying a common superscript differ ($P < 0.05$)

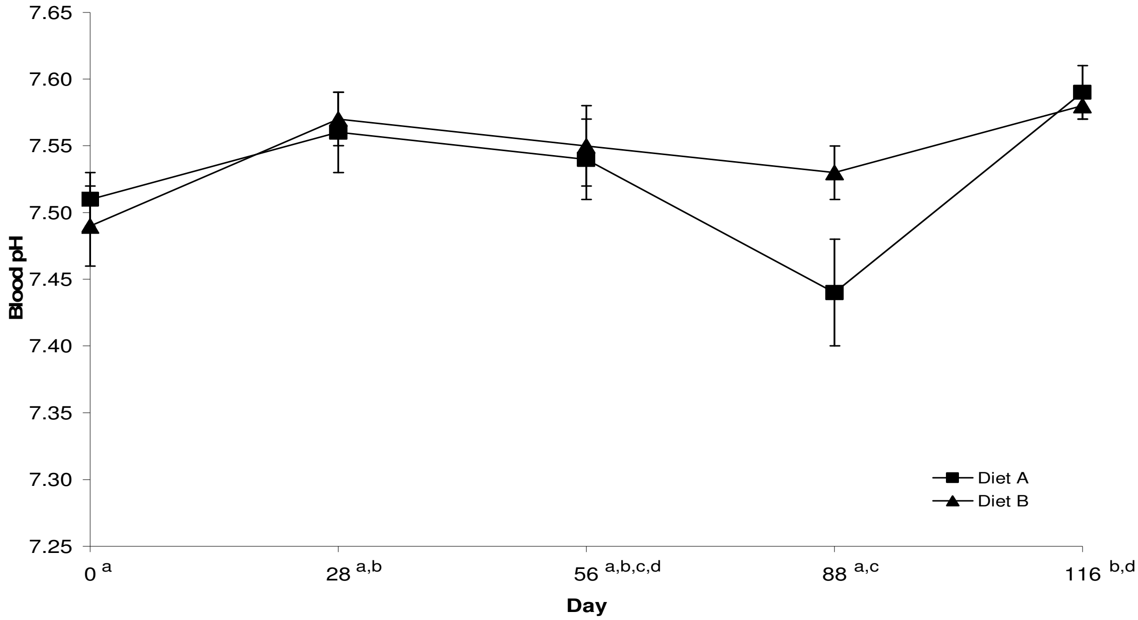

Figure 4. Blood pH by diet and day.

[a,b,c,d]Means across diets for days not sharing a common superscript differ (P < 0.001)

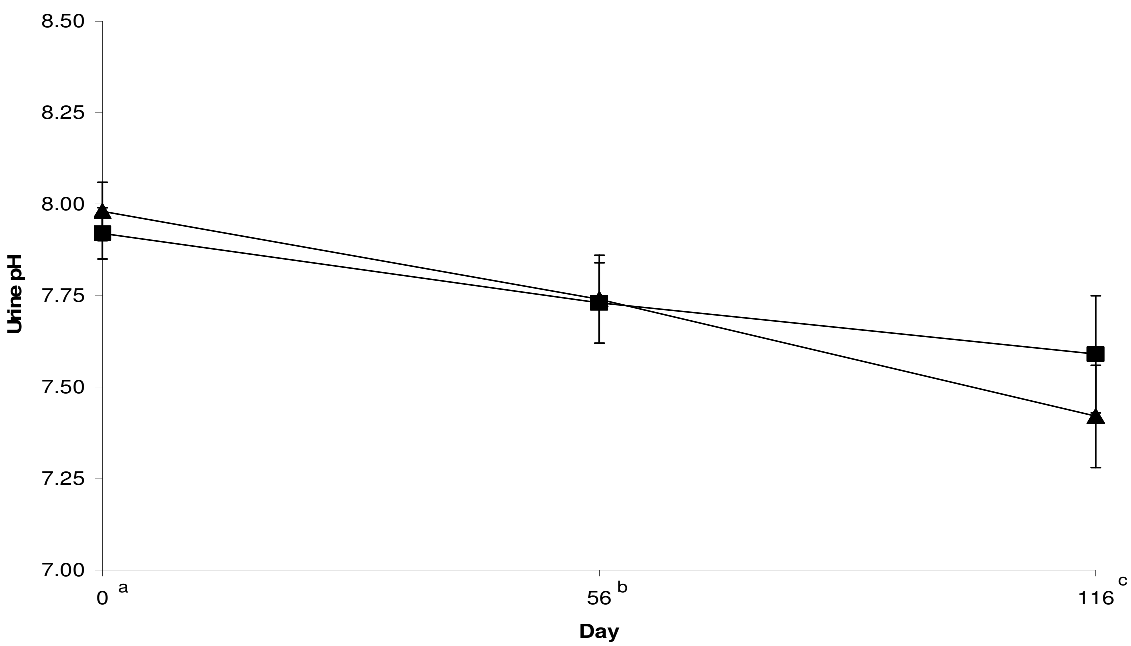

Figure 5. Urine pH by diet and day.

[a,b,c]Means across diets for days not sharing a common superscript differ (P< 0.001)

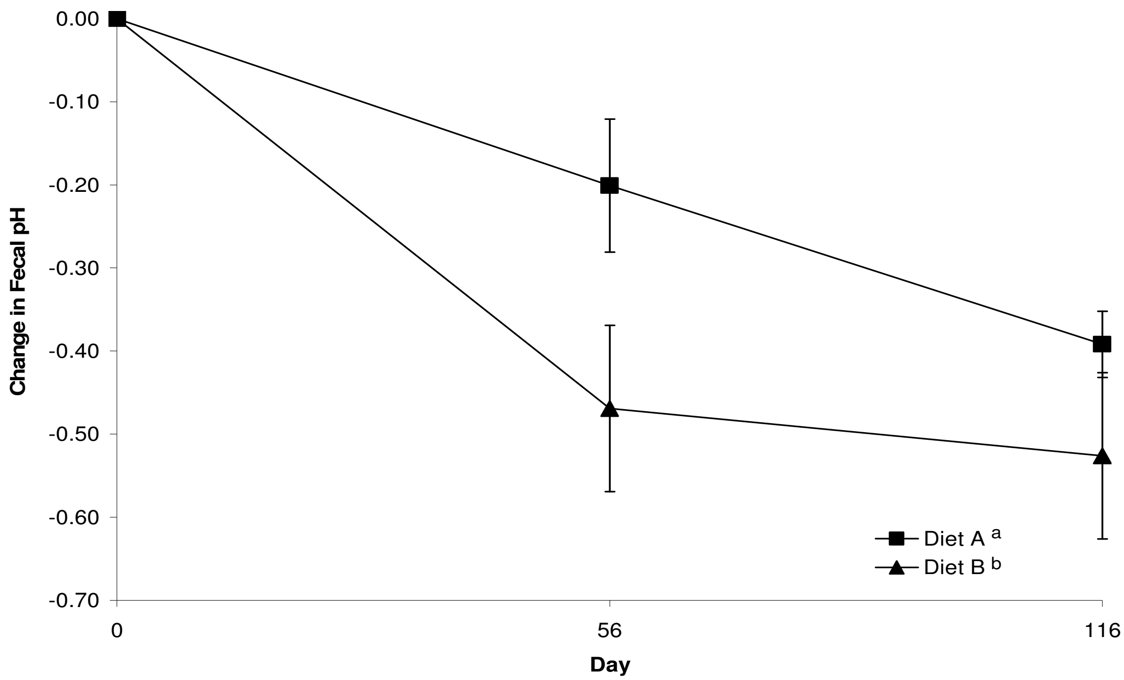

Figure 6. Normalized fecal pH by diet and day.

[a,b]Diets not sharing a common superscript differ ($P < 0.02$)

Calcium Balance

Calcium intake averaged 183.08 g/mgBW/d at d0, decreased to 129.33 mg/kgBW/d at d56, then further decreased to 117.66 g/mgBW/d at d112 (Table 6). The initial decrease is the result of a decrease in concentrate Ca content of the concentrate portion of the diet from the pre-study ration to dietary treatments, where as the decrease from d56 to d112 is mostly the result of a decrease in Ca content in the hay. Still, there was no difference in Ca intake between dietary treatments. NRC (1989) recommendations for Ca intake for these horses range from 18.7–19.6 g/day, based on an average rate of gain of 0.21 kg/day (the average rate of gain over the study duration). Therefore, Ca intake averaged 293% of NRC at d0 and 194% at d112. Still, Ca content in the dietary treatments is similar to that commonly found in commercial horse rations.

Fecal Ca over both treatments decreased to d56 ($p < 0.0001$) then remained unchanged through d116 (Figure 7). This is in sharp contrast to previous exercise studies where an increase in fecal Ca at d64 was observed and attributed to decreased absorption resulting from elevated systemic Ca levels due to the resorption phase of bone remodeling (Nielsen et al, 1998). Since the horses in this study were not exercised to an extent to stimulate remodeling, an increase in fecal Ca here would not be expected. Conversely, the decrease in fecal Ca observed in this study can easily be attributed to decreased Ca intake over the same period.

Table 6. Calcium balance by diet and day

Day	0	56	112	Mean
Ca intake (mg/kgBW/day)				
Diet A	182.75	138.18	120.24	147.06
SEM	1.81	9.81	9.08	6.96
Diet B	183.40	120.47	115.08	139.65
SEM	4.40	4.44	5.60	6.97
Mean	183.08[a]	129.33[b]	117.66[c]	
SEM	2.14	5.68	5.20	
Fecal Ca (mg/kgBW/day)				
Diet A[c]	112.67	64.87	58.93	78.83
SEM	6.93	2.85	2.01	5.59
Diet B[d]	140.00	71.29	75.18	95.49
SEM	13.34	2.82	3.84	7.97
Mean	126.34[a]	68.08[b]	67.06[b]	
SEM	8.07	2.11	2.96	
Urine Ca (mg/kgBWday)				
Diet A	3.87	2.96	1.83	2.88
SEM	1.02	1.25	0.83	0.60
Diet B	3.09	2.42	1.72	2.41
SEM	0.71	0.84	0.69	0.43
Mean	3.48	2.69	1.78	
SEM	0.61	0.73	0.52	
Ca absorbed (mg/kgBW/day)				
Diet A[c]	70.08	73.32	61.30	68.23
SEM	6.56	11.35	8.58	8.58
Diet B[d]	43.40	49.19	39.90	44.16
SEM	15.57	3.31	4.06	5.29
Mean	56.74[a]	61.25[b]	50.607[b]	
SEM	8.86	6.50	5.35	
Ca absorbed as % of intake				
Diet A	38.39	61.41	60.68	53.49
SEM	3.59	3.62	2.41	2.86
Diet B	33.73	53.95	49.06	46.66
SEM	5.92	1.73	1.80	2.49
Mean	36.39	57.68	54.87	
SEM	3.18	2.16	2.09	
Ca retained (mg/kgBW/day)				
Diet A[c]	66.21	70.36	59.48	65.35
SEM	6.73	12.03	8.91	5.31
Diet B[d]	40.31	46.76	38.17	41.75
SEM	15.21	3.99	4.24	5.24
Mean	53.26[a]	58.56[b]	48.82[b]	
SEM	8.70	6.84	5.50	
Ca retained as % of intake				
Diet A[e]	36.29	59.59	59.38	51.75
SEM	3.72	4.18	2.86	3.03
Diet B[f]	31.95	52.29	47.90	45.15
SEM	5.65	2.30	2.06	2.55
Mean	34.43[a]	55.93[b]	53.64[b]	
SEM	3.14	2.49	2.26	
Ca retained as % of absorption				
Diet A	93.71	96.43	97.62	95.92
SEM	1.90	2.01	1.37	1.04
Diet B	94.86	96.66	97.48	96.47
SEM	1.09	1.28	1.07	0.68
Mean	94.21	96.54	97.55	
SEM	1.16	1.15	0.84	

[a,b]Row means not carrying a common superscript differ ($P < 0.0001$)
[c,d]Diets not carrying a common superscript differ ($P < 0.01$)
[e,f]Diets not carrying a common superscript differ ($P < 0.05$)

Fecal Ca was also different due to diet ($p < 0.005$) (Table 6, Figure 7). Diet A averaged 78.83 ± 5.59 mg/kgBW/day while diet B averaged 95.49 ± 7.97 mg/kgBW/d. This was likely the result of decreased Ca absorption in diet B resulting in more unabsorbed Ca remaining in the feces. No diet*day interaction was determined from the ANOVA.

Urinary excretion of Ca was not significantly different at any time during the study and was not different by diet. These findings are in contrast with Nielsen et al. (1998), who reported a 7-fold decrease in urinary Ca in horses undergoing training. This is also in contrast to previous research into the effects of excess dietary protein on Ca balance and bone density. It is commonly believed that excess dietary protein leads to hypercalciuria (Kersetter and Allen, 1990), and that excessively high protein diets in humans may increase urinary Ca excretion by as much as 800% (Schryver et al., 1987).

Calcium absorption did not change due to day even when corrected for body weight. Absorption averaged 17.45 ± 1.23 g/day or 56.20 ± 4.04 mg/kgBW/d. This finding is surprising in that Ca absorption is generally expected to increase as Ca intake decreases, such as in the decrease that occurred from d0 to d56. Absorption as a percent of intake when corrected to body weight (mg/kgBW/d) increased to d56 ($p < .001$). This is easily explainable mathematically by a decrease in intake corresponding to no change in absorption values.

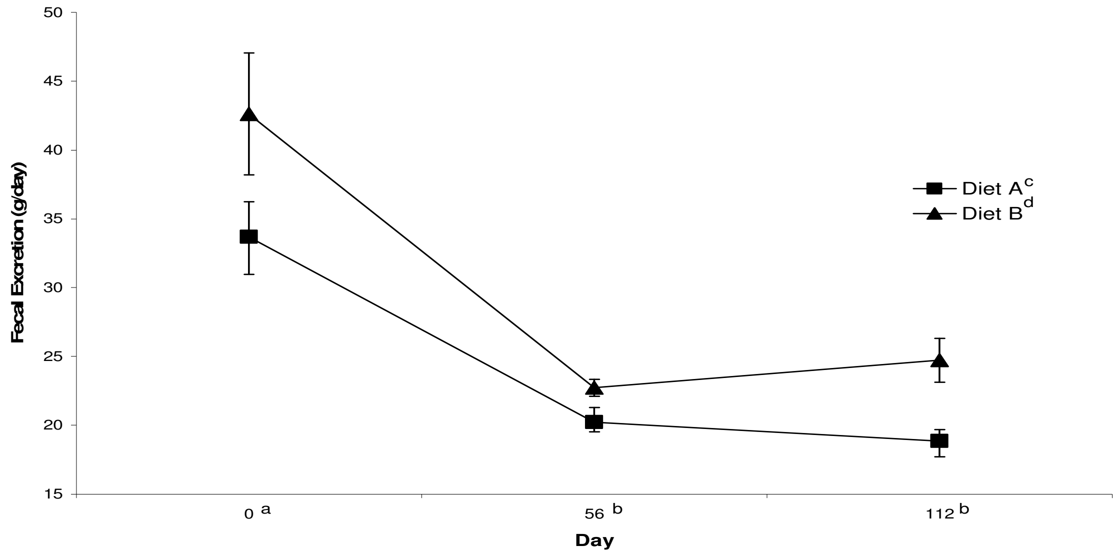

Figure 7. Fecal Ca excretion by diet and day.

a,b Means across diets differ for days not sharing a common superscript ($P < 0.0001$)
c,d Diets not sharing a common superscript differ ($P < 0.005$)

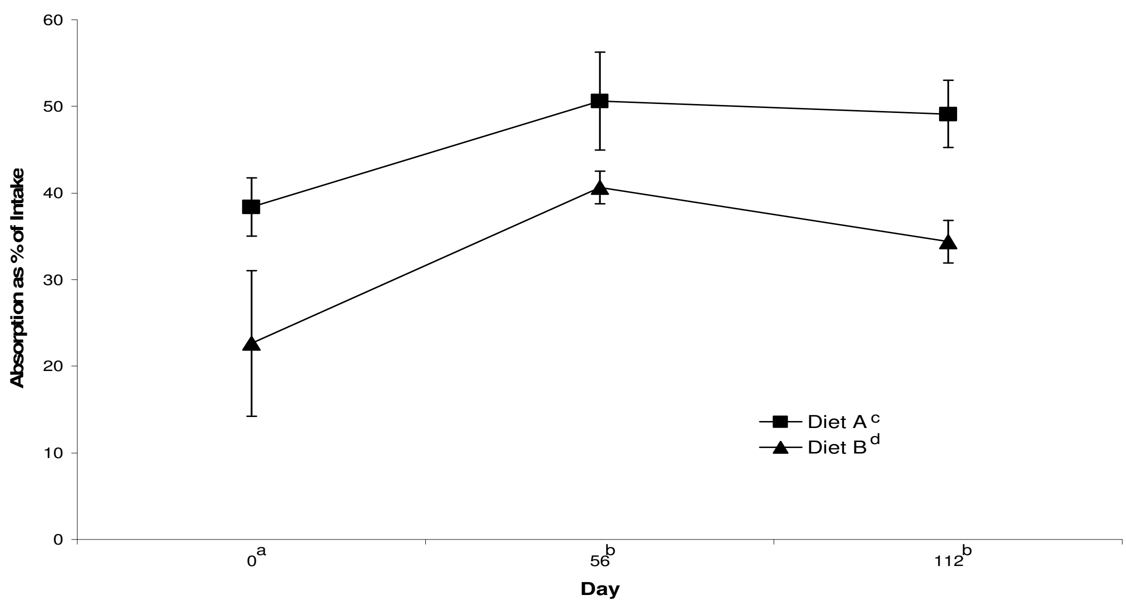

Figure 8. Ca absorption as a percent of intake by diet and day.

a,b Means across diets differ for days not sharing a common superscript ($P < 0.01$)
c,d Diets not sharing a common superscript differ ($P < 0.002$)

Over the course of the study, Ca absorption was higher in diet A than in diet B, the high protein treatment (Table 6, $p < 0.005$). Absorption averaged 68.23 ± 5.10 mg/kgBW/d and 44.16 ± 5.29 mg/kgBW/d in diets A and B, respectively. Then, as expected with similar Ca intake between diets, absorption as a percent of intake was also higher in diet A (Figure 8, $p < 0.002$).

Calcium retention (mg/kgBW/d) was very similar to absorption. Both retention and retention as a percent of intake were lower in the high protein group (diet B) ($p < .001$, $p < 0.002$ respectively). As urinary Ca excretion was not different between diets, the differences in retention values must be attributed to differences in absorption. Consequently, retention as a percent of absorption did not differ between diets or by day. There were no significant diet*day interactions for any measured variables of Ca balance.

These findings are surprising in that only one study on the effect of excess dietary protein on Ca balance has reported changes in Ca absorption or retention. In that particular study, the results opposite to those found here were observed; the high protein diet had increased intestinal absorption compared to a moderate protein intake (26.2 ± 1.9%, 18.5 ± 1.6%,
$P < 0.0001$) (Kerstetter et al., 2005). This increase in absorption paralleled an increase in urinary Ca excretion and led the authors to conclude that the increase in urinary Ca is the result of increased intestinal absorption with no contribution from bone resorption. Other studies have failed to reach the same conclusions and instead have found no effect of dietary protein level on Ca absorption (Heaney, 2000). Thus, it is difficult to say with any certainty that the differences in absorption between the dietary treatments in this study are the absolute result of dietary protein levels.

Phosphorus Balance

Phosphorus intake decreased over time ($p < 0.001$), from 106.56 ± 1.22 mg/kgBW/d at d0 to 80.77 ± 1.73 mg/kgBW/d on d56, as a result of the change from the pre-study diet to dietary treatments (Table 7). There was no change in P intake from d56 to d112. P intake was also slightly but significantly different between diets (Figure 9) ($p < 0.001$) as a result of diet formulations. P intake in diet A averaged 86.09 ± 3.07 mg/kgBW/d while diet B averaged 91.32 ± 2.58 mg/kgBW/d. Because P intake was not significantly different between diets at d0, there was also a diet*day interaction ($p < 0.02$) (Appendix 7A).

Fecal P decreased to d56 ($p < 0.0001$) as a result of a decrease in P intake, but remained unchanged from d56 to d112 (Table 7). Fecal P tended to be higher in diet B, although this difference was not considered significant ($p < 0.10$). Nevertheless, this is most likely a result of the greater intake in diet B. No diet*day interaction was observed for fecal P.

Urine P excretion averaged 0.46 ± 0.1 mg/kgBW/d over the course of the study. While there was a trend for Urine P to increase from d56 to d112 ($p < 0.07$), there was no difference between diets even with differences in intake. The tendency for increased P excretion from d56-d112 is difficult to explain, as intake did not vary over this time.

Phosphorus absorption was not different due to diet or day, and averaged 11.73 ± 2.25 mg/kgBW/d. Correspondingly, P retention also did not vary due to diet or day and averaged 11.27 ± 2.25 mg/kgBW/d. Variation in P absorption and retention between individual horses was quite large, with many horses having both negative absorption and retention values (Appendix 7B). As a result of such negative numbers, P absorption as a percent of intake, retention as a percent of intake, and retention as a percent of absorption were not calculated.

Because dietary P intake differed between the control (diet A) and high-protein diet (diet B), it is possible that the increased P intake in the high protein diet is responsible for the decreased Ca absorption observed. It has been shown that increased P content in the diet may negatively impact calcium absorption perhaps as a result of the P forming insoluble complexes with Ca (Schryver, 1978). Studies in humans, however, have shown little or no effect of variation in P intake on overall Ca balance (Heaney and Recker, 1982). Still, it is commonly accepted that the ratio of Ca to P is more important than simple P intake. The Ca:P ratio in this study remained greater than 1:1 for both diets at all times.

Table 7. Phosphorus balance by diet and day

Day	0	56	112	Mean
P intake (mg/kgBW/d)				
Diet A[c]	106.26	75.25	76.75	86.09
SEM	1.05	1.67	1.40	3.07
Diet B[d]	106.87	86.28	80.81	91.32
SEM	2.29	1.19	2.26	2.58
Mean	106.56[a]	80.77[b]	78.78[b]	
SEM	1.22	1.73	1.38	
Fecal P (mg/kgBW/d)				
Diet A	100.68	56.84	63.320	73.61
SEM	8.16	2.23	1.86	4.88
Diet B	96.38	66.03	78.59	80.34
SEM	6.09	3.55	4.22	3.69
Mean	98.52[a]	61.44[b]	70.96[b]	
SEM	4.95	2.35	2.98	
Urine P (mg/kgBW/d)				
Diet A	0.21	0.32	0.81	0.44
SEM	0.1	0.2	0.4	0.2
Diet B	0.28	0.33	0.80	0.47
SEM	0.1	0.2	0.4	0.1
Mean	0.24	0.32	0.80	
SEM	0.1	0.1	0.3	
P absorbed (mg/kgBW/d)				
Diet A	5.59	18.42	13.44	12.48
SEM	8.1	3.0	2.3	3.2
Diet B	18.42	20.25	2.21	10.98
SEM	3.0	4.2	4.4	3.4
Mean	8.04	19.33	7.82	
SEM	5.3	2.5	2.8	
P retained (mg/kgBW/d)				
Diet A	5.38	18.10	12.63	12.04
SEM	8.0	3.0	2.4	3.0
Diet B	10.22	19.92	1.42	10.52
SEM	7.2	4.2	4.5	3.4
Mean	7.80	19.01	7.02	
SEM	5.2	2.5	2.9	

[a,b]Days not sharing a common superscript differ ($P < 0.0001$)
[c,d]Diets not sharing a common superscript differ ($P < 0.02$)

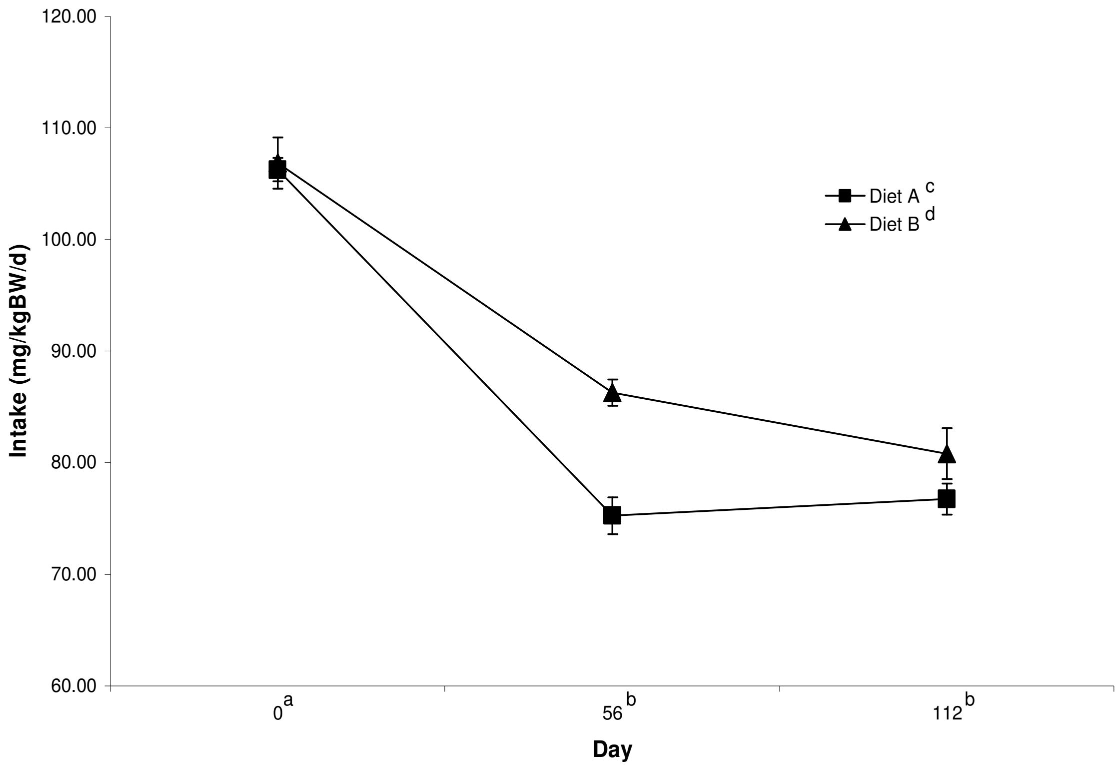

Figure 9. Phosphorus intake by diet and day.

[a,b]Means across diets for days not sharing a common superscript differ ($P < 0.0001$)
[c,d]Diets not sharing a common superscript differ ($P < 0.001$)

RBAE

Few radiographic abnormalities were identified. Horse 8A was found to exhibit radiographic signs of physitis. Because the area used to determine RBAE was not affected and no lameness was observed, the horse remained on the study and data were included. Additionally, horse 6B was observed radiographically to have a bone chip in his fetlock. Since no lameness was present, the horse continued on the project and data were included.

RBAE values for medial and lateral cortices did not differ according to day (Table 8, Appendix 4A, 4C). When means were pooled across treatment groups a significant difference ($p < 0.001$) was observed in RBAE of the dorsal cortex due to day, with mean values increasing from 13.17 ± 0.3 mm Al at d0 to 15.05 ± 0.2 mm Al at d112 (Figure 10). Similarly, RBAE of the palmar cortex increased ($p < 0.001$) from 11.16 ± 0.4 mm Al at d0 to 12.89 ± 0.26 mm Al at d112, when means were pooled across groups (Figure 11). Total RBAE did not change from d0 values.

There were no significant changes in RBAE due to diet, but because RBAE is highly variable across different horses, but very repeatable in individual horses, and because d0 values were significantly different for diet A and diet B ($p < 0.05$), RBAE was normalized to d0 values (Appendix 4A, 4B, 4C). Even with normalization of the data, no significant differences were observed by diet, day, or diet*day for any measured variable.

Table 8. Cortical and total RBAE by diet and day

Day	0	56	112
Lateral RBAE (mm Al)			
Diet A	15.96	16.46	17.24
SEM	0.42	0.48	0.59
Diet B	16.75	16.8	16.98
SEM	0.23	0.45	0.37
Mean	16.35	16.63	17.1
SEM	0.25	0.32	0.34
Medial RBAE (mm Al)			
Diet A	18.16	18.99	18.72
SEM	0.39	0.20	0.34
Diet B	18.31	18.54	18.89
SEM	0.31	0.35	0.37
Mean	18.23	18.76	18.80
SEM	0.24	0.20	0.24
Palmar RBAE (mm Al)			
Diet A	10.78	12.23	12.91
SEM	.43	0.44	0.38
Diet B	11.55	12.48	12.88
SEM	.64	0.33	0.37
Mean	11.16[a]	12.36[b]	12.90[c]
SEM	0.39	0.26	0.26
Dorsal RBAE (mm Al)			
Diet A	12.89	14.45	15.12
SEM	0.49	0.32	0.28
Diet B	13.44	14.38	14.98
SEM	0.50	0.24	0.22
Mean	13.17[a]	14.41[b]	15.05[c]
SEM	0.35	0.19	0.17
Total RBAE (mm^2 Al)			
Diet A	661.38	676.11	687.73
SEM	28.71	56.70	24.59
Diet B	723.61	698.24	699.66
SEM	48.97	21.42	16.88
Mean	692.50	687.91	693.69
SEM	28.57	27.82	13.76

[a,b,c]Days not sharing a common superscript differ ($P < .001$)

Although previous work in this laboratory has identified training effects on bone, there was no pattern of bone demineralization in early training evident in this project. This was expected as the level of exercise the horses were given was most likely not enough to illicit a bone modeling response. Hiney et al. (2001) demonstrated that short bouts of intense exercise (galloping) were needed to stimulate bone modeling in young horses. The horses in this project were exercised over a soft ground surface at a walk and strong trot which resulted in low torsional stress on the bone. Still, the overall increase in RBAE of the dorsal cortex is similar to that seen in animals subjected to more strenuous exercise. RBAE findings were in contrast, however, to a study conducted by Hiney and colleagues (2004), where in young growing horses, RBAE values over time increased in the medial and lateral cortices but failed to show an increase in the dorsal or palmar cortices. This difference may be related to the age of the animals, as those used in the Hiney (2001) study averaged 4 months. Furthermore, it is also important to realize that total RBAE is a result of not only bone density but also of bone size. As it would be expected that the size of the bone would be increasing in these animals, the fact that total RBAE remained unchanged may indicate lower bone mineral per area of bone.

Given that hypercalciuria was not a result of the high protein diet, it is not surprising that no changes in RBAE were observed due to diet as Ca was apparently not being pulled from skeletal stores. The lack of effect of diet was could have also been the result of the length of the time of the study or the amount of forced exercise. Perhaps if the horses consumed the high protein diet over a longer period of time, the resulting longer-term decrease in Ca absorption could have resulted in changes to bone mineral content. Furthermore, if the horses were exercised to the point of stimulating bone remodeling, where it is expected that mineral demands within the animal would increase, then changes in Ca absorption and retention could be expected to affect bone mineral content.

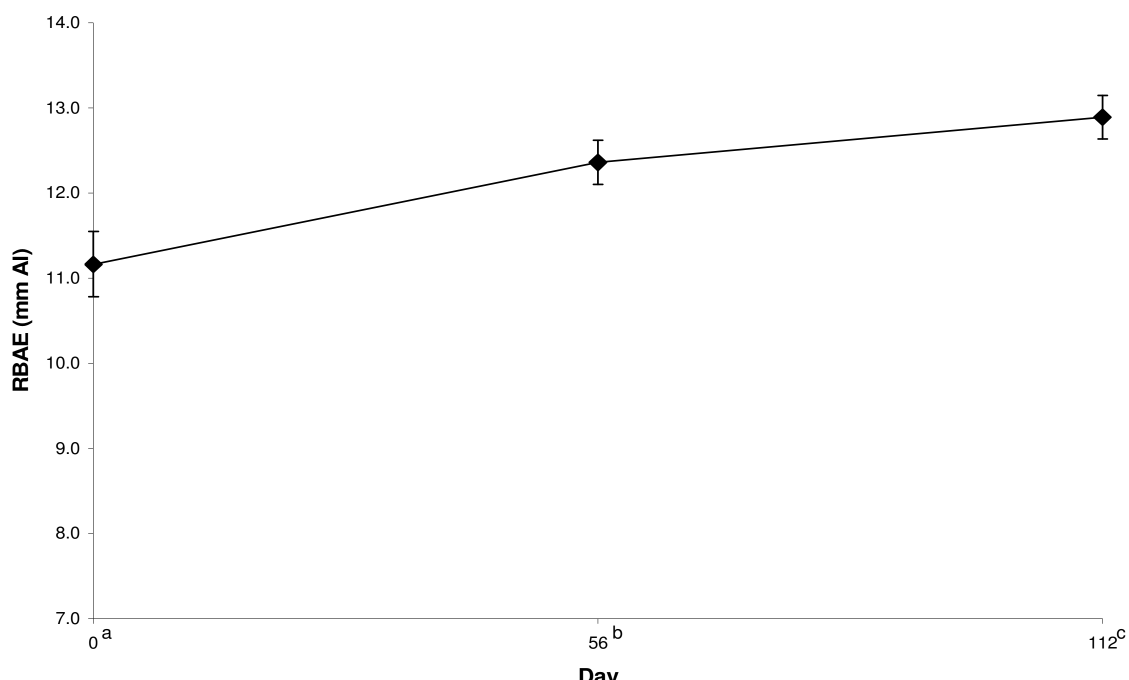

Figure 10. Palmar RBAE by day.

[a,b,c]Days not sharing a common superscript differ (P< 0.001)

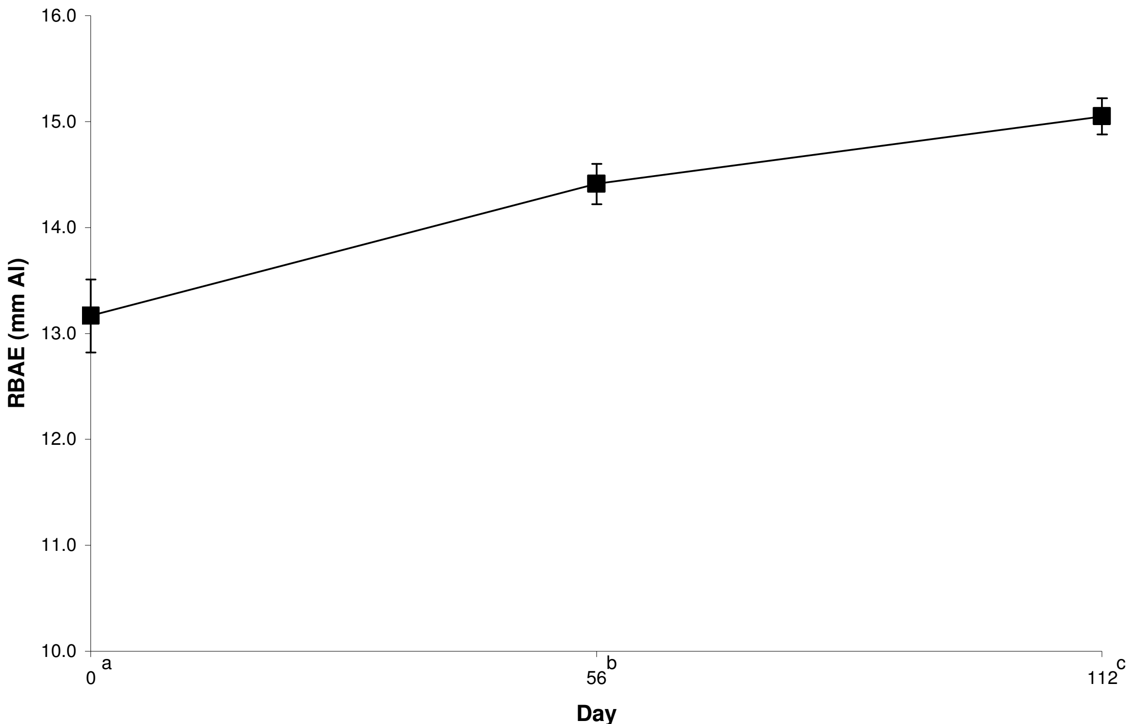

Figure 11. Dorsal RBAE by day.

[a,b,c]Days not sharing a common superscript differ (P< 0.001)

Bone Geometry

Measurements of bone geometry taken in this study agree with previous research on the shape of the third metacarpal (MCIII) in the equine. The dorsal and medial cortices have greater width when compared to their palmar and lateral counterparts (Appendix 5B, 5C) enabling them to withstand the greater stresses placed upon them by locomotion (Welch, 1999). Overall bone width is greater in medial-lateral (ML) direction than the dorsal-palmar (DP), as is the width of the respective medullary cavities. This supports the concept that as a foal grows, the round cross-section of MCIII becomes more oval with an elongated, elliptical medullary cavity.

Due to variation in bone width of the third metacarpal between treatments (Appendix 5A, 5B) and individual animals (Appendix 5C) data were normalized to d0 for analysis. In the medial-lateral (ML) direction a difference was identified due to diet ($p < 0.05$); total bone width for diet A increased an average of 0.224 $\pm$ 0.42 mm, while diet B decreased by -0.863 $\pm$ 0.33 mm (Figure 12). Similarly, the change in width of the medial cortex approached significance ($p < 0.09$) with a mean of 0.517 $\pm$ 0.26 mm for diet A and -0.152 $\pm$ 0.27 mm for diet B. It can be assumed then that the change observed in total ML bone width can be attributed to change in the medial cortex. No other variables exhibited significant differences due to diet, day, or diet*day.

It may be that the increase in total ML width and trend toward increased width of the medial cortex in the control diet compared to the high protein can be attributed to availability of Ca within the body for use in the bone. If Ca absorption and retention are impacted by acidogenic effects of high protein and the available Ca pool is reduced, bone growth may be adversely affected. This could be a bigger concern in younger or more intensely exercised horses where bone turnover is greater.

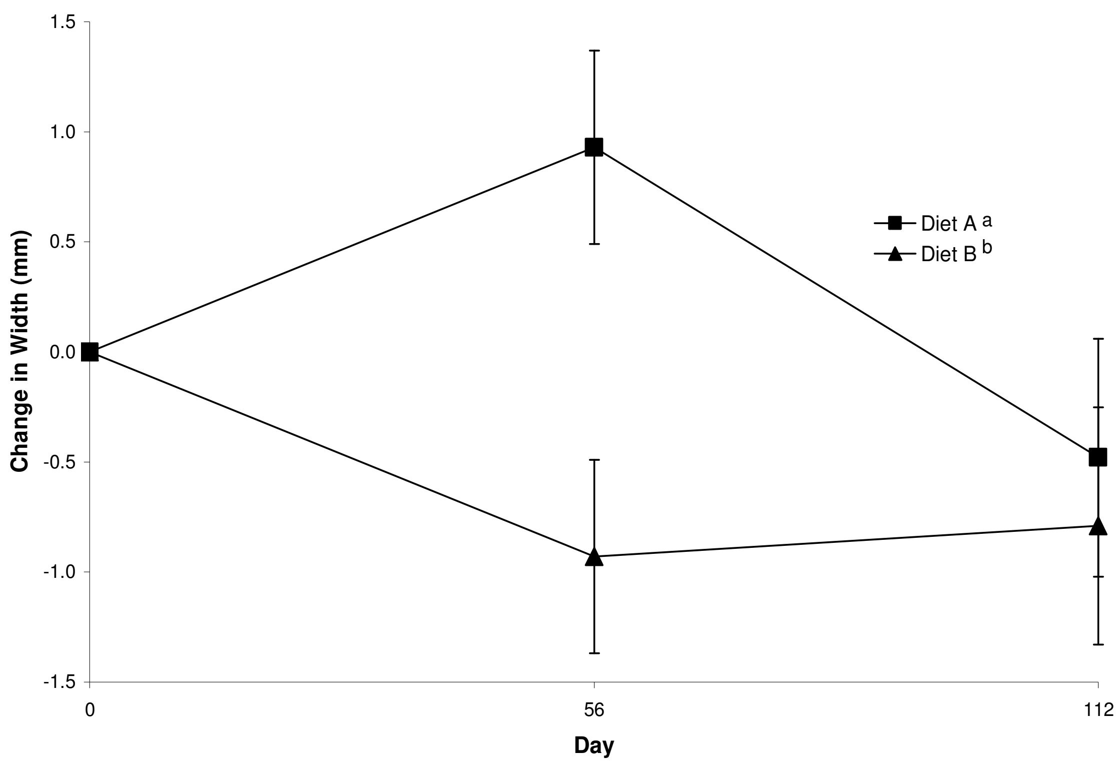

Figure 12. Normalized total ML width by diet and day.

[a,b]Diets not sharing a common superscript differ ($P < 0.05$)

CHAPTER V

GENERAL DISCUSSION

The physical demands placed on two and three-year-old horses in race or performance training has escalated over the past few decades as purses in both juvenile races and performance horse futurities have escalated. While economics continue to drive trainers to work horses harder at a younger age, the horses' welfare must be considered, especially as the horse industry works to market itself to mainstream America. Still, rates of injury in the immature horse are quite high. To reduce the risk of such injuries, research is being conducted to identify management practices, both training and nutritional, which may be altered.

It has been demonstrated that peak bone mass in the horse is not reached until approximately 6 years of age (Lawrence et al., 1994). At the same time, intense exercise has been shown to initiate a bone remodeling response culminating in reduced bone mineral density approximately 60 days after the initiation of an exercise regimen (Nielsen et al., 1997). Unfortunately, this corresponds to the time when most trainers begin asking for sprint work or increased performance, setting the stage for increased bone injury and failure. Management practices for increasing bone mass initially as well as avoiding factors which may further decrease bone density are currently being identified.

Calcium requirements have been shown to increase dramatically in horses undergoing training, as Ca and type-1 collagen make up the majority of the structural component of the bone. NRC (1989) recommendations for Ca in exercised horses may be inadequate to support the modeling/remodeling occurring, particularly early in training (Nielsen et al., 1997; Stephens et al., 2004). As a result, higher Ca concentrations are being incorporated into the diets of young, exercising animals.

Still, research in humans and other species indicates that factors other than Ca intake may play a role in the amount of Ca present in the body and thus available for incorporation within bone. Dietary protein intake is one such factor. High protein intake specifically has been associated with hypercalciuria and reduced bone density (Kersetter and Allen, 1990), purportedly as a result of an acidogenic effect of the diet.

As protein intakes above NRC (1989) recommendations are commonplace in the horse industry, and the potential for excess amino acid metabolism to cause metabolic acidosis exists, this study was initiated to determine the effects of excess dietary protein on physiological parameters including pH, bone density, bone geometry, and mineral balance in young horses.

This study did not reveal metabolic acidosis or hypercalciuria as a result of the high protein diet. While fecal pH was significantly lower after feeding the high protein diet, no changes in urine or blood pH were attributed to feeding the high protein diet. However, urinary pH was abnormally high at d0, most likely as a result of the pre-study diet. Perhaps if horses would have started the study with lower urine pH values, an effect of the diet might have been observed.

Serum Ca and serum P concentrations were also unaffected by dietary treatment. This was expected for Ca as a result of the body's tight homeostatic mechanisms. On the other hand, serum blood urea nitrogen (BUN) was higher in the high protein group. This has been previously reported (Schryver, 1986) and can serve as an indicator of the deamination of excess dietary protein, likely due to excess fermentation of protein in the hindgut and resulting absorption of ammonia.

Differences in fecal Ca, as well as Ca absorption and retention, were observed between dietary treatments. Horses consuming the high protein diet were found to have increased fecal Ca excretion. As Ca intakes were similar between diets, absorption, then, was lower in the high protein group. Urine Ca excretion, while found to be up to 800% higher in studies of high protein diets versus normal intake (Schryver et al., 1987), was not different in this study. Calcium retention was also lower in the high protein horses, but with similar urine Ca excretion, this is simply the result of decreased absorption. It may be that the decrease in fecal pH observed in this study resulted in reduced Ca absorption, although no previous research indicating such could be identified. It is also possible that an unknown factor in the high protein diet is responsible for both reduced pH and decreased absorption. Still, previous research has failed to identify an inhibiting effect of high protein on Ca absorption, and, in fact, one study identified opposite results (Kerstetter et al., 2005).

Phosphorus balance was similar between diets, although the high protein diet had increased dietary P intake. Still, absorption and retention remained similar for both treatments, while fecal excretion tended to be higher in the high protein group. Previous research has indicated a negative effect of P on Ca absorption. It may be possible then, that the decrease in Ca absorption is the result of higher P intake. Still, this possibility seems unlikely, as the differences in P intake, although significant, were quite small and the calcium to phosphorus ratio remained greater than 1.

No differences in bone density (RBAE) were attributed to the high protein diet. The RBAE in the dorsal and palmar cortices increased over time for both treatment groups, most likely as a result of normal growth.

While most measures of bone geometry were unaffected by diet, when values were normalized to each animal's day 0 readings, total bone width in the medial-lateral direction was found to be reduced in horses consuming the high protein diet, with the majority of change occurring in the medial cortex.
It may be that this is the result of decreased calcium availability for incorporation into new bone. Still, as no other cortices or total bone widths were different due to diet, it is difficult to confirm that the diet was in fact the causative agent.

Overall, it appears that feeding a high protein diet to immature horses does not negatively impact bone density perhaps as a result of the horse's unique digestive system. Calcium absorption, however, may be affected by overfeeding of dietary protein, or by a decreased pH in the hindgut. Further research is necessary to determine the effects of reduced absorption and decreased hindgut pH over a longer period and in exercising horses.

Still, it is important to remember that there have been no demonstrated benefits of protein intakes above NRC recommendations. As dietary protein is economically more expensive in terms of feedstuffs and more metabolically expensive for the horse, feeding protein at levels above NRC recommendations, while not found to be harmful to bone density, remains an ineffective management practice.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This study was conducted to determine the influence of excess dietary protein on physiological parameters including blood, urine, and fecal pH, calcium balance, and bone density in immature horses. While previous work has identified mineral requirements in the young and exercising horse, work in other species indicates that other dietary factors, such as protein intake, may play a role in absorption and availability of Ca.

Overall, findings in this study regarding consumption of excess dietary protein in the immature horse are conflicting. While the animals appeared to grow normally over time, no differences were observed in bone density, although bone size may have been negatively impacted as indicated by reduced medial-lateral width of the third metacarpal in horses consuming the high protein diet. Still, horses in this study were not exercised to the extent that appears to be necessary to induce bone modeling/remodeling. Perhaps if Ca requirements were greater as a result of increased modeling/remodeling due to exercise a difference would have been observed.

Metabolic acidosis as evidenced by blood and urine pH was not observed, but fecal pH proved to be lower in horses consuming the high protein diet. Calcium absorption and retention was also lower in the high protein treatment but differences in P intake may be to blame as previous research into the effect of excess dietary protein has failed to show decreased Ca absorption.

Further research is necessary to confirm the negative impact, if any, of excess dietary protein in the immature horse.

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APPENDICES

APPENDIX 1A. ANOVA TABLE FOR PHYSICAL MEASUREMENTS

Source	df	Partial SS	MS	F-value	P-value
Body Weight					
Total	79	177101.888	2241.79604		
Model	9	34055.7625	3783.97361	1.85	0.0740
Residual	70	143046.125	2043.51607		
Diet	1	5200.3125	5200.3125	2.54	0.1152
Day	4	28356.7	7089.175	3.47	0.0121
Diet*Day	4	498.75	124.6875	0.06	0.9930
Wither Height					
Total	79	178.0125	2.25332278		
Model	9	14.496875	1.61076389	0.69	0.7159
Residual	70	163.515625	2.3359375		
Diet	1	0.903125	0.903125	0.39	0.5361
Day	4	13.121875	3.28046875	1.40	0.2415
Diet*Day	4	0.471875	0.11796875	0.05	0.9951
Hip Height					
Total	79	219.511719	2.77862935		
Model	9	39.3789063	4.37543403	1.70	0.1053
Residual	70	180.132813	2.57332589		
Diet	1	0.17578125	0.17578125	0.07	0.7946
Day	4	34.90625	8.7265625	3.39	0.0136
Diet*Day	4	4.296875	1.07421875	0.42	0.7955
Heart Girth					
Total	79	1535.02394	19.4306827		
Model	9	462.190202	51.3544668	3.35	0.0018
Residual	70	1072.83373	15.3261962		
Diet	1	64.980136	64.980136	4.24	0.0432
Day	4	388.014573	97.0036434	6.33	0.0002
Diet*Day	4	9.19549213	2.29887303	0.15	0.9624

APPENDIX 1A. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Body Length					
Total	79	5682.89893	71.9354295		
Model	9	1273.67017	141.518908	2.25	0.0286
Residual	70	4409.22876	62.9889823		
Diet	1	313.236137	313.236137	4.97	0.0290
Day	4	838.24577	209.561442	3.33	0.0149
Diet*Day	4	122.188265	30.5470661	0.48	0.7467
Forearm Circumference					
Total	79	410.628872	5.19783383		
Model	9	16.5751234	1.84168038	0.33	0.9633
Residual	70	394.053749	5.62933927		
Diet	1	2.21112475	2.1112475	0.39	.5329
Day	4	4.77574956	1.19393739	0.21	0.9309
Diet*Day	4	9.58824908	2.39706227	0.43	0.7895
Gaskin Circumference					
Total	79	131.294885	1.66196057		
Model	9	9.99613071	1.11068119	0.64	0.7583
Residual	70	121.298754	1.73283934		
Diet	1	0.003125	0.003125	0.00	0.9662
Day	4	8.63675571	2.15918893	1.25	0.2996
Diet*Day	4	1.35625	0.3390625	0.20	0.9399
Rump Fat 1					
Total	79	302.75	3.83227848		
Model	9	148.75	16.5277778	7.51	0.0000
Residual	70	154	2.2		
Diet	1	0.2	0.2	0.09	0.7639
Day	4	147.125	36.78125	16.72	0.0000
Diet*Day	4	1.425	0.35625	0.16	0.9569
Rump Fat 2					
Total	79	219.6875	2.78085443		
Model	9	124.5625	13.8402778	10.18	0.0000
Residual	70	95.125	1.35892857		
Diet	1	0.125	0.125	0.01	0.9239
Day	4	124.125	31.03125	22.84	0.0000
Diet*Day	4	0.425	0.10625		

APPENDIX 1B. PHYSICAL MEASUREMENTS OF HORSES

Horse	Diet	Day	Body Weight (kg)	Wither Height (cm)	Hip Height (cm)	Heart Girth (cm)	Body Length (cm)	Forearm (cm)	Gaskin (cm)	Rump Fat 1 (mm)	Rump Fat 2 (mm)
1A	A	0	704	53.25	56.25	151.2	137	53	41.5	1	1
2A	A	0	572	51.5	53.25	145	134.7	49	39	1	1
3A	A	0	664	53.5	56	154	130	50	41.6	1	1
4A	A	0	656	53	54.5	152	139	49.5	41.5	1	1
5A	A	0	662	51.75	54.5	153.4	136	52	42.1	1	1
6A	A	0	592	49.5	51.5	146.5	129	49.5	42	1	1
7A	A	0	704	53.75	55.75	154	146	54.5	42	2	2
8A	A	0	682	52.5	55.25	154	140	53.5	42.5	1	1
1B	B	0	644	52.5	54	150.2	139.5	50	40.1	1	2
2B	B	0	652	52	53.75	152	137	46	40.1	2	1
3B	B	0	686	50.5	54	154	193	53	42	2	2
4B	B	0	702	55.5	58.25	155	138.5	52.5	43	1	1
5B	B	0	656	54	56.5	154	134.5	49.2	40	1	1
6B	B	0	594	51.75	53.75	142	136	47	39.5	1	1
7B	B	0	678	54	55	152	141	50	42	2	1
8B	B	0	718	51.25	54.25	155	142	51	42	1	1
1A	A	28	696	54.5	57	153	141.5	53	42	1	1
2A	A	28	602	52.75	54.5	146.5	144	51	40	1	1
3A	A	28	682	55.25	57	156.5	136	53	42	1	1
4A	A	28	680	53.25	55	154	138.5	48.5	41.5	2	2
5A	A	28	688	52.25	55.25	154	139	51	42	2	2
6A	A	28	613	49	52	146	129	49	42	1	2
7A	A	28	724	53.5	56.5	153.5	140.5	50.5	41	3	3
8A	A	28	710	53	55.5	153.5	142	52.5	42	3	2
1B	B	28	648	53.25	54.75	152.5	139.5	51	41	1	1
2B	B	28	675	52.75	55.25	157.5	143.5	47.5	41	1	1
3B	B	28	714	51.75	53.75	155.5	140	53.5	42.5	2	1
4B	B	28	720	55.75	58.5	157	146.5	54	44	2	1
5B	B	28	682	54.5	57	156	144	49	40	2	2
6B	B	28	598	52.25	54.25	144	133.5	47.5	40	2	2
7B	B	28	700	54.25	56.5	153.5	146	50	42	3	2
8B	B	28	730	52.25	54.75	156	148	51.5	42.5	3	2
1A	A	56	718	54.25	57	153.5	140	52	42	2	2
2A	A	56	602	53	54.5	146.5	139	57.5	39.5	2	1
3A	A	56	716	54.5	55	156.5	146	51	42.5	4	2
4A	A	56	710	52.75	55.5	156	143	49	42	3	2
5A	A	56	678	52.25	55	155	140.5	49.5	41	3	3
6A	A	56	624	50	51.5	145.5	136.5	48	41	3	3
7A	A	56	714	54.5	56.5	153.5	151	51	41.5	3	3
8A	A	56	716	53.5	56.25	155.5	148	52	42.5	2	2

APPENDIX 1B. CONTINUED

Horse	Diet	Day	Body Weight (kg)	Wither Height (cm)	Hip Height (cm)	Heart Girth (cm)	Body Length (cm)	Forearm (cm)	Gaskin (cm)	Rump Fat 1 (mm)	Rump Fat 2 (mm)
1B	B	56	696	53.75	55.75	154	149	53	41.5	2	2
2B	B	56	720	53	54	156	140	48	41.5	2	2
3B	B	56	746	51	54.5	156.5	143	54.5	43	3	2
4B	B	56	772	55.25	58.75	160	154	53	44	2	3
5B	B	56	686	54	56.5	155.5	143.5	50.5	40	3	3
6B	B	56	606	52	54.25	147	141	48.5	40.5	3	2
7B	B	56	690	54.25	56	155	142.5	50	43	3	2
8B	B	56	722	52.75	55.5	158	149	51.5	41.5	4	3
1A	A	84	712	54.5	57.25	155.5	149.5	51	42.5	3	3
2A	A	84	630	53.5	55.25	148	137	50.5	39.5	3	2
3A	A	84	706	55	57.25	158.5	139	50.5	41	6	5
4A	A	84	716	53.75	56.25	158.5	146.5	50.5	43	1	2
5A	A	84	700	52.25	55.5	158.5	139.5	49.5	42	1	2
6A	A	84	642	50.25	53.25	152.5	137	49.5	42	3	1
7A	A	84	722	55	57.25	156	154.5	52.5	43.5	4	4
8A	A	84	734	54	56.5	157.5	149	53.5	44	4	4
1B	B	84	686	54.25	56	155	147	51.5	41	2	1
2B	B	84	732	52.75	56.25	159	138	49	42	2	4
3B	B	84	744	52	54.75	158	143.5	54	42.5	2	4
4B	B	84	784	56.25	60.25	162.5	153.5	53	44.5	1	1
5B	B	84	702	55	57	159	146	51.5	41	1	1
6B	B	84	634	52	53	152	140	48	40.5	5	4
7B	B	84	716	54.5	57	157	149	51	43.5	6	4
8B	B	84	742	53	56	159.5	150.5	53.5	43	9	5
1A	A	112	732	54.75	57.5	156	153.5	52.5	43	3	2
2A	A	112	618	53	55	149.5	142	49.5	39.5	4	4
3A	A	112	726	55.75	57.5	160	146	50	42.5	8	5
4A	A	112	720	53.5	56.75	160.5	144.5	51.5	43	6	6
5A	A	112	710	53.25	56	159.5	139	50.5	41	5	5
6A	A	112	636	49.75	59.5	152	148	49	42	7	6
7A	A	112	737	54.5	57.5	155.5	154	50	42	4	4
8A	A	112	746	54.5	57	158	157	52.5	43.5	6	6
1B	B	112	692	53.5	56	156.5	148.5	51	41	4	5
2B	B	112	732	53.5	55.5	160	149	47.5	42	3	1
3B	B	112	748	53.25	55.25	157.5	151	56	42.5	3	4
4B	B	112	794	55.5	59.25	163	155.5	53	45	4	4
5B	B	112	696	54	57.5	161	146	53.5	40	6	6
6B	B	112	634	52	54.75	150	149.5	43.5	39.5	4	4
7B	B	112	718	54	55.75	158	151	50.5	42.5	6	5
8B	B	112	752	53	56.25	161	158	51	44	10	9

APPENDIX 2A. BLOOD PARAMETERS BY DIET AND DAY

Day	0	28	56	88	116	Mean
Serum Ca						
Diet A[a]	10.94	11.18	11.31	10.96	11.10	11.10
SEM	0.17	0.08	0.07	0.27	0.11	0.07
Diet B[b]	8.54	8.41	8.53	7.96	8.75	8.44
SEM	0.50	0.37	0.28	0.25	0.39	0.16
Mean	9.74	9.79	9.92	9.46	9.93	
SEM	0.40	0.40	0.39	0.43	0.36	
Serum P						
Diet A[a]	4.99	5.41	5.23	5.35	5.45	5.29
SEM	0.12	0.15	0.13	0.13	0.16	0.06
Diet B[b]	4.2	4.55	4.56	4.36	4.24	4.38
SEM	0.14	0.14	0.16	0.11	0.12	0.06
Mean	4.59	4.98	4.89	4.86	4.84	
SEM	0.14	0.15	0.13	0.15	0.18	
Serum BUN						
Diet A[c]	17.81	16.2	16.68	16.61	18.125	17.09
SEM	1.57	1.23	1.62	1.19	1.40	0.61
Diet B[d]	16.38	17.2	20.4	20.48	20.31	18.95
SEM	0.84	0.98	0.89	0.91	1.07	0.49
Mean	17.09	16.7	18.54	18.54	19.22	
SEM	0.88	0.70	1.01	0.88	0.90	

[a,b]Diets not carrying a common superscript differ ($P<0.0001$)
[c,d]Diets not carrying a common superscript differ ($P<0.02$)

APPENDIX 2B. ANOVA TABLE FOR BLOOD PARAMETERS

Source	df	Partial SS	MS	F-value	P-value
Serum ca					
Total	79	189.855497	2.40323414		
Model	9	145.012996	16.1125551	25.15	0.0000
Residual	70	44.8425014	0.640607163		
Diet	1	141.511995	141.511995	220.90	0.0000
Day	4	2.27675063	.569189657	0.89	0.4755
Diet*Day	4	1.22425018	.306062545	0.48	0.7519
Normalized serum ca					
Total	79	79.007999	1.00010125		
Model	9	4.85299999	0.539222221	0.51	0.8633
Residual	70	74.154999	1.05935713		
Diet	1	1.35199994	1.35199994	1.28	0.2625
Day	4	2.27675003	.306062503	0.54	0.7088
Diet*Day	4	1.22425001	.306062503	0.29	0.8842
Serum P					
Total	79	28.9988734	0.367074346		
Model	9	18.3301231	2.03668034	13.36	0.0000
Residual	70	10.6687503	0.152410719		
Diet	1	16.2901223	16.2901223	106.88	0.0000
Day	4	1.33700021	0.334250052	2.19	0.0786
Diet*Day	4	.703000518	.175750129	1.15	0.3390
Normalized Serum P					
Total	79	16.412002	0.207746838		
Model	9	2.3045004	0.25605556	1.27	0.2684
Residual	70	14.1075002	0.201535717		
Diet	1	0.26450002	0.26450002	1.31	0.2559
Day	4	1.33700002	0.334250006	1.66	0.1694
Diet*Day	4	0.703000014	0.175750003	0.87	0.4852
Blood Urea N					
Total	79	1026.28189	12.99091		
Model	9	219.85565	24.4284056	2.12	0.0389
Residual	70	806.426239	11.5203748		
Diet	1	69.75113	69.75113	6.05	0.0163
Day	4	73.2712513	18.3178128	1.59	0.1866
Diet*Day	4	76.8332668	19.2083172	1.67	0.1673

APPENDIX 2C. BLOOD PARAMETERS

Horse	Diet	Day	Serum Ca (mg/ml)	Normalized Serum Ca	Serum P (mg/dl)	Normalized Serum P	Serum BUN (mg/dl)	Normalized Serum BUN
1A	A	0	10.9	0	4.6	0	18.1	0
2A	A	0	11.5	0	4.7	0	14.1	0
3A	A	0	10.8	0	4.9	0	15.8	0
4A	A	0	11.2	0	4.7	0	17.4	0
5A	A	0	11	0	5.6	0	22.6	0
6A	A	0	11	0	5.1	0	26.1	0
7A	A	0	9.9	0	5.1	0	13.2	0
8A	A	0	11.2	0	5.2	0	15.2	0
1B	B	0	10.1	0	4.6	0	18.3	0
2B	B	0	7.3	0	3.9	0	11.6	0
3B	B	0	7.6	0	3.9	0	18.1	0
4B	B	0	9.1	0	4.1	0	18.8	0
5B	B	0	7.3	0	3.7	0	17.1	0
6B	B	0	11.1	0	4.8	0	16.8	0
7B	B	0	7.9	0	4	0	15.4	0
8B	B	0	7.9	0	4.6	0	14.9	0
1A	A	28	11.2	0.3	5.2	0.6	19.7	1.6
2A	A	28	11	-0.5	5.1	0.4	13.4	-0.7
3A	A	28	10.9	0.1	4.7	-0.2	14.2	-1.6
4A	A	28	11	-0.2	5.8	1.1	16.1	-1.3
5A	A	28	11.3	0.3	5.6	0	19.2	-3.4
6A	A	28	11.4	0.4	6	0.9	21.4	-4.7
7A	A	28	11.1	1.2	5.4	0.3	13.5	0.3
8A	A	28	11.5	0.3	5.5	0.3	12.1	-3.1
1B	B	28	10.2	0.1	4.6	0	16.7	-1.6
2B	B	28	8	0.7	4.3	0.4	13	1.4
3B	B	28	7.7	0.1	4.8	0.9	17.5	-0.6
4B	B	28	8.7	-0.4	5.1	1	22.7	3.9
5B	B	28	7.4	0.1	4.5	0.8	17.9	0.8
6B	B	28	9.1	-2	5	0.2	18.2	1.4
7B	B	28	9.1	1.2	4	0	16.2	0.8
8B	B	28	7.1	-0.8	4.1	-0.5	15.4	0.5
1A	A	56	11.5	0.6	5.1	0.5	19.9	1.8
2A	A	56	11.3	-0.2	5.7	1	12.1	-2
3A	A	56	11.3	0.5	5.6	0.7	14.7	-1.1
4A	A	56	11.1	-0.1	5.5	0.8	14.5	-2.9
5A	A	56	11.6	0.6	4.7	-0.9	21.3	-1.3
6A	A	56	11.4	0.4	4.7	-0.4	24.4	-1.7
7A	A	56	11	1.1	5.3	0.2	14.3	1.1
8A	A	56	11.3	0.1	5.2	0	12.2	-3

APPENDIX 2C. CONTINUED

Horse	Diet	Day	Serum Ca (mg/ml)	Normalized Serum Ca	Serum P (mg/dl)	Normalized Serum P	Serum BUN (mg/dl)	Normalized Serum BUN
1B	B	56	9.9	-0.2	5.1	0.5	19.2	0.9
2B	B	56	9	1.7	4.5	0.6	16.1	4.5
3B	B	56	7.9	0.3	4	0.1	21.9	3.8
4B	B	56	8.8	-0.3	4.8	0.7	24.7	5.9
5B	B	56	9	1.7	4.9	1.2	19.9	2.8
6B	B	56	8.3	-2.8	4.3	-0.5	21.7	4.9
7B	B	56	7.4	-0.5	3.9	-0.1	19.2	3.8
8B	B	56	7.9	0	5	0.4	20.5	5.6
1A	A	84	11.1	0.2	5.1	0.5	18.1	0
2A	A	84	9.2	-2.3	5.2	0.5	13.2	-0.9
3A	A	84	10.9	0.1	5.1	0.2	14.1	-1.7
4A	A	84	10.9	-0.3	5.2	0.5	15	-2.4
5A	A	84	11.5	0.5	5.7	0.1	18.8	-3.8
6A	A	84	11.3	0.3	6.1	1	23.5	-2.6
7A	A	84	11.3	1.4	5	-0.1	14.7	1.5
8A	A	84	11.5	0.3	5.4	0.2	15.5	0.3
1B	B	84	6.8	-3.3	4.6	0	22.4	4.1
2B	B	84	7.1	-0.2	4.5	0.6	16.4	4.8
3B	B	84	8.7	1.1	4.9	1	22.2	4.1
4B	B	84	8.5	-0.6	4.2	0.1	24.8	6
5B	B	84	7.7	0.4	4	0.3	19.6	2.5
6B	B	84	8.3	-2.8	4	-0.8	19.6	2.8
7B	B	84	8.1	0.2	4.4	0.4	19.2	3.8
8B	B	84	8.5	0.6	4.3	-0.3	19.6	4.7
1A	A	116	11.5	0.6	5.1	0.5	20.6	2.5
2A	A	116	11.5	0	5.8	1.1	13	-1.1
3A	A	116	11.1	0.3	5.7	0.8	18.7	2.9
4A	A	116	10.8	-0.4	5.1	0.4	17.7	0.3
5A	A	116	11.3	0.3	5.2	-0.4	21.7	-0.9
6A	A	116	11	0	5.8	0.7	24.3	-1.8
7A	A	116	10.6	0.7	4.8	-0.3	14.7	1.5
8A	A	116	11	-0.2	6.1	0.9	14.3	-0.9
1B	B	116	7.5	-2.6	4.6	0	22	3.7
2B	B	116	8.1	0.8	4.1	0.2	19.3	7.7
3B	B	116	8.2	0.6	3.9	0	21.5	3.4
4B	B	116	9.8	0.7	4.6	0.5	25	6.2
5B	B	116	8.2	0.9	4.2	0.5	21.7	4.6
6B	B	116	8.8	-2.3	4.2	-0.6	20.1	3.3
7B	B	116	8.5	0.6	3.7	-0.3	14.8	-0.6
8B	B	116	10.9	3	4.6	0	18.1	3.2

APPENDIX 3A. ANOVA TABLE FOR PH

Source	df	Partial SS	MS	F-value	P-value
Blood					
Total	79	0.481675289	0.006097156		
Model	9	0.145825259	0.016202807	3.38	0.0017
Residual	70	0.335850029	0.004797858		
Diet	1	0.006844978	0.006844978	1.43	0.2363
Day	4	0.110037698	0.007235646	1.51	0.2092
Diet*Day	4	0.028942583	0.007235646	1.51	0.2092
Urine					
Total	153	53.2145954	0.347807813		
Model	5	5.70295421	1.14059084	3.55	0.0046
Residual	148	47.5116412	0.321024603		
Diet	1	0.048964976	0.048964976	0.15	0.6967
Day	2	5.530070277	2.65035139	8.26	0.0004
Diet*Day	2	0.368198841	0.18409942	0.57	0.5648
Fecal					
Total	153	23.0850249	0.150882516		
Model	5	6.74897526	1.34979505	12.23	0.0000
Residual	148	16.3360496	0.110378714		
Diet	1	0.401324953	0.401324953	3.64	0.0585
Day	2	5.84898622	2.92449311	26.50	0.0000
Diet*Day	2	0.428622858	0.214311429	1.94	0.1471
Normalized Fecal pH					
Total	47	3.68535294	0.078411765		
Model	5	2.16307875	0.432615749	11.94	0.0000
Residual	42	1.5222742	0.036244624		
Diet	1	0.215003252	0.215003252	5.93	0.0192
Day	2	1.80429242	0.90214621	24.89	0.0000
Diet*Day	2	0.143783075	0.071891538	1.98	0.1503

APPENDIX 3B. PH MEASUREMENTS

Horse	Diet	Day	Blood pH	Urine pH	Fecal pH
1A	A	0	7.50	7.71	6.72
2A	A	0	7.47	8.41	6.61
3A	A	0	7.44	8.09	6.50
4A	A	0	7.51	7.67	7.05
5A	A	0	7.46	8.04	6.55
6A	A	0	7.51	7.93	6.69
7A	A	0	7.58	7.81	6.72
8A	A	0	7.57	7.70	6.63
1B	B	0	7.52	8.11	6.87
2B	B	0	7.43	8.28	6.70
3B	B	0	7.45	8.24	6.53
4B	B	0	7.56	8.01	7.15
5B	B	0	7.38	8.13	6.83
6B	B	0	7.59	7.35	6.75
7B	B	0	7.54	8.00	6.89
8B	B	0	7.51	7.68	7.53
1A	A	28	7.44		
2A	A	28	7.53		
3A	A	28	7.57		
4A	A	28	7.57		
5A	A	28	7.60		
6A	A	28	7.53		
7A	A	28	7.55		
8A	A	28	7.69		
1B	B	28	7.56		
2B	B	28	7.48		
3B	B	28	7.55		
4B	B	28	7.56		
5B	B	28	7.53		
6B	B	28	7.68		
7B	B	28	7.66		
8B	B	28	7.68		
1A	A	56	7.60	7.76	6.26
2A	A	56	7.64	7.17	6.06
3A	A	56	7.65	8.26	6.36
4A	A	56	7.52	7.57	6.73
5A	A	56	7.48	7.94	6.04
6A	A	56	7.53	8.02	6.67
7A	A	56	7.47	7.88	6.75
8A	A	56	7.46	7.22	6.74

APPENDIX 3B. CONTINUED

Horse	Diet	Day	Blood pH	Urine pH	Fecal pH
1B	B	56	7.61	8.07	6.43
2B	B	56	7.62	7.29	6.59
3B	B	56	7.63	7.90	6.42
4B	B	56	7.53	7.86	6.73
5B	B	56	7.60	7.81	6.04
6B	B	56	7.43	7.79	6.23
7B	B	56	7.49	7.91	6.26
8B	B	56	7.48	7.25	6.69
1A	A	84	7.48		
2A	A	84	7.54		
3A	A	84	7.52		
4A	A	84	7.43		
5A	A	84	7.43		
6A	A	84	7.20		
7A	A	84	7.47		
8A	A	84	7.46		
1B	B	84	7.54		
2B	B	84	0.50		
3B	B	84	7.57		
4B	B	84	7.49		
5B	B	84	7.42		
6B	B	84	7.64		
7B	B	84	7.51		
8B	B	84	7.52		
1A	A	116	7.57	7.85	6.32
2A	A	116	7.63	7.48	6.06
3A	A	116	7.66	7.47	6.17
4A	A	116	7.53	7.37	6.59
5A	A	116	7.61	7.63	6.07
6A	A	116	7.58	8.57	6.44
7A	A	116	7.58	7.96	6.45
8A	A	116	7.52	7.67	6.26
1B	B	116	7.57	7.70	6.22
2B	B	116	7.59	7.03	6.64
3B	B	116	7.59	8.05	6.50
4B	B	116	7.62	7.79	6.78
5B	B	116	7.63	7.27	6.17
6B	B	116	7.51	7.36	5.94
7B	B	116	7.56	7.63	6.17
8B	B	116	7.59	6.49	6.80

APPENDIX 4A. ANOVA TABLE FOR CALCIUM BALANCE

Source	df	Partial SS	MS	F-value	P-value
Ca intake (mg/kgBW/d)					
Total	47	54288.1381	1155.06677		
Model	5	40322.5933	8064.51867	24.25	0.0000
Residual	42	13965.5447	332.51297		
Diet	1	658.156167	658.156167	1.98	0.1668
Day	2	38959.8696	19479.9348	58.58	0.0000
Diet*Day	2	704.567605	352.283802	1.06	0.3557
Fecal Ca (mg/kgBW/d)					
Total	47	55663.7948	1184.33606		
Model	5	41059.9833	8211.99666	23.62	0.0000
Residual	42	14603.8115	347.709798		
Diet	1	3332.00048	3332.00048	9.58	0.0035
Day	2	36852.0707	18426.0354	52.99	0.0000
Diet*Day	2	875.912118	437.956059	1.26	0.2943
Urine ca (mg/kgBW/d)					
Total	47	305.243762	6.49454813		
Model	5	26.8541913	537083827	0.81	0.5490
Residual	42	278.389571	6.62832311		
Diet	1	2.66020835	2.66020835	0.40	0.5298
Day	2	23.2438785	11.6219393	1.75	0.1856
Diet*Day	2	0.95010447	0.475052235	0.07	0.9310
Ca absorbed (mg/kgBW/d)					
Total	47	36770.8898	782.359357		
Model	5	7922.4436	1584.48872	2.31	0.0612
Residual	42	28848.4462	686.867767		
Diet	1	6952.61981	6952.61981	10.12	0.0028
Day	2	914.23054	457.115177	0.67	0.5193
Diet*Day	2	55.5934368	27.7967184	0.04	0.9604

APPENDIX 4A. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Ca abs. as % of intake (mg/kgBW/d)					
Total	45	1.20519161	0.25642375		
Model	5	0.423766632	0.084753326	4.56	0.0021
Residual	40	0.781424981	0.018605357		
Diet	1	0.218699977	0.218699977	11.75	0.0014
Day	2	0.197516657	0.098758329	5.31	0.0088
Diet*Day	2	0.007549998	0303774999	0.20	0.8172
Ca retained (mg/kgBW/d)					
Total	47	37426.4633	796.307729		
Model	5	7485.00567	1497.00113	2.10	0.0843
Residual	42	29941.4576	712.891847		
Diet	1	6682.34018	6682.34018	9.37	0.0038
Day	2	760.552216	380.276108	0.53	0.5905
Diet*Day	2	42.1132714	21.0566357	0.03	0.9709
Ca ret. as % of intake (mg/kgBW/d)					
Total	45	7882.16996	175.159332		
Model	5	1906.39129	381.278257	2.55	0.0427
Residual	40	5975.77867	149.394467		
Diet	1	1036.40625	1036.40625	6.94	0.0119
Day	2	645.901549	322.950775	2.16	0.1284
Diet*Day	2	198.37586	99.1879298	0.66	0.5204
Ca ret. as % of absorption					
Total	45	3995.3125	88.7847221		
Model	5	124.042547	24.8085095	0.26	0.9341
Residual	40	3871.26995	96.7817487		
Diet	1	24.6045428	24.6045428	0.25	0.6169
Day	2	65.6287494	32.813747	0.34	0.7145
Diet*Day	2	33.7322968	16.8661484	0.17	0.8407

APPENDIX 4B. CALCIUM BALANCE

Horse	Diet	Day	Conc. DM intake (kg/day)	Ca content in conc.	Ca intake from conc. (g/day)	Hay DM intake (kg/day)	Ca content in hay	Ca intake from hay (g/day)	Total Ca intake (g/day)
1A	A	0	3.66	1.32%	48.33	1.76	0.54%	9.51	57.84
2A	A	0	3.04	1.32%	40.13	1.65	0.54%	8.92	49.05
3A	A	0	3.53	1.32%	46.60	1.87	0.54%	10.12	56.72
4A	A	0	3.35	1.32%	44.23	1.73	0.54%	9.36	53.59
5A	A	0	3.51	1.32%	46.38	1.87	0.54%	10.11	56.49
6A	A	0	2.89	1.32%	38.11	1.60	0.54%	8.64	46.75
7A	A	0	3.74	1.32%	49.40	1.63	0.54%	8.78	58.19
8A	A	0	3.62	1.32%	47.79	1.58	0.54%	8.53	56.32
1B	B	0	3.46	1.32%	45.70	1.86	0.54%	10.04	55.74
2B	B	0	3.50	1.32%	46.25	1.81	0.54%	9.80	56.04
3B	B	0	3.69	1.32%	48.64	1.98	0.54%	10.71	59.35
4B	B	0	3.77	1.32%	49.74	1.74	0.54%	9.40	59.14
5B	B	0	3.52	1.32%	46.46	1.87	0.54%	10.07	56.54
6B	B	0	3.19	1.32%	42.10	1.34	0.54%	7.24	49.34
7B	B	0	3.64	1.32%	48.10	1.57	0.54%	8.46	56.56
8B	B	0	3.22	1.32%	42.44	1.58	0.54%	8.53	50.97
1A	A	56	3.74	1.30%	48.66	1.55	0.47%	7.27	55.92
2A	A	56	3.04	1.30%	39.52	1.26	0.47%	5.90	45.42
3A	A	56	3.53	1.30%	45.89	1.46	0.47%	6.85	52.75
4A	A	56	3.48	0.83%	28.89	1.44	0.51%	7.34	36.23
5A	A	56	2.86	0.83%	23.74	1.18	0.51%	6.03	29.77
6A	A	56	3.15	0.83%	26.11	1.30	0.51%	6.63	32.74
7A	A	56	3.74	0.94%	35.26	1.55	0.73%	11.29	46.54
8A	A	56	3.62	0.94%	34.10	1.50	0.73%	10.92	45.02
1B	B	56	3.46	0.85%	29.43	1.43	0.47%	6.72	36.15
2B	B	56	3.50	0.85%	29.78	1.45	0.47%	6.80	36.58
3B	B	56	3.69	0.85%	31.32	1.52	0.47%	7.16	38.48
4B	B	56	3.77	0.78%	29.39	1.56	0.51%	7.94	37.33
5B	B	56	3.52	0.78%	27.46	1.45	0.51%	7.42	34.87
6B	B	56	3.19	0.86%	27.43	1.32	0.73%	9.62	37.05
7B	B	56	3.64	0.86%	31.34	1.51	0.73%	10.99	42.33
8B	B	56	3.86	0.86%	33.19	1.59	0.73%	11.64	44.82
1A	A	112	3.82	1.10%	41.98	1.58	0.50%	7.88	49.86
2A	A	112	3.20	1.10%	35.15	1.32	0.50%	6.60	41.75
3A	A	112	3.81	1.10%	41.89	1.57	0.50%	7.87	49.76
4A	A	112	3.78	0.64%	24.16	1.56	0.50%	7.80	31.96
5A	A	112	3.46	0.64%	22.15	1.43	0.50%	7.15	29.30
6A	A	112	3.32	0.64%	21.23	1.37	0.50%	6.85	28.09
7A	A	112	3.58	0.84%	30.08	1.48	0.55%	8.14	38.22
8A	A	112	3.59	0.84%	30.15	1.48	0.55%	8.15	38.30

APPENDIX 4B. CONTINUED

Horse	Diet	Day	Conc. DM intake (kg/day)	Ca content in conc.	Ca intake from conc. (g/day)	Hay DM intake (kg/day)	Ca content in hay	Ca intake from hay (g/day)	Total Ca intake (g/day)
1B	B	112	3.73	0.88%	32.87	1.54	0.50%	7.71	40.58
2B	B	112	3.87	0.88%	34.03	1.60	0.50%	7.99	42.02
3B	B	112	4.01	0.88%	35.27	1.66	0.50%	8.28	43.54
4B	B	112	4.15	0.87%	36.09	1.71	0.50%	8.57	44.66
5B	B	112	3.69	0.87%	32.06	1.52	0.50%	7.61	39.67
6B	B	112	3.07	0.67%	20.60	1.27	0.55%	6.99	27.59
7B	B	112	3.50	0.67%	23.48	1.45	0.55%	7.96	31.44
8B	B	112	3.66	0.67%	24.52	1.51	0.55%	8.32	32.84

APPENDIX 4B. CONTINUED

Horse	Diet	Day	Fecal DM (kg/day)	Ca content in feces	Fecal ca (g/day)	Urine (l/day)	Ca content urine (mg/dl)	Urine Ca (g/day)
1A	A	0	2.62	1.15%	30.17	4.71	8.5	0.40
2A	A	0	2.11	1.15%	24.23	12.48	19.1	2.38
3A	A	0	2.43	1.40%	33.90	8.14	10.0	0.81
4A	A	0	2.27	1.39%	31.63	4.85	12.6	0.61
5A	A	0	2.65	1.68%	44.65	5.80	30.6	1.77
6A	A	0	1.97	1.33%	26.18	3.66	15.3	0.56
7A	A	0	3.28	1.30%	42.44	4.95	41.0	2.03
8A	A	0	2.51	1.45%	36.33	8.84	5.2	0.46
1B	B	0	2.85	1.04%	29.47	8.82	14.1	1.24
2B	B	0	2.31	1.37%	31.67	4.92	31.6	1.55
3B	B	0	2.26	1.42%	32.12	7.51	11.0	0.83
4B	B	0	3.05	1.56%	47.72	4.42	18.9	0.84
5B	B	0	2.80	2.14%	59.90	5.40	24.6	1.33
6B	B	0	2.34	1.41%	32.87	3.28	41.1	1.35
7B	B	0	2.86	1.72%	49.10	8.91	1.0	0.09
8B	B	0	3.03	1.92%	58.09	4.20	1.7	0.07
1A	A	56	2.11	0.97%	20.58	6.91	3.7	0.26
2A	A	56	1.74	0.92%	15.95	37.07	0.6	0.22
3A	A	56	2.52	0.88%	22.08	17.30	0.4	0.07
4A	A	56	2.07	0.98%	20.33	6.41	1.6	0.10
5A	A	56	2.39	1.04%	24.91	17.35	15.8	2.74
6A	A	56	1.91	0.91%	17.43	4.76	1.6	0.08
7A	A	56	2.22	1.02%	22.67	9.61	21.5	2.07
8A	A	56	2.19	0.81%	17.78	11.88	16.4	1.95
1B	B	56	2.39	0.94%	22.42	21.92	5.9	1.29
2B	B	56	2.34	1.01%	23.61	8.89	20.0	1.78
3B	B	56	2.57	0.98%	25.22	16.77	12.3	2.06
4B	B	56	2.57	0.78%	19.93	17.88	1.3	0.23
5B	B	56	2.47	0.83%	20.39	25.96	0.5	0.13
6B	B	56	1.98	1.18%	23.37	11.55	3.1	0.36
7B	B	56	2.42	0.96%	23.18	29.54	0.9	0.27
8B	B	56	2.55	0.93%	23.62	8.72	2.1	0.18
1A	A	112	2.32	0.88%	20.35	7.56	6.3	0.48
2A	A	112	1.88	0.91%	17.13	59.14	1.3	0.77
3A	A	112	2.61	0.82%	21.50	25.67	0.6	0.15
4A	A	112	2.41	0.88%	21.30	6.40	4.7	0.30
5A	A	112	2.56	0.78%	19.88	45.38	5.2	2.36
6A	A	112	1.83	0.80%	14.62	6.24	1.3	0.08
7A	A	112	2.21	0.79%	17.46	8.22	2.3	0.19
8A	A	112	2.64	0.70%	18.58	38.68	0.8	0.31

APPENDIX 4B. CONTINUED

Horse	Diet	Day	Fecal DM (kg/day)	Ca content in feces	Fecal ca (g/day)	Urine (l/day)	Ca content urine (mg/dl)	Urine Ca (g/day)
1B	B	112	2.91	0.89%	25.87	31.98	0.9	0.29
2B	B	112	2.51	1.22%	30.73	8.02	25.1	2.01
3B	B	112	2.78	1.04%	28.97	11.59	2.3	0.27
4B	B	112	3.28	0.85%	27.89	17.62	1.1	0.19
5B	B	112	2.67	0.77%	20.51	47.75	1.3	0.62
6B	B	112	1.79	0.99%	17.71	7.89	1.1	0.09
7B	B	112	2.45	0.90%	22.08	40.30	0.4	0.16
8B	B	112	2.65	0.91%	24.06	8.36	11.4	0.95

APPENDIX 4B. CONTINUED

Horse	Diet	Day	Ca Absorbed (g/day)	Ca Abs. as % of Intake	Ca Retained (g/day)	Ca Ret. as % of Intake	Ca Ret. as % of Abs.
1A	A	0	27.66	47.83	27.26	47.14	98.55
2A	A	0	24.82	50.60	22.44	45.74	90.40
3A	A	0	22.82	40.24	22.01	38.80	96.43
4A	A	0	21.96	40.97	21.34	39.83	97.22
5A	A	0	11.84	20.95	10.06	17.81	85.01
6A	A	0	20.57	44.00	20.01	42.80	97.28
7A	A	0	15.75	27.07	13.72	23.58	87.11
8A	A	0	19.99	35.49	19.53	34.67	97.70
1B	B	0	26.27	47.12	25.02	44.89	95.27
2B	B	0	24.37	43.49	22.82	40.72	93.63
3B	B	0	27.23	45.88	26.41	44.49	96.97
4B	B	0	11.42	19.31	10.58	17.90	92.69
5B	B	0	-3.36	--	-4.69	--	--
6B	B	0	16.48	33.39	15.13	30.66	91.81
7B	B	0	7.46	13.18	7.37	13.03	98.80
8B	B	0	-7.12	--	-7.20	--	--
1A	A	56	35.34	63.20	35.09	62.74	99.28
2A	A	56	29.47	64.88	29.25	64.39	99.25
3A	A	56	30.67	58.15	30.60	58.02	99.77
4A	A	56	15.90	43.89	15.80	43.60	99.36
5A	A	56	4.85	16.31	2.11	7.10	43.51
6A	A	56	15.31	46.76	15.23	46.53	99.50
7A	A	56	23.88	51.30	21.81	46.86	91.35
8A	A	56	27.24	60.51	25.29	56.18	92.85
1B	B	56	13.73	37.97	12.43	34.39	90.58
2B	B	56	12.97	35.46	11.19	30.59	86.29
3B	B	56	13.26	34.47	11.20	29.11	84.45
4B	B	56	17.40	46.61	17.17	45.99	98.66
5B	B	56	14.48	41.53	14.35	41.16	99.10
6B	B	56	13.68	36.93	13.32	35.96	97.38
7B	B	56	19.15	45.23	18.88	44.60	98.61
8B	B	56	21.20	47.31	21.02	46.90	99.14
1A	A	112	29.51	59.18	29.03	58.23	98.39
2A	A	112	24.62	58.98	23.85	57.14	96.88
3A	A	112	28.25	56.78	28.10	56.47	99.45
4A	A	112	10.67	33.37	10.37	32.43	97.18
5A	A	112	9.41	32.13	7.06	24.08	74.94
6A	A	112	13.46	47.94	13.38	47.65	99.40
7A	A	112	20.76	54.33	20.57	53.83	99.09
8A	A	112	19.72	51.49	19.41	50.69	98.43

APPENDIX 4B. CONTINUED

Horse	Diet	Day	Ca Absorbed (g/day)	Ca Abs. as % of Intake	Ca Retained (g/day)	Ca Ret. as % of Intake	Ca Ret. as % of Abs.
1B	B	112	14.72	36.26	14.43	35.55	98.04
2B	B	112	11.29	26.86	9.27	22.07	82.16
3B	B	112	14.57	33.46	14.30	32.85	98.17
4B	B	112	16.76	37.54	16.57	37.11	98.84
5B	B	112	19.16	48.29	18.54	46.73	96.76
6B	B	112	9.87	35.79	9.79	35.47	99.12
7B	B	112	9.36	29.76	9.20	29.25	98.28
8B	B	112	8.78	26.74	7.83	23.84	89.15

APPENDIX 5A. ANOVA FOR PHOSPHORUS BALANCE

Source	df	Partial SS	MS	F-value	P-value
P intake (mg/kgBW/d)					
Total	47	9226.1185	196.300394		
Model	5	8240.16892	1648.03378	70.20	0.0000
Residual	42	985.949576	23.4749899		
Diet	1	328.496311	328.496311	13.99	0.0005
Day	2	7686.67532	3843.33766	163.72	0.0000
Diet*Day	2	224.997291	112.498646	4.79	0.0133
Fecal P (mg/kgBW/day)					
Total	47	21200.1411	451.066832		
Model	5	13217.274	2643.4548	13.91	0.0000
Residual	42	7982.86708	190.068264		
Diet	1	543.178309	543.178309	2.86	0.0983
Day	2	11871.8263	5935.91317	31.23	0.0000
Diet*Day	2	802.269355	401.134678	2.11	0.1338
Urine P (mg/kgBW/day)					
Total	47	23.332397	0.496433979		
Model	5	2.9303103	0.58606206	1.21	0.3228
Residual	42	20.4020867	0.485763969		
Diet	1	0.005852086	0.005852086	0.01	0.9131
Day	2	2.91155405	1.45577702	3.00	0.0607
Diet*Day	2	0.12904166	0.006452083	0.01	0.9868
P absorbed (mg/kgBWday)					
Total	47	11456.9386	243.764651		
Model	5	2000.14857	400.029714	1.78	0.1386
Residual	42	9456.79002	225.161667		
Diet	1	26.8801382	26.8801382	0.12	0.7314
Day	2	1386.61416	693.307078	3.08	0.0565
Diet*Day	2	586.654276	293.327138	1.630	0.2825

APPENDIX 5A. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
P retained (mg/kgBW/day)					
Total	47	11466.4821	243.967704		
Model	5	2050.91217	410.182435	1.83	0.1278
Residual	42	9415.56994	224.180237		
Diet	1	27.6792311	27.6792311	0.12	0.7271
Day	2	1441.19453	720.597263	3.21	0.0502
Diet*Day	2	582.038417	291.019209	1.30	0.2838

APPENDIX 5B. PHOSPHORUS BALANCE

Horse	Diet	Day	Conc. DM intake (kg/day)	P content in conc.	P intake from conc. (g/day)	Hay DM intake (kg/day)	P content in hay	P intake from hay (g/day)	Total P intake (mg/kgBW/d)
1A	A	0	3.66	0.85%	31.12	1.76	0.15%	2.64	105.50
2A	A	0	3.04	0.85%	25.84	1.65	0.15%	2.48	108.92
3A	A	0	3.53	0.85%	30.01	1.87	0.15%	2.81	108.74
4A	A	0	3.35	0.85%	28.48	1.73	0.15%	2.60	104.23
5A	A	0	3.51	0.85%	29.87	1.87	0.15%	2.81	108.59
6A	A	0	2.89	0.85%	24.54	1.60	0.15%	2.40	100.11
7A	A	0	3.74	0.85%	31.81	1.63	0.15%	2.44	107.04
8A	A	0	3.62	0.85%	30.77	1.58	0.15%	2.37	106.91
1B	B	0	3.46	0.85%	29.43	1.86	0.15%	2.79	110.06
2B	B	0	3.50	0.85%	29.78	1.81	0.15%	2.72	109.66
3B	B	0	3.69	0.85%	31.32	1.98	0.15%	2.97	110.00
4B	B	0	3.77	0.85%	32.03	1.74	0.15%	2.61	108.55
5B	B	0	3.52	0.85%	29.92	1.87	0.15%	2.80	109.72
6B	B	0	3.19	0.85%	27.11	1.34	0.15%	2.01	107.86
7B	B	0	3.64	0.85%	30.97	1.57	0.15%	2.35	108.13
8B	B	0	3.22	0.85%	27.33	1.58	0.15%	2.37	90.99
1A	A	56	3.74	0.61%	22.83	1.70	0.18%	2.78	78.48
2A	A	56	3.04	0.61%	18.54	1.38	0.18%	2.26	76.03
3A	A	56	3.53	0.61%	21.53	1.60	0.18%	2.63	74.24
4A	A	56	3.48	0.62%	21.58	1.58	0.18%	2.59	74.90
5A	A	56	2.86	0.62%	17.73	1.30	0.18%	2.13	64.44
6A	A	56	3.15	0.62%	19.51	1.43	0.18%	2.34	77.02
7A	A	56	3.74	0.61%	22.83	1.70	0.20%	3.09	79.88
8A	A	56	3.62	0.61%	22.08	1.64	0.20%	2.99	77.04
1B	B	56	3.46	0.70%	24.23	1.57	0.18%	2.57	84.74
2B	B	56	3.50	0.70%	24.52	1.59	0.18%	2.61	82.90
3B	B	56	3.69	0.70%	25.80	1.67	0.18%	2.74	84.16
4B	B	56	3.77	0.69%	26.00	1.71	0.18%	2.80	82.07
5B	B	56	3.52	0.69%	24.29	1.60	0.18%	2.62	86.29
6B	B	56	3.19	0.69%	22.01	1.45	0.20%	2.64	89.46
7B	B	56	3.64	0.69%	25.14	1.65	0.20%	3.01	89.77
8B	B	56	3.86	0.69%	26.63	1.75	0.20%	3.19	90.84
1A	A	112	3.82	0.61%	23.28	1.73	0.20%	3.15	79.44
2A	A	112	3.20	0.61%	19.49	1.45	0.20%	2.64	78.78
3A	A	112	3.81	0.61%	23.23	1.73	0.20%	3.15	79.93
4A	A	112	3.78	0.61%	23.03	1.71	0.20%	3.12	79.90
5A	A	112	3.46	0.61%	21.11	1.57	0.20%	2.86	74.28
6A	A	112	3.32	0.61%	20.24	1.51	0.20%	2.74	79.49
7A	A	112	3.58	0.59%	21.13	1.63	0.19%	2.81	71.46
8A	A	112	3.59	0.59%	21.17	1.63	0.19%	2.82	70.75

APPENDIX 5B. CONTINUED

Horse	Diet	Day	Conc. DM intake (kg/day)	P content in conc.	P intake from conc. (g/day)	Hay DM intake (kg/day)	P content in hay	P intake from hay (g/day)	Total P intake (mg/kgBW/d)
1B	B	112	3.73	0.66%	24.65	1.70	0.20%	3.09	88.18
2B	B	112	3.87	0.66%	25.52	1.76	0.20%	3.20	86.31
3B	B	112	4.01	0.66%	26.45	1.82	0.20%	3.31	87.53
4B	B	112	4.15	0.54%	22.40	1.88	0.20%	3.43	71.56
5B	B	112	3.69	0.54%	19.90	1.67	0.20%	3.05	72.53
6B	B	112	3.07	0.67%	20.60	1.40	0.19%	2.41	79.86
7B	B	112	3.50	0.67%	23.48	1.59	0.19%	2.75	80.36
8B	B	112	3.66	0.67%	24.52	1.66	0.19%	2.87	80.14

APPENDIX 5B. CONTINUED

Horse	Diet	Day	Fecal DM	P content in feces	Fecal P (mg/kgBW/d)	Urine (l/day)	P content urine (mg/dl)	Urine P (mg/kgBW/d)
1A	A	0	2.62	1.1727%	96.18	4.71	0.20	0.03
2A	A	0	2.11	1.1005%	89.48	12.48	1.00	0.48
3A	A	0	2.43	0.8705%	70.00	8.14	2.80	0.75
4A	A	0	2.27	1.3141%	100.19	4.85	0.40	0.07
5A	A	0	2.65	1.7011%	150.06	5.80	0.30	0.06
6A	A	0	1.97	1.2619%	92.57	3.66	0.30	0.04
7A	A	0	3.28	1.0861%	111.21	4.95	0.90	0.14
8A	A	0	2.51	1.1823%	95.65	8.84	0.40	0.11
1B	B	0	2.85	0.8827%	85.81	8.82	0.30	0.09
2B	B	0	2.31	1.1715%	91.25	4.92	3.10	0.51
3B	B	0	2.26	1.2716%	92.01	7.51	1.00	0.24
4B	B	0	3.05	1.0977%	104.96	4.42	1.10	0.15
5B	B	0	2.80	0.6658%	62.55	5.40	5.90	1.07
6B	B	0	2.34	1.3030%	112.80	3.28	0.30	0.04
7B	B	0	2.86	1.2267%	113.68	8.91	0.20	0.06
8B	B	0	3.03	1.1633%	107.96	4.20	0.50	0.06
1A	A	56	2.11	0.8967%	58.09	6.91	0.10	0.02
2A	A	56	1.74	0.7388%	46.95	37.07	0.10	0.14
3A	A	56	2.52	0.7088%	54.80	17.30	0.40	0.21
4A	A	56	2.07	0.7995%	51.37	6.41	0.70	0.14
5A	A	56	2.39	0.8223%	63.75	17.35	0.60	0.34
6A	A	56	1.91	0.7995%	53.94	4.76	0.10	0.02
7A	A	56	2.22	0.9632%	65.78	9.61	0.70	0.21
8A	A	56	2.19	0.8930%	60.01	11.88	4.00	1.46
1B	B	56	2.39	0.9296%	70.14	21.92	0.20	0.14
2B	B	56	2.34	0.9367%	67.08	8.89	5.20	1.41
3B	B	56	2.57	1.1229%	85.14	16.77	0.50	0.25
4B	B	56	2.57	0.8692%	63.60	17.88	0.40	0.20
5B	B	56	2.47	0.7004%	55.50	25.96	0.20	0.17
6B	B	56	1.98	1.0101%	72.52	11.55	0.30	0.13
7B	B	56	2.42	0.7626%	58.87	29.54	0.20	0.19
8B	B	56	2.55	0.7124%	55.42	8.72	0.40	0.11
1A	A	112	2.32	0.9863%	68.72	7.56	0.40	0.09
2A	A	112	1.88	0.8510%	56.89	59.14	1.20	2.53
3A	A	112	2.61	0.7938%	62.71	25.67	0.60	0.47
4A	A	112	2.41	0.8925%	65.69	6.40	0.80	0.16
5A	A	112	2.56	0.8653%	68.69	45.38	0.20	0.28
6A	A	112	1.83	0.9566%	60.55	6.24	0.80	0.17
7A	A	112	2.21	0.8426%	55.54	8.22	0.50	0.12
8A	A	112	2.64	0.8690%	67.77	38.68	2.30	2.62

APPENDIX 5B. CONTINUED

Horse	Diet	Day	Fecal DM	P content in feces	Fecal P (mg/kgBW/d)	Urine (l/day)	P content urine (mg/dl)	Urine P (mg/kgBW/d)
1B	B	112	2.91	0.9798%	90.51	31.98	0.80	0.81
2B	B	112	2.51	1.1077%	83.70	8.02	0.70	0.17
3B	B	112	2.78	1.0858%	88.91	11.59	2.10	0.72
4B	B	112	3.28	1.0204%	92.79	17.62	0.70	0.34
5B	B	112	2.67	0.9001%	75.97	47.75	2.20	3.32
6B	B	112	1.79	1.0532%	65.49	7.89	1.10	0.30
7B	B	112	2.45	0.8444%	63.34	40.30	0.40	0.49
8B	B	112	2.65	0.8791%	68.06	8.36	0.90	0.22

APPENDIX 5B. CONTINUED

Horse	Diet	Day	P Absorbed (mg/kgBW/d)	P Retained (mg/kgBW/d)
1A	A	0	9.32	9.30
2A	A	0	19.44	18.96
3A	A	0	38.74	37.99
4A	A	0	4.04	3.97
5A	A	0	-41.47	-41.53
6A	A	0	7.54	7.50
7A	A	0	-4.17	-4.31
8A	A	0	11.26	11.14
1B	B	0	24.24	24.15
2B	B	0	18.41	17.90
3B	B	0	17.99	17.75
4B	B	0	3.60	3.44
5B	B	0	47.17	46.10
6B	B	0	-4.94	-4.98
7B	B	0	-5.55	-5.61
8B	B	0	-16.97	-17.03
1A	A	56	20.40	20.37
2A	A	56	29.08	28.94
3A	A	56	19.44	19.23
4A	A	56	23.53	23.39
5A	A	56	0.69	0.36
6A	A	56	23.08	23.07
7A	A	56	14.09	13.89
8A	A	56	17.04	15.58
1B	B	56	14.60	14.46
2B	B	56	15.82	14.41
3B	B	56	-0.98	-1.23
4B	B	56	18.47	18.27
5B	B	56	30.79	30.62
6B	B	56	16.94	16.82
7B	B	56	30.90	30.71
8B	B	56	35.43	35.32
1A	A	112	10.72	10.63
2A	A	112	21.89	19.36
3A	A	112	17.22	16.75
4A	A	112	14.22	14.06
5A	A	112	5.58	5.30
6A	A	112	18.94	18.76
7A	A	112	15.93	15.80
8A	A	112	2.99	0.36

APPENDIX 5B. CONTINUED

Horse	Diet	Day	P Absorbed (mg/kgBW/d)	P Retained (mg/kgBW/d)
1B	B	112	-2.33	-3.15
2B	B	112	2.61	2.44
3B	B	112	-1.38	-2.09
4B	B	112	-21.23	-21.58
5B	B	112	-3.44	-6.76
6B	B	112	14.37	14.07
7B	B	112	17.02	16.53
8B	B	112	12.08	11.86

APPENDIX 6A. NORMALIZED RBAE BY DIET AND DAY

Day	0	56	112
Lateral RBAE (mm Al)			
Diet A	0	0.50	1.28
SEM		0.30	0.59
Diet B	0	0.06	0.23
SEM		0.40	0.37
Mean	0	0.28	0.76
SEM		0.25	0.36
Medial RBAE (mm Al)			
Diet A	0	0.83	0.56
SEM		0.33	0.38
Diet B	0	0.23	0.58
SEM		0.24	0.20
Mean	0	0.53	0.57
SEM		0.21	0.20
Palmar RBAE (mm Al)			
Diet A	0	1.61	2.14
SEM		0.83	0.62
Diet B	0	0.93	1.32
SEM		0.48	0.41
Mean	0	1.25	1.73
SEM		0.45	0.37
Dorsal RBAE (mm Al)			
Diet A	0	1.66	2.23
SEM		0.83	0.67
Diet B	0	0.94	1.54
SEM		0.40	0.40
Mean	0	1.28	1.88
SEM		0.44	0.39
Total RBAE (mm^2 Al)			
Diet A	0	23.35	26.34
SEM		60.70	44.08
Diet B	0	-25.38	-23.95
SEM		54.17	55.33
Mean	0	-2.64	1.19
SEM		39.51	34.78

APPENDIX 6B. ANOVA TABLE FOR RBAE

Source	df	Partial SS	MS	F-value	P-value
Lateral					
Total	47	71.8303012	1.52830428		
Model	5	7.93158313	1.58631663	1.04	0.4055
Residual	42	63.8987181	1.52139805		
Diet	1	1.04260254	1.04260254	0.69	0.4124
Day	2	4.66668025	2.33340125	1.53	0.2276
Diet*Day	2	2.2221781	1.11108905	0.73	0.4878
Normalized Lateral					
Total	47	51.3366694	1.09226956		
Model	5	9.87562712	1.97512542	2.00	0.0983
Residual	42	41.4610423	0.987167674		
Diet	1	2.98664755	2.98664755	3.03	0.0893
Day	2	4.6668015	2.33340075	2.36	0.1065
Diet*Day	2	2.22217806	1.11108903	1.13	0.3341
Medial					
Total	47	41.252653	.877716022		
Model	5	4.24036544	0.848073088	0.96	0.4517
Residual	42	37.0122876	0.881244943		
Diet	1	0.23144205	0.23144205	0.03	0.8720
Day	2	3.24001627	1.62000813	1.84	0.1717
Diet*Day	2	0.977204968	0.488602484	0.55	0.5785
Normalized Medial					
Total	47	24.3776423	0.51867324		
Model	5	4.64861285	0.92972257	1.98	0.1016
Residual	42	19.7290294	0.469738796		
Diet	1	0.431392785	0.431392785	0.92	0.3434
Day	2	3.24001432	1.62000716	3.45	0.0410
Diet*Day	2	0.977205747	0.488602873	1.04	0.3623
Palmar					
Total	46	90.9003904	1.97609544		
Model	5	27.8180296	5.56360592	3.62	0.0084
Residual	41	63.0823608	1.53859417		
Diet	1	1.25639374	1.2563974	0.82	0.3715
Day	2	25.1405741	12.570287	8.17	0.0010
Diet*Day	2	1.34703067	0.673515334	0.44	0.6485

APPENDIX 6B. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
Normalized Palmar					
Total	46	102.223126	2.22224188		
Model	5	29.8686229	5.97372459	3.39	0.0119
Residual	41	72.3545035	1.76474399		
Diet	1	2.88752976	2.88752976	1.64	0.2080
Day	2	25.7007892	12.8503946	7.28	0.0020
Diet*Day	2	1.50160227	0.750801135	0.43	0.6563
Dorsal					
Total	46	72.5600604	1.57739262		
Model	5	30.6361997	6.12723994	5.99	0.0003
Residual	41	41.9238607	1.02253319		
Diet	1	0.155399957	0.155399957	0.15	0.6987
Day	2	29.3397174	14.6698587	14.35	0.0000
Diet*Day	2	1.13997916	0.56998958	0.56	0.5770
Normalized Dorsal					
Total	46	105.876012	2.30165243		
Model	5	33.3393051	6.6786101	3.77	0.0067
Residual	41	72.5367068	1.76918797		
Diet	1	2.57660647	2.57660647	1.46	0.2344
Day	2	29.6815056	14.8407528	8.39	0.0009
Diet*Day	2	1.30352062	0.651760311	0.37	0.6941
Total RBAE					
Total	46	409157.88	8894.73651		
Model	5	18174.8365	3634.9573	0.38	0.8588
Residual	41	390983.043	9536.17178		
Diet	1	12075.9115	12075.9115	1.27	0.2670
Day	2	368.629457	184.314728	0.02	0.9809
Diet*Day	2	5628.85161	2814.42581	0.30	0.7460
Normalized Total RBAE					
Total	46	618403.157	13443.5469		
Model	5	19101.54	3820.30799	0.26	0.9315
Residual	41	599301.617	14617.1126		
Diet	1	12770.2346	12770.2346	0.87	0.3554
Day	2	37.8358496	18.9179248	0.00	0.9987
Diet*Day	2	6474.69566	3237.34783	0.22	0.8023

APPENDIX 6C. RBAE MEASUREMENTS OF THE MCIII

Horse	Diet	Day	Lateral (mm Al)	Normalized Lateral	Medial (mm Al)	Normalized Medial
1A	A	0	15.44	0.00	16.36	0.00
2A	A	0	16.94	0.00	17.64	0.00
3A	A	0	15.21	0.00	17.84	0.00
4A	A	0	16.42	0.00	18.34	0.00
5A	A	0	15.27	0.00	18.28	0.00
6A	A	0	13.96	0.00	17.72	0.00
7A	A	0	16.89	0.00	18.91	0.00
8A	A	0	17.51	0.00	20.19	0.00
1B	B	0	17.27	0.00	18.58	0.00
2B	B	0	16.90	0.00	16.80	0.00
3B	B	0	17.06	0.00	17.85	0.00
4B	B	0	16.18	0.00	19.78	0.00
5B	B	0	16.68	0.00	18.63	0.00
6B	B	0	16.46	0.00	17.97	0.00
7B	B	0	15.66	0.00	17.87	0.00
8B	B	0	17.79	0.00	18.96	0.00
1A	A	56	17.10	1.66	19.26	2.89
2A	A	56	17.79	0.86	18.42	0.78
3A	A	56	16.47	1.27	18.79	0.95
4A	A	56	16.25	-0.17	19.28	0.94
5A	A	56	16.49	1.21	19.08	0.79
6A	A	56	13.42	-0.54	18.05	0.33
7A	A	56	16.60	-0.29	19.16	0.25
8A	A	56	17.55	0.04	19.86	-0.33
1B	B	56	17.28	0.01	19.43	0.85
2B	B	56	18.16	1.26	17.13	0.32
3B	B	56	17.57	0.51	19.27	1.42
4B	B	56	16.16	-0.01	20.01	0.23
5B	B	56	18.42	1.74	18.59	-0.04
6B	B	56	14.80	-1.66	17.54	-0.44
7B	B	56	15.48	-0.18	18.08	0.20
8B	B	56	16.57	-1.23	18.28	-0.68
1A	A	112	17.59	2.15	19.30	2.94
2A	A	112	16.79	-0.15	18.34	0.70
3A	A	112	18.36	3.15	17.64	-0.20
4A	A	112	18.91	2.49	17.73	-0.61
5A	A	112	18.63	3.36	18.96	0.68
6A	A	112	13.66	-0.31	17.91	0.19
7A	A	112	17.16	0.27	19.65	0.74
8A	A	112	16.79	-0.72	20.22	0.03

APPENDIX 6C. CONTINUED

Horse	Diet	Day	Lateral (mm Al)	Normalized Lateral	Medial (mm Al)	Normalized Medial
1B	B	112	18.56	1.30	19.94	1.36
2B	B	112	17.95	1.04	17.22	0.41
3B	B	112	17.70	0.65	19.01	1.16
4B	B	112	16.93	0.75	20.36	0.59
5B	B	112	15.97	-0.71	19.31	0.69
6B	B	112	15.48	-0.97	17.80	-0.18
7B	B	112	16.77	1.11	18.74	0.86
8B	B	112	16.47	-1.32	18.72	-0.24

APPENDIX 6C. CONTINUED

Horse	Diet	Day	Palmar (mm Al)	Normalized Palmar	Dorsal (mm Al)	Normalized Dorsal	Total (mm^2Al)	Normalized Total
1A	A	0	8.31	0.00	9.94	0.00	798.81	0.00
2A	A	0	10.27	0.00	12.44	0.00	712.87	0.00
3A	A	0	11.44	0.00	14.39	0.00	631.69	0.00
4A	A	0	10.79	0.00	13.45	0.00	632.60	0.00
5A	A	0	11.25	0.00	13.84	0.00	659.08	0.00
6A	A	0	12.10	0.00	13.28	0.00	539.66	0.00
7A	A	0	10.21	0.00	12.21	0.00	594.60	0.00
8A	A	0	11.84	0.00	13.60	0.00	721.75	0.00
1B	B	0	12.04	0.00	14.07	0.00	811.92	0.00
2B	B	0	12.69	0.00	14.16	0.00	677.15	0.00
3B	B	0	12.34	0.00	13.87	0.00	728.47	0.00
4B	B	0	12.83	0.00	15.21	0.00	700.74	0.00
5B	B	0	9.93	0.00	12.81	0.00	720.00	0.00
6B	B	0	9.34	0.00	11.26	0.00	522.91	0.00
7B	B	0	14.04	0.00	14.55	0.00	630.56	0.00
8B	B	0	9.19	0.00	11.63	0.00	997.16	0.00
1A	A	56	14.35	6.03	16.00	6.06	550.86	-247.95
2A	A	56	11.14	0.87	13.54	1.10	822.23	109.36
3A	A	56	11.75	0.31	13.77	-0.62	549.16	-82.53
4A	A	56	11.09	0.30	14.25	0.81	873.20	240.59
5A	A	56	12.06	0.80	14.51	0.67	793.79	134.72
6A	A	56	12.07	-0.03	13.98	0.70	510.91	-28.75
7A	A	56	13.19	2.98	15.08	2.88	632.60	38.00
8A	A	56						
1B	B	56	11.23	-0.81	13.73	-0.33	710.43	-101.49
2B	B	56	13.43	0.75	14.78	0.63	727.27	50.12
3B	B	56	12.93	0.59	14.85	0.99	595.38	-133.09
4B	B	56	13.02	0.19	14.99	-0.21	634.28	-66.46
5B	B	56	12.77	2.84	15.20	2.39	726.88	6.88
6B	B	56	11.60	2.26	13.53	2.27	687.12	164.21
7B	B	56	13.48	-0.56	14.40	-0.15	792.52	161.96
8B	B	56	11.37	2.17	13.58	1.95	712.02	-285.14
1A	A	112	14.40	6.09	16.60	6.66	652.35	-146.46
2A	A	112	11.35	1.08	14.12	1.68	629.18	-83.69
3A	A	112	13.17	1.73	14.88	0.49	615.91	-15.78
4A	A	112	12.66	1.86	15.45	2.00	657.70	25.10
5A	A	112	12.98	1.72	15.11	1.27	781.74	122.67
6A	A	112	12.21	0.11	14.39	1.11	690.05	150.39
7A	A	112	12.18	1.97	14.75	2.54	805.74	211.13
8A	A	112	14.37	2.53	15.67	2.07	669.13	-52.62

APPENDIX 6C. CONTINUED

Horse	Diet	Day	Palmar (mm Al)	Normalized Palmar	Dorsal (mm Al)	Normalized Dorsal	Total (mm^2Al)	Normalized Total
1B	B	112	12.74	0.69	14.82	0.76	617.40	-194.52
2B	B	112	12.27	-0.41	14.73	0.58	782.07	104.92
3B	B	112	13.89	1.55	15.45	1.58	697.93	-30.55
4B	B	112	13.83	1.01	15.64	0.44	664.12	-36.62
5B	B	112	12.27	2.34	15.25	2.43	712.54	-7.46
6B	B	112	11.32	1.98	13.65	2.40	691.21	168.30
7B	B	112	14.38	0.34	15.14	0.60	729.40	98.84
8B	B	112	12.34	3.14	15.15	3.52	702.62	-294.54

APPENDIX 7A. BONE GEOMETRY BY DIET AND DAY

Day	0	56	112	Mean
Total ML Width (mm)				
Diet A	33.77	34.70	33.28	33.92
SEM	0.75	0.58	0.38	0.35
Diet B	34.90	33.97	34.10	34.32
SEM	0.59	0.53	0.66	0.34
Mean	34.33	34.34	33.69	
SEM	0.48	0.39	0.38	
Lateral Cortex (mm)				
Diet A	7.33	7.89	7.35	7.53
SEM	0.38	0.34	0.22	0.19
Diet B	8.13	7.77	7.86	7.92
SEM	0.36	0.35	0.35	0.20
Mean	7.73	7.83	7.61	
SEM	0.27	0.24	0.21	
Medial Cortex (mm)				
Diet A	9.27	9.95	9.63	9.62
SEM	0.45	0.54	0.55	0.29
Diet B	9.83	9.63	9.74	9.73
SEM	0.42	0.56	0.56	0.28
Mean	9.55	9.79	9.68	
SEM	0.31	0.38	0.38	
ML Medullary Cavity (mm)				
Diet A	17.16	16.86	16.30	16.77
SEM	0.55	0.45	0.58	0.30
Diet B	16.94	16.58	16.50	16.67
SEM	0.82	0.76	0.77	0.44
Mean	17.05	16.72	16.40	
SEM	0.48	0.43	0.47	
Total DP Width (mm)				
Diet A[a]	28.65	28.65	28.82	28.71
SEM	0.20	0.34	0.66	0.24
Diet B[b]	27.73	27.96	27.96	27.89
SEM	0.30	0.45	0.33	0.20
Mean	28.19	28.31	28.39	
SEM	0.21	0.29	.37	
Palmar Cortex (mm)				
Diet A	6.04	6.42	6.50	6.32
SEM	0.24	0.23	0.27	0.14
Diet B	6.00	6.23	6.87	6.37
SEM	0.39	0.26	0.35	0.20
Mean	6.02	6.33	6.68	
SEM	0.22	0.17	0.22	

APPENDIX 7A. CONTINUED

Day	0	56	112	Mean
Dorsal Cortex (mm)				
Diet A	9.64	9.69	9.59	9.59
SEM	0.29	0.39	0.20	0.20
Diet B	10.01	10.12	9.81	9.98
SEM	0.39	0.45	0.35	0.22
Mean	9.82	9.91	9.63	
SEM	0.24	0.29	0.25	
DP Medullary Cavity (mm)				
Diet A[a]	12.97	12.54	12.87	12.79
SEM	0.43	0.41	0.55	0.26
Diet B[b]	11.72	11.61	11.29	11.54
SEM	0.38	0.29	0.32	0.19
Mean	12.34	12.08	12.08	
SEM	0.32	0.27	0.37	

[a,b]Diets not carrying a common subscript differ ($P < 0.02$)

APPENDIX 7B. ANOVA TABLE FOR BONE GEOMETRY

Source	df	Partial SS	MS	F-value	P-value
Total ML Width					
Total	47	131.889614	2.79878372		
Model	5	14.3406979	2.86813957	1.02	0.4155
Residual	42	117.548916	2.79878372		
Diet	1	2.01310674	2.01310674	0.72	0.4012
Day	2	4.40547375	2.20273687	0.79	0.4618
Diet*Day	2	7.92211738	3.96105869	1.42	0.2542
Normalized Total ML Width					
Total	47	80.7027456	1.71707969		
Model	5	18.6314736	3.72629472	2.52	0.0439
Residual	42	62.071272	1.47788743		
Diet	1	6.30387548	6.30387548	4.27	0.0451
Day	2	4.40547211	2.20273606	1.49	0.2369
Diet*Day	2	7.92212601	3.96106301	2.68	0.0803
Lateral Cortex					
Total	47	42.4793634	.903816243		
Model	5	4.05326544	0.810653088	0.89	0.4990
Residual	42	38.426098	0.914907094		
Diet	1	1.85653298	1.85653088	2.03	0.1617
Day	2	0.41305386	0.20652693	0.23	0.7989
Diet*Day	2	1.7836786	0.891839299	0.97	0.3856
Normalized Lateral Cortex					
Total	47	38.4936982	0.819014856		
Model	5	4.14483557	0.828967114	1.01	0.4218
Residual	42	34.3488626	0.817830063		
Diet	1	1.94810217	1.94810217	2.28	0.1302
Day	2	0.413054159	0.206527079	0.25	0.7780
Diet*Day	2	1.78367924	0.891839618	1.09	0.3454
Medial Cortex					
Total	47	91.1966756	1.9403548		
Model	5	2.14697733	0.429195466	0.20	0.9597
Residual	42	89.0506983	2.12025472		
Diet	1	0.158125479	0.158125479	0.07	0.7861
Day	2	0.440344432	0.220172216	0.10	0.9016
Diet*Day	2	1.54750742	0.77375371	0.36	0.6964

APPENDIX 7B. CONTINUED

Source	Df	Partial SS	MS	F-value	P-value
Normalized Medial Cortex					
Total	47	37.2367174	0.793370582		
Model	5	4.3730605	0.8746121	1.12	0.3657
Residual	42	32.8636569	.782468021		
Diet	1	2.38520838	2.38520838	3.05	0.0881
Day	2	0.440344817	0.220172409	0.28	0.7562
Diet*Day	2	1.5475073	0.77375362	0.99	0.3805
ML Medullary Width					
Total	47	155.586718	3.31035571		
Model	5	4.04113614	0.808227228	0.22	0.9501
Residual	42	151.545582	3.60822814		
Diet	1	0.116526062	0.116526062	0.03	0.8582
Day	2	3.35832169	1.67916085	0.47	0.6311
Diet*Day	2	0.566288385	0.283144193	0.08	0.9247
Normalized ML Medullary Width					
Total	47	22.0041457	0.468173312		
Model	5	4.1089984	0.821799679	1.93	0.1098
Residual	42	17.8951473	0.426074935		
Diet	1	0.184387998	0.184387998	0.43	0.5142
Day	2	3.35832191	1.67916095	3.94	0.0270
Diet*Day	2	0.566288492	0.283144246	0.66	0.5198
Total DP Width					
Total	47	64.1765656	1.36545884		
Model	5	8.52515955	1.70503191	1.29	0.2877
Residual	42	55.6514061	1.32503348		
Diet	1	8.07290165	8.07290165	6.09	0.177
Day	2	0.337778755	0.168889378	0.13	0.8807
Diet*Day	2	0.11447915	0.057239575	0.04	0.9548

APPENDIX 7B. CONTINUED

Source	Df	Partial SS	MS	F-value	P-value
Normalized Total DP					
Total	47	44.5245156	0.94733012		
Model	5	0.567310398	0.11346208	0.11	0.9899
Residual	42	43.9572052	1.04660012		
Diet	1	0.115052072	0.115052072	0.11	0.7419
Day	2	0.337779167	0.16889583	0.16	0.8515
Diet*Day	2	0.114479159	0.057239579	0.05	0.9468
Palmar Cortex					
Total	47	34.0496969	0.724461637		
Model	5	4.26928518	0.853857035	1.20	0.3238
Residual	42	29.7804118	0.709057423		
Diet	1	0.24299932	0.24299932	0.03	0.8540
Day	2	3.54041334	1.77020667	2.50	0.0945
Diet*Day	2	0.704571903	0.352285951	0.50	0.6120
Normalized Palmar Cortex					
Total	47	17.2905118	0.367883229		
Model	5	4.32299009	0.864598017	2.80	0.0286
Residual	42	12.9675217	0.308750516		
Diet	1	0.078004683	0.078004683	0.25	0.6178
Day	2	3.54041352	1.77020676	5.73	0.0063
Diet*Day	2	0.704571887	0.352285943	1.14	0.3292
Dorsal Cortex					
Total	47	50.1462546	1.06694159		
Model	5	2.48607188	0.497214377	0.44	0.8193
Residual	42	47.6601827	1.13476626		
Diet	1	1.81935498	1.81935498	1.60	0.2124
Day	2	0.652476262	0.326238131	0.29	0.7516
Diet*Day	2	0.014240645	0.007120322	0.01	0.9937
Normalized Dorsal Cortex					
Total	47	10.3785495	0.220820201		
Model	5	1.16995887	0.233991774	1.07	0.3921
Residual	42	9.20859059	0.219252157		
Diet	1	0.000229688	0.000229688	0.00	0.9743
Day	2	1.12792606	0.563963028	2.57	0.0883
Diet*Day	2	0.041803125	0.020901562	0.10	0.9093

APPENDIX 7B. CONTINUED

Source	df	Partial SS	MS	F-value	P-value
DP Medullary Width					
Total	47	75.8220662	1.61323545		
Model	5	20.5309091	4.10618183	3.12	0.0175
Residual	42	55.291157	1.31645612		
Diet	1	18.8877481	18.8877481	14.35	0.0005
Day	2	0.753715645	0.376857823	0.29	0.7525
Diet*Day	2	0.88944544	0.44472272	0.34	0.7152
Normalized DP Medullary Width					
Total	47	32.3144956	0.68754246		
Model	5	1.64316094	0.328632188	0.45	0.8108
Residual	42	30.6713347	0.730269873		
Diet	1	0.000052081	0.000052081	0.00	0.9993
Day	2	0.753715636	0.376857818	0.52	0.6006
Diet*Day	2	0.889444783	0.444722392	0.61	0.5486

APPENDIX 7C. GEOMETRIC MEASUREMENTS OF MCIII

Horse	Diet	Day	Total ML Width (mm)	Norm. Total ML Width	Lateral Cortex Width (mm)	Norm. Lateral Width	Medial Width (mm)	Norm. Medial Width	ML Medullary Width (mm)	Norm. ML Medullary Width
1A	A	0	36.57	0.00	8.65	0.00	9.11	0.00	18.81	0.00
2A	A	0	34.64	0.00	7.93	0.00	8.81	0.00	17.91	0.00
3A	A	0	35.63	0.00	7.74	0.00	9.07	0.00	18.82	0.00
4A	A	0	32.18	0.00	6.90	0.00	8.47	0.00	16.82	0.00
5A	A	0	30.37	0.00	5.52	0.00	7.14	0.00	17.72	0.00
6A	A	0	33.41	0.00	6.09	0.00	11.47	0.00	15.85	0.00
7A	A	0	35.27	0.00	7.99	0.00	10.08	0.00	17.21	0.00
8A	A	0	32.07	0.00	7.86	0.00	10.06	0.00	14.16	0.00
1B	B	0	35.21	0.00	9.23	0.00	11.73	0.00	14.25	0.00
2B	B	0	38.03	0.00	8.91	0.00	8.25	0.00	20.88	0.00
3B	B	0	35.66	0.00	7.99	0.00	10.84	0.00	16.84	0.00
4B	B	0	34.82	0.00	8.66	0.00	9.72	0.00	16.45	0.00
5B	B	0	33.04	0.00	5.92	0.00	8.80	0.00	18.33	0.00
6B	B	0	33.54	0.00	8.59	0.00	9.84	0.00	15.12	0.00
7B	B	0	35.69	0.00	7.74	0.00	8.92	0.00	19.04	0.00
8B	B	0	33.21	0.00	8.01	0.00	10.60	0.00	14.61	0.00
1A	A	56	36.50	-0.06	8.33	-0.32	10.69	1.58	17.49	-1.33
2A	A	56	33.80	-0.84	8.33	0.40	8.50	-0.31	16.98	-0.93
3A	A	56	37.07	1.44	9.28	1.54	8.77	-0.31	19.03	0.21
4A	A	56	32.28	0.10	6.42	-0.48	8.19	-0.27	17.67	0.85
5A	A	56	34.28	3.91	8.19	2.67	9.80	2.66	16.30	-1.42
6A	A	56	35.08	1.67	6.49	0.40	12.89	1.42	15.71	-0.14
7A	A	56	35.46	0.19	8.09	0.10	10.55	0.47	16.83	-0.38
8A	A	56	33.14	1.07	8.06	0.21	10.21	0.16	14.87	0.70
1B	B	56	34.96	-0.26	9.38	0.15	12.78	1.05	12.80	-1.45
2B	B	56	34.69	-3.34	7.19	-1.72	8.04	-0.21	19.47	-1.42
3B	B	56	35.67	0.01	8.90	0.92	9.67	-1.18	17.10	0.27
4B	B	56	32.56	-2.26	8.14	-0.52	7.98	-1.74	16.45	0.00
5B	B	56	32.67	-0.38	6.32	0.40	8.40	-0.40	17.95	-0.38
6B	B	56	32.71	-0.84	7.52	-1.07	9.98	0.15	15.21	0.09
7B	B	56	36.01	0.32	7.25	-0.49	10.24	1.32	18.53	-0.51
8B	B	56	32.54	-0.68	7.47	-0.54	9.96	-0.64	15.11	0.50
1A	A	112	33.78	-2.79	7.36	-1.29	8.99	-0.12	17.43	-1.38
2A	A	112	32.53	-2.11	6.40	-1.53	8.32	-0.49	17.82	-0.09
3A	A	112	35.25	-0.38	7.22	-0.52	10.00	0.93	18.03	-0.79
4A	A	112	32.39	0.21	8.31	1.41	8.23	-0.23	15.85	-0.97
5A	A	112	31.99	1.62	7.76	2.24	8.06	0.92	16.18	-1.54
6A	A	112	34.22	0.81	6.60	0.52	11.05	-0.42	16.58	0.72
7A	A	112	33.01	-2.26	7.49	-0.51	9.97	-0.11	15.56	-1.65
8A	A	112	33.09	1.02	7.68	-0.18	12.47	2.42	12.94	-1.22

APPENDIX 7C. CONTINUED

Horse	Diet	Day	Total ML Width (mm)	Norm. Total ML Width	Lateral Cortex Width (mm)	Norm. Lateral Width	Medial Width (mm)	Norm. Medial Width	ML Medullary Width (mm)	Norm. ML Medullary Width
1B	B	112	35.73	0.51	9.47	0.24	12.14	0.41	14.12	-0.14
2B	B	112	35.34	-2.69	6.92	-1.99	8.23	-0.02	20.20	-0.68
3B	B	112	35.70	0.04	8.81	0.82	10.14	-0.70	16.76	-0.08
4B	B	112	31.39	-3.44	8.37	-0.29	7.33	-2.39	15.69	-0.76
5B	B	112	33.01	-0.03	7.56	1.64	9.15	0.35	16.31	-2.02
6B	B	112	32.22	-1.32	7.36	-1.23	9.19	-0.65	15.67	0.56
7B	B	112	36.21	0.52	6.47	-1.28	10.57	1.66	19.17	0.14
8B	B	112	33.23	0.02	7.95	-0.06	11.15	0.56	14.13	-0.48

APPENDIX 7C. CONTINUED

Horse	Diet	Day	Total DP Width (mm)	Norm. Total DP Width	Palmar Width (mm)	Norm. Palmar Width	Dorsal Width (mm)	Norm. Dorsal Width	DP Medullary Width (mm)	Norm. DP Medullary Width
1A	A	0	29.02	0.00	5.85	0.00	9.29	0.00	13.89	0.00
2A	A	0	27.83	0.00	5.15	0.00	11.50	0.00	11.19	0.00
3A	A	0	29.08	0.00	5.89	0.00	9.72	0.00	13.48	0.00
4A	A	0	28.25	0.00	6.42	0.00	9.01	0.00	12.82	0.00
5A	A	0	27.90	0.00	5.70	0.00	9.51	0.00	12.69	0.00
6A	A	0	29.30	0.00	5.42	0.00	8.78	0.00	15.11	0.00
7A	A	0	28.95	0.00	6.72	0.00	9.49	0.00	12.75	0.00
8A	A	0	28.86	0.00	7.17	0.00	9.84	0.00	11.85	0.00
1B	B	0	28.97	0.00	8.53	0.00	10.06	0.00	10.39	0.00
2B	B	0	27.59	0.00	5.17	0.00	8.40	0.00	14.02	0.00
3B	B	0	28.00	0.00	6.00	0.00	9.75	0.00	12.26	0.00
4B	B	0	27.31	0.00	5.73	0.00	10.50	0.00	11.08	0.00
5B	B	0	28.66	0.00	4.91	0.00	11.93	0.00	11.82	0.00
6B	B	0	27.20	0.00	6.23	0.00	9.67	0.00	11.30	0.00
7B	B	0	26.26	0.00	5.52	0.00	9.01	0.00	11.74	0.00
8B	B	0	27.85	0.00	5.94	0.00	10.79	0.00	11.13	0.00
1A	A	56	28.44	-0.58	5.83	-0.01	10.31	1.02	12.30	-1.59
2A	A	56	28.53	0.70	5.68	0.53	11.14	-0.36	11.71	0.52
3A	A	56	29.09	0.01	5.97	0.08	8.42	-1.30	14.70	1.22
4A	A	56	29.45	1.21	7.23	0.81	9.85	0.85	12.38	-0.44
5A	A	56	28.38	0.48	6.57	0.88	10.08	0.57	11.73	-0.96
6A	A	56	27.04	-2.26	5.94	0.52	7.69	-1.09	13.41	-1.70
7A	A	56	30.21	1.26	7.07	0.36	10.11	0.62	13.03	0.28
8A	A	56	28.10	-0.76	7.16	-0.01	9.91	0.06	11.04	-0.81
1B	B	56	28.77	-0.20	7.61	-0.91	10.51	0.46	10.65	0.26
2B	B	56	27.45	-0.15	5.71	0.54	8.34	-0.06	13.40	-0.63
3B	B	56	28.32	0.31	6.19	0.19	10.33	0.58	11.80	-0.46
4B	B	56	27.01	-0.30	5.05	-0.69	10.51	0.01	11.45	0.37
5B	B	56	30.32	1.67	6.35	1.44	12.21	0.28	11.77	-0.05
6B	B	56	26.06	-1.14	6.01	-0.22	8.80	-0.88	11.26	-0.04
7B	B	56	27.49	1.23	6.68	1.16	9.26	0.26	11.56	-0.19
8B	B	56	28.31	0.46	6.23	0.29	11.04	0.25	11.05	-0.08
1A	A	112	28.31	-0.71	5.53	-0.32	10.48	0.17	12.31	-1.58
2A	A	112	28.05	0.22	5.59	0.44	10.13	-1.01	12.33	1.15
3A	A	112	29.59	0.51	6.31	0.43	8.46	0.04	14.83	1.35
4A	A	112	29.33	1.08	7.09	0.67	9.21	-0.64	13.03	0.21
5A	A	112	27.53	-0.37	6.06	0.36	9.86	-0.22	11.61	-1.08
6A	A	112	26.60	-2.70	6.47	1.06	7.33	-0.37	12.81	-2.30
7A	A	112	32.79	3.84	7.49	0.78	9.94	-0.17	15.36	2.61
8A	A	112	28.37	-0.49	7.45	0.28	10.20	0.30	10.73	-1.13

APPENDIX 7C. CONTINUED

Horse	Diet	Day	Total DP Width (mm)	Norm. Total DP Width	Palmar Width (mm)	Norm. Palmar Width	Dorsal Width (mm)	Norm. Dorsal Width	DP Medullary Width (mm)	Norm. DP Medullary Width
1B	B	112	28.18	-0.80	8.37	-0.15	9.88	-0.63	9.93	-0.47
2B	B	112	28.74	1.15	7.50	2.33	8.47	0.13	12.78	-1.25
3B	B	112	27.41	-0.59	5.94	-0.06	9.67	-0.66	11.81	-0.45
4B	B	112	27.51	0.20	5.87	0.14	10.20	-0.32	11.45	0.37
5B	B	112	29.12	0.46	5.56	0.65	11.47	-0.74	12.09	0.27
6B	B	112	26.66	-0.54	6.85	0.62	9.16	0.37	10.66	-0.64
7B	B	112	27.04	0.78	7.43	1.91	8.90	-0.36	10.72	-1.03
8B	B	112	29.06	1.21	7.47	1.53	10.72	-0.32	10.88	-0.25

VITA

Holly Sue (Kenimond) Spooner is the daughter of Charles and Kathy Kenimond. She was born in Kokomo, Indiana, on May 14, 1981.

Following graduation from Carroll Jr./Sr. High School in Flora, Indiana, she attended Texas A&M University as a National Merit Scholar and Presidential Endowed Scholar. At Texas A&M University, she earned her B.S. in Agricultural Development, graduating Magna Cum Laude in May of 2003. Holly began her graduate career in August 2003 with Dr. Gary Potter at Texas A&M University, graduating with a M.S. in Animal Science in August of 2005. Her research focused on equine nutrition and exercise physiology, with a publication in the Equine Science Society Symposium. Holly served as a Regent's Fellow, was a graduate teaching assistant, and assisted the horse judging team.

Holly was married to Scott C. Spooner in August, 2004. Her permanent address is 4090 E 350 S, Bringhurst, IN 46913.