THE EFFECT OF DIETARY CATION-ANION DIFFERENCE ON GROWTH AND MINERAL BALANCE IN GROWING HORSES

By

STEVEN ROBERT COOPER

Bachelor of Science
Texas Tech University
Lubbock, Texas
1992

Master of Science
University of Illinois
Urbana, Illinois
1995

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 1998

THE EFFECT OF DIETARY CATION-ANION
DIFFERENCE ON GROWTH AND
MINERAL BALANCE IN
GROWING HORSES

Thesis Approved:

Don R. Topliff
Thesis Adviser

Rodney Gisert

David W Freeman

F G Brazile

Wayne B. Powell
Dean of Graduate College

ACKNOWLEDGMENTS

I would first like to thank Dr. Don Topliff and Dr. David Freeman for their endless hours of professional and personal support throughout my graduate career at OSU. Their friendship and guidance will always be appreciated and never forgotten. Furthermore, I want to extend my sincere appreciation to Dr. Rodney Geisert and Dr. James E. Breazile for their service on my graduate committee and for their valued expertise and support of this dissertation.

A very special thanks goes to my parents Mickey and Nancy, my brother Chris, and my grandparents Hiram, Lorene, Bess and Woodrow for all of their love and support throughout my college endeavor as well as my life. Without these people I would have never had the strength to attain all of my personal and professional aspirations. I am also very grateful to my friends Zeb, Jody, Bilynn, Ron, Bobbi, Mike, Matt, Brett and Kari for always being there for me and standing behind me. I would also like to thank the farm managers Andy and Clay for providing me with their invaluable assistance and for being two of my closest friends.

Finally, I will forever be grateful to my wonderful wife Emily whose unconditional love, friendship and support were unwavering through the hardships of both my graduate programs. Thank you Emily for all that you have given me throughout these years together.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In the past decade, researchers have extensively evaluated the effects of dietary cation-anion difference (DCAD) on acid-base status, mineral metabolism and performance variables in cattle, sheep, swine, horses and poultry. The DCAD of the diet, calculated as meq (Na+K)-(Cl+S)/kg diet DM, can influence the physiological state of the animal by altering acid-base balance in the blood, fecal and urinary mineral excretion, dry matter digestibility and parameters of growth and athletic performance. Most of the experiments conducted in horses to date have studied only mature horses over a short period of time and merely suggested how DCAD might affect growth. Currently, only one study in the young growing horse has been conducted to evaluate the long term effects of DCAD on mineral balance and growth performance. It is also interesting to note that this study contradicts the findings of the previous short term studies concerning mineral balance, specifically calcium. Also of importance in the growing horse is the effect of DCAD on bone metabolism. Previous studies have suggested that the lowered calcium balance observed in horses consuming a diet low in DCAD may lead to an osteoporotic

weakening of the skeletal system. Due to these results, it is important that further research be conducted to analyze the effects of DCAD in the young growing horse over an extended period of time. If in fact an optimum DCAD does significantly enhance mineral balance and helps producers optimize performance, then there may be a need for the NRC (1989) to establish specific recommendations on DCAD for different classes of horses.

The purpose of this study was to determine the effects of DCAD on young, growing horses and establish the use of either a method or biological marker which would accurately assess changes in bone mineralization. The objectives of this study were: 1) To determine the effects of varying cation-anion difference on growth parameters by measuring changes in body weight and skeletal growth; 2) To determine the effects of varying cation-anion difference on mineral balance, especially calcium; 3) To determine the effects of varying cation-anion difference on bone metabolism by measuring changes in serum osteocalcin concentrations.

CHAPTER II

LITERATURE REVIEW

Dietary Cation-Anion Difference and Acid-Base Physiology

The influence of dietary cation-anion difference (DCAD) on acid-base physiology has been extensively studied in several species and equations have been developed to describe the balance of cations to anions in the diet. Recently, nutritionists (Mongin, 1981; Patience et al., 1987, Tucker et al., 1988a; and Baker et al., 1992) have formulated diets based on an equation assuming complete dietary availability of sodium (Na^+), potassium (K^+) and chloride (Cl^-) described as follows: meq (Na + K) - Cl / kg dietary DM. The divalent anion sulfur also may be included in the equation [meq (Na + K) - (Cl + S) / kg of dietary DM] since sulfur has effects similar to Cl on acid-base status in lactating dairy cows (Tucker et al., 1991). But because apparent absorption of sulfur in ruminants is only 52% to 61% (Spears et al., 1985), effects of S on acid-base balance would be less than Cl. Nevertheless, differences between Cl and S for all acid-base parameters were nonsignificant supporting the concept of a similar acidogenic effect of Cl and S (Tucker et al., 1991). Supporting evidence has come from human studies with increased acid excretion in the urine when methionine or sulfur containing foods are

consumed (Hunt, 1956). Furthermore, Kass (1957) related sulfur metabolism to urine acidification by demonstrating that the administration of methionine was effective in maintaining an acid urine in patients with chronic urinary tract infection. Because manipulation of the DCAD can have profound effects on the homeostatic environment within the body, it potentially could affect growth and performance.

Acid-base status, in most living organisms, is tightly regulated. To maintain pH equilibrium, anions and cations are retained or excreted in a homeostatic system; concentrations are not significantly changed by slower metabolic processes such as growth. However, when the diet or the environment disrupts acid-base homeostasis, performance of the animal may be reduced (Mongin, 1980).

The relatively constant extracellular fluid (ECF) pH is the result of a balance between acids and bases. An acid is any chemical that can donate protons (hydrogen ions) to a solution, and a base is any substance that accepts hydrogen ions from solution. The buffer system of body fluids which is comprised of weak acids and the bases of these weak acids (buffer pair) minimizes changes in hydrogen ion concentration. The total amount of base in whole blood including HCO_3^-, hemoglobin and other bases is called buffer base. These bases constitute the metabolic component that determines blood pH. An acid-base disturbance that involves an abnormal decrease or increase of these bases is known as metabolic acidosis and alkalosis, respectively. The pH of the ECF is maintained at a pH of 7.4 ± .02 in arterial plasma and 7.38 ± .02 in mixed venous plasma. Since $pH = -\log [H^+]$, then the hydrogen ion concentration can be calculated by the

relation $[H^+]= 10^{-pH}$. The pH of the ECF is determined by the ratio of conjugate bases to their weak acids, as expressed in the Henderson-Hasselbalch equation:

$$pH = pKa + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

The pKa value is the pH at which the ratio of the base (HCO_3^-) to the acid (H_2CO_3) is 1/1, and is therefore a negative logarithm. The pKa values are a measure of the strength of the acid component of the buffer pair where increasing strength of an acid corresponds to a lower pKa value.

The anion gap is often used to evaluate acid-base disorders such as metabolic acidosis (Swenson and Reece, 1993). When the concentrations of the major monovalent cations (Na^+ and K^+) and major monovalent anions (Cl^- and HCO_3^-) are measured, the sum of the cation concentration usually exceeds the sum of the anions which is known as the anion gap. This yields plasma that normally is slightly alkaline. The anion gap is the difference in milliequivalents between sodium plus potassium minus chloride plus bicarbonate in whole blood or plasma (Swenson and Reece, 1993). The anion gap is a measure of the non Cl^- and HCO_3^- anions associated with Na^+ and K^+ in the ECF and an increase above normal (≤ 20) is a measure of the severity of the metabolic acidosis.

The level of Na^+ and K^+ in the diet can effect acid-base equilibrium in the animal (Stewart, 1981). Sodium and potassium are absorbed from the gastrointestinal tract in exchange for a hydrogen ion. Sodium ions are reabsorbed from kidney tubules in exchange for hydrogen ions that are secreted into the tubular lumen. Generally, one sodium ion is

reabsorbed for each hydrogen ion that is secreted. This exchange maintains an appropriate electrical balance between the anions and cations in both the tubular fluid and the ECF. Sodium ions are released into the extracellular fluid in place of the H^+ involved in the reaction. At the same time, a bicarbonate ion, formed in the process of secreting a H^+, is also released into the extracellular fluid. The net effect of this reaction is to increase the amount of sodium bicarbonate in the ECF; this is the kidney's way of reducing acidosis of body fluids. When excess chloride is provided in the diet, these Cl^- ions are absorbed from the GI tract in exchange for a bicarbonate ion which tends to reduce ECF pH producing a metabolic acidosis. Excretion by the kidney is the main route of chloride loss from the body. In a state of chronic metabolic acidosis, one of the routes of excretion of excess hydrogen ions is through the combination of hydrogen with chloride in the renal tubule lumen which combines with ammonia to form ammonium chloride. Thus, NH_4Cl is then excreted in the urine which causes the urinary pH to be reduced.

In the process of controlling the pH of the body fluids, the renal acid-base system also regulates the concentration of HCO_3^- in plasma. The kidney regulates the amount of HCO_3^- recovered and reabsorbed from the glomelular filtrate and generates new bicarbonate to replace that lost in buffering the strong acids formed in the body. In systemic metabolic acidosis, the kidney helps return the ECF pH back to normal values through acidification of the urine. Inside the absorptive epithelial cell, H_2CO_3 dissociates to form H^+ and HCO_3^-, and the H^+ is then secreted into the tubular lumen. This secretion of H^+ is coupled to passive movement of Na^+ from the tubular lumen into

the epithelial cell (Na^+ - H^+ antiport). The HCO_3^- formed in the dissociation diffuses across the baso-lateral membrane and into the peritubular fluid. Thus, for each H^+ secreted, a bicarbonate ion simultaneously enters the extracellular fluid. The ammonia buffer system plays an important role in removal of these excess H^+ ions from the tubules. The hydrogen ions combine with chloride in the tubule lumen. This HCl then combines with ammonia to form ammonium chloride (NH_4Cl), a much weaker acid than HCL and less damaging to the kidneys (Baker et al., 1993). Conversely, when the body is alkalotic, the ammonia system becomes inoperative; bicarbonate ions instead of chloride ions pass into the urine so a concomitant excess of chloride is reabsorbed. The elements sodium, potassium, chloride and sulfur play major roles in the regulation of acid-base homeostasis and addition of these strong ions to the diet can have an impact on the acid-base balance of the animal.

Influence of DCAD on Acid-Base Homeostasis in the Animal

Horses consuming a highly anionic diet often experience a nutritionally-induced metabolic acidosis (Popplewell et al., 1993; Baker et al., 1992; and Wall et al., 1992). Mean arterial and venous blood pH, P_{CO_2} and HCO_3^- values were significantly lower in sedentary geldings when fed a highly anionic diet (Baker et al., 1992). It has been shown by Tucker et al. (1988a) that altering the DCAD can markedly affect blood acid-base measures, as pH, P_{CO_2}, and HCO_3^- levels decreased in cows consuming a low DCAD diet. Tucker et al. (1988b) further demonstrated that blood pH tended to be lower for cows consuming a high chloride diet than for cows consuming a diet with normal levels of

chloride. Lambs that were fed a diet supplemented with sodium bicarbonate had higher pH and base excess values than those given a diet supplemented with ammonium chloride (Abu Damir et al., 1990). Studies in adult cats have shown that an addition of ammonium chloride (Ching et al., 1989) or phosphoric acid (Fetteman et al., 1992) to the diet produced a metabolic acidosis as evidenced by a decrease in blood bicarbonate and urinary pH values.

Patience et al. (1987) altered dietary electrolyte balance for growing swine and showed linear and quadratic effects ($p<.05$) on whole blood bicarbonate concentration and base excess, as well as on average daily gain and average daily feed. As the diet became increasingly acidogenic through addition of calcium chloride, a metabolic acidosis, accompanied by a reduced growth rate, was observed in the pigs. In a later study, Patience et al. (1989) demonstrated that lowering the dietary undetermined anion (dUA), calculated as (sodium+potassium+calcium+magnesium)-(chloride+sulfate+phosphate), from 345 to 81meq/kg resulted in a reduced average daily gain and average daily feed. Furthermore, the acid-base parameters of blood pH, bicarbonate, and base excess all were reduced as acidity of the diet increased.

Scott and Buchan (1981) established in sheep that infusion of hydrochloric acid produced a metabolic acidosis evidenced by a fall in blood pH ($p<.001$) and a reduction in blood base excess. Moreover, sheep that were infused with sodium bicarbonate exhibited a metabolic alkalosis shown by a rise in blood pH ($p<.001$) and increase in blood base excess values. The alkalotic sheep also showed signs of slight respiratory compensation indicated by a small increase in blood P_{CO_2} values ($p<.05$). In an earlier study, Scott and

McIntosh (1975) were able to show the same effects on blood pH in pigs by infusing either hydrochloric acid and sodium bicarbonate which produced a metabolic acidosis and alkalosis, respectively.

Maintaining a constant arterial blood pH is critical for normal body function, so homeostatic mechanisms have been developed to maintain the concentration of blood bicarbonate to blood P_{CO_2} at a constant ratio. This balance is accomplished by adjusting respiratory rate to control blood P_{CO2} and adjusting renal excretion of bicarbonate to control blood bicarbonate concentration (Best, 1985). Changes in arterial pH produce corresponding changes in intracellular pH in that hydrogen ion secretion increases in acidosis and decreases with alkalosis. A decrease in the plasma [HCO_3^-] or an increase in arterial P_{CO2} could cause an acidosis. The resulting increase in H^+ secretion caused by the acidosis generates additional bicarbonate for the body which tends to return the arterial pH to normal. As the serum cation-anion balance increases, more bicarbonate ion is generated and released into the system. Therefore, it appears likely that the mechanism by which DCAD affects blood pH is via altering blood bicarbonate concentration (Tucker et al., 1988a). However, venous blood pH is indicative of changes in tissue acid-base balance and cannot undergo compensation by the body. The venous blood must first circulate through the heart before compensation can take place which would explain the lower venous blood pH, P_{CO2} and HCO_3^- values found in animals consuming low DCAD diets. This decrease in pH enhances respiratory ventilation in arterial blood and lowers CO_2 concentration until the bicarbonate can be restored to normal values. This hyperventilation, referred to as respiratory compensation for metabolic acidosis,

maintains the ratio between $NaHCO_3$ and H_2CO_3 at 20/1 which is required to sustain a pH at 7.4.

When comparing blood pH to serum cation-anion balance, Tucker et al. (1988a) revealed that the relationship at the lower balance values was fairly good but tended to deteriorate as serum cation-anion balance increased. In the previously mentioned study, they postulated that the quantity of acid or base generated at the lower balance values was such that normal homeostatic mechanisms (e.g., chemical buffers and regulation of respiration rate) were adequate to compensate and maintain fairly constant pH. However, as serum cation-anion balance increased, the relative proportion of bicarbonate generated increased to the point that other homeostatic mechanisms were activated which increased variation in blood pH.

Jackson et al. (1992) has shown in dairy calves that blood pH, P_{CO_2} and HCO_3^- all increased linearly with increasing DCAD. Moreover, calves fed the 0-meq diet had lower pH, P_{CO_2} and HCO_3^- values than those calves fed the $^+210$, $^+370$ and $^+520$ meq diets. In lactating dairy cows, Tucker et al. (1988a) reported that as DCAD was increased from -10 to $^+20$ meq/100g diet DM, blood pH increased linearly from 7.369 to 7.427. This change apparently was a result of a linear increase in blood HCO_3^- from 19.3 to 23.2 meq/L. This increase in blood HCO_3^- was correlated closely (r^2=.925) with the cation-anion balance of the serum which in turn was closely and positively correlated with the cation-anion balance of the diet.

Physiological responses to DCAD appear to be related to alterations in systemic acid-base status accompanying the absorption of monovalent ions (e.g., Na^+, K^+ and Cl^-) from the gastrointestinal tract (Tucker and Hogue, 1990). Diets that contain the same DCAD, whether achieved by altering concentrations of dietary Na^+, K^+ and Cl^-, should yield similar proportions of HCO_3^- and H^+ and should have similar effects on systemic acid-base status (Tucker and Hogue, 1990). To test this theory, Tucker and Hogue (1990) conducted a study to evaluate the response of lactating dairy cows to differences in dietary fixed ion concentrations while holding cation-anion balance constant at 32 meq/100g diet DM. No significant differences in pH, P_{CO_2} and HCO_3^- were detected across treatments. These findings indicated that, at a constant DCAD, the balance of Na^+ and K^+ to Cl^- in the diet was a more important determinant of the dietary impact on acid-base homeostasis than was the actual dietary concentration of these minerals (Tucker and Hogue, 1990). Likewise, Patience et al. (1989) found that elevated levels of chloride in the diet had no significant effect on growth or acid-base status in pigs which were fed diets with similar dUA.

The cation-anion difference in the diet obviously plays a major role in the acid-base status of the animal and should be considered when formulating rations. Feed ingredients have various levels of minerals which, depending on the percent to which they are added to the diet, will significantly affect the DCAD of the ration. Commonly fed grains such as corn, barley and oats typically have a low cation-anion balance [approx. 80-120 meq (Na^+ K)-Cl] / kg diet DM] as compared to Bermuda, Timothy and Alfalfa

hays [approx. 400-600 meq (Na^+ K) - Cl / kg diet DM] depending on the quality and stage of maturity of the hay (Baker et al., 1992). Ralston et al. (1993) found that mature mares consuming a 60% concentrate ration had lower fecal and venous blood pH, and higher urinary excretion of calcium and phosphorus than when fed a 10% grain ration with the same DCAD (200meq/kg DM). When horses were fed approximately 3 kg of a high starch feed, their venous pH was reduced from 7.41 to 7.388 ($p<.05$) within 1 hour relative to when the horses were fed only hay (Ralston, 1994). Furthermore, this reduction in venous pH was followed by a decrease in urinary pH within 4 to 6 hours after feeding and an increased excretion of both calcium and phosphorus. Differences in blood and urine pH also have been reported in ruminants given either cereal or forage diets (Scott, 1976; and Roby et al., 1987). Both blood and urine pH levels were lower in animals given cereal diets versus those fed a forage diet. Cereal diets have a cation deficit and are acidogenic, whereas forage diets have cation excesses and are alkalogenic (Freeden et al., 1988b). Consequently, as the grain portion of the diet increases or as a lower quality hay is fed, the DCAD of the diet may fall; this may result in a nutritionally-induced metabolic acidosis.

Interactions of Acid-Base Balance with Mineral Metabolism

One of the most important considerations in balancing rations deals with mineral requirements. Minerals must be supplied to the animal in adequate amounts and in the correct ratios for proper growth and performance. Sodium, potassium, calcium, phosphorus and chloride serve individually and collectively in the body fluids in order to carry out general physiological functions. In the young growing horse, skeletal

mineralization plays a major role in determining the future performance and soundness of the animal. The young horse is very susceptible to changes in the diet, so it is critical that diets be formulated which will provide and maintain optimal balances of the major minerals.

Sedentary horses consuming a high DCAD diet excrete more Na^+ and K^+ in the urine than those consuming a low DCAD diet (Baker et al., 1993). This study also found levels of Ca^{2+} and Cl^- in the urine were higher for horses on an anionic diet versus horses on a cationic diet. In exercising horses, Topliff et al. (1989) demonstrated that urinary excretion of Ca^{2+} and Cl^- was higher ($p<.05$) for horses fed a diet high in anions than for those fed a diet high in cations. Furthermore, Wall et al. (1992; 1993) found that excretion of Na^+ was greater in the urine when the diet was high in DCAD than when it was low in DCAD.

Abu Damir et al. (1990) suggested that dietary-induced changes in blood acid-base status were a major factor contributing to the lower rates of retention of Ca^{2+}, P and Mg^{2+} in the body composition of lambs given cereal-based diets. Lambs given a diet containing $NaHCO_3$ retained proportionately 1.46, 1.26 and 1.21 times the Ca^{2+}, P and Mg^{2+} retained by those given a diet containing NH_4Cl. Plasma Ca^{2+} and P levels also were lower in lambs given the NH_4Cl diet when compared to those given the $NaHCO_3$ diet.

Differences in mineral balance have been reported in ruminants given either cereal or forage diets with excretion of Ca^{2+}, P and Mg^{2+} in the urine being higher in those given

cereal diets (Roby et al., 1987). Increased losses of Ca^{2+} from the skeleton have been reported in adult goats made acidotic through changes in dietary fixed ion balance (Freeden et al., 1988 a; b). Those goats fed an anionic diet had higher intestinal absorption and bone resorption of Ca^{2+} and higher excretion of Ca^{2+} than goats fed a cationic diet . In lactating dairy cows, supplementation of $NaHCO_3$ resulted in a lowering of Na^+ in plasma and caused an increase in urinary Na^+ excretion (Tucker et al., 1988b). Urinary Cl^- and Ca^{2+} excretion were higher in cows on a high Cl^- diet as opposed to cows on a normal Cl^- diet. It is likely that as excess dietary Na^+ was absorbed into the blood, plasma osmolality rose which affected the flow of water from within cells into the extracellular fluid, yielding a Na^+-induced hypervolemia. Expanded extracellular fluid volume can be expected to increase the glomelular filtration rate, reduce aldosterone release, and increase the release of natriuretic hormone, all of which will increase urinary sodium excretion (Rose, 1984).

Jackson et al. (1992) reported that in dairy calves plasma Ca^{2+} increased linearly and Mg^{2+} and Cl^- decreased linearly with increasing cation-anion balance. In addition, urinary Ca^{2+}, Mg^{2+} and Cl^- excretions decreased linearly while urinary P, Na^+ and K^+ excretion increased linearly with increasing dietary cation-anion difference. There were no differences found in plasma Na^+ and P across treatments in this particular study. But no significant difference in plasma Na^+, Mg^{2+}, and K^+ concentrations was found between

cats fed a highly anionic diet and those fed a basal diet (Ching et al., 1989 and Fetteman et al., 1992).

Effect of dietary treatment on plasma K^+ has met with confounding results. In previous research, plasma K^+ has increased (Escabosa et al., 1984) or remained unchanged (Kilmer et al., 1981) in response to dietary $NaHCO_3$. Further research demonstrated that plasma K^+ was higher for the diets with supplemental NaCl and KCl than for the basal diet (Tucker and Hogue, 1990). A depression of plasma K^+ was shown for cows consuming an anionic diet when compared to concentrations for cows consuming a cationic diet (Block, 1984). Tucker et al. (1988a) found no significant effect of DCAD on plasma K^+. In contrast, research in sheep and pigs (Scott and McIntosh, 1975; and Scott and Buchan, 1981) has found that infusion of HCl increased the concentration of plasma K^+ while $NaHCO_3$ treatment resulted in reduced K^+ levels in the plasma.

Horses consuming a highly anionic diet may be in a net negative calcium balance due to the increased calcium excretion; and if prolonged, this condition could cause osteoporotic weakening of the skeletal system (Topliff et al., 1989; Baker et al., 1993; and Wall et al., 1993). Similar effects on urinary Ca^{2+} excretion have been reported in man due to an increased metabolic acid load induced by diets high in sulfate (Lemann and Relman, 1959). Petito and Evans (1984) detected increases in urinary Ca^{2+} excretion and lowered bone density in rats consuming a diet supplemented with NH_4Cl. Plasma Ca^{2+} and hydroxyproline (an index of bone Ca^{2+} resorption activity) also were elevated in these

rats in response to NH_4Cl ingestion, indicating a dissolution of bone. Thus, it appears that with acidosis caused by the NH_4Cl, bone was resorbed as evidenced by an increase in hydroxyproline, a concomitant increase in plasma Ca^{2+}, and a decrease in dry weight ash and specific gravity of the femur. Sauveur and Mongin (1978) found that metabolic acidosis resulting from excessive dietary chloride or NH_4Cl supplementation increased the incidence of tibial dyschondroplasia, while Na^+ and K^+ reduced the incidence. In broiler chicks, narrowing the cation-anion ratios increased the incidence of both dyschondroplasia and varus deformation (Halley et al., 1987). Long term dietary acidification has been shown to significantly increase blood ionized calcium levels and average daily urinary excretion of calcium in cats fed NH_4Cl (Ching et al., 1989). Also, the percent digestible calcium and the total daily calcium balance were lower ($p<.05$) in the treated cats compared to the control group.

The mechanism through which metabolic acidosis induces a negative Ca^{2+} balance is not yet clear. Lemann et al. (1967) and Beck and Webster (1976) proposed that acid stress inhibits the reabsorption of Ca^{2+} via kidney tubules by a direct effect of the acidosis on metabolic processes within renal tubular cells This inhibition, coupled with the pH lowering effect of the acid ingestion, may explain how urinary Ca^{2+} excretion is elevated with a lowering of the DCAD. Barzel (1969a) also postulated that lowering of pH per se increases calcium mobilization from the bone.

On the other hand, Wachman and Benstein (1970) proposed that metabolic acidosis augments calcium mobilization from bone either by increasing parathyroid

hormone secretion or augmenting the action of PTH on bone. Calcium balance is affected by absorption of Ca^{2+} from the gastrointestinal tract and excretion via the kidneys as well as resorption from the bone. Active transport of Ca^{2+} across the intestine is mediated by 1,25 dihydroxyvitamin D [1,25-$(OH)_2$D] and PTH regulates both bone Ca^{2+} resorption and renal production of 1,25-$(OH)_2$D. Goff et al. (1991) demonstrated that renal production of 1,25-$(OH)_2$D and osteoclastic bone resorption were temporarily refractory to PTH stimulation in cows fed cationic diets. Also, reducing the alkalinity of the diet by addition of anions increased the ability of the animal to produce 1,25-$(OH)_2$D and resorb bone calcium. Plasma hydroxyproline concentrations also were higher in cows fed an anionic diet, suggesting an increased mobilization of Ca^{2+} from the bone. Beck and Webster (1976) further verified in rats that acute metabolic acidosis directly raised serum Ca^{2+} concentrations and augmented the effects of PTH to raise serum Ca^{2+} levels. These data indicate that bone is refractory to the effects of PTH in the alkaline state, but that PTH is stimulated during an acidotic state. Other research has shown that ingestion of NH_4Cl in the absence of PTH increased bone resorption, suggesting that increased release of Ca^{2+} from the bone occurs independently of the action of PTH and may be due to the acidosis itself (Kraut et al., and Kunkel et al., 1986). Ching et al. (1989) found that even though chronic metabolic acidosis increased blood ionized calcium levels in the cat, PTH was unaffected while plasma 1,25 $(OH)_2$D concentrations were significantly reduced.

This data suggests that 1, 25$(OH)_2D$ plays a minimal role in bone mineral mobilization during this time.

Metabolic acidosis may affect Ca^{2+} homeostasis through multiple interactions of these hormones in a variety of organs and tissues. Therefore, it is difficult to construe the mechanism involved in a negative Ca^{2+} balance brought forth by a state of acidosis. However, it is clear that if this condition is prolonged, it could lead to an osteoporotic weakening of the skeletal system which may hinder the future performance of the animal.

Role of Osteocalcin in Bone Metabolism

The previous studies present conflicting data concerning the ability of 1,25 dihydroxycholicalciferol and PTH to accurately assess skeletal mineralization during alteration of acid-base balance. The assessment of bone metabolism *in vivo* however, can be accomplished through the use of biochemical markers. Bone formation and resorption processes can be quantified by measuring certain enzymes and protein products that are released by osteoblasts and osteoclasts, respectively. One alternative biological marker that has long been used as an index for bone formation is osteocalcin or bone Gla protein (Robins, 1994). This bone specific protein, synthesized by osteoblasts, contains three γ-carboxyglutamic acid residues which bind calcium ions and form complexes with bone mineral (Swenson and Reece, 1993). The osteoblasts are directly involved in bone formation on the surface of bone at specific areas, each of which is termed a bone metabolism unit (BMU). Osteoblastic cells have an extensive network of rough endoplasmic reticulum which synthesizes bone matrix. These cells replace bone matrix

that was eroded by osteoclasts at BMU sites. Osteocalcin is the major non-collagenous component of bone (Robins, 1994); once secreted from osteoblasts, it is incorporated into new bone or enters the circulation (Hope et al., 1993). Serum osteocalcin concentration may be a reflection of osteoid production (Malluche et al., 1984) and is greater during disease (Paget's disease, hyperthyroidism, and renal osteodystrophy) which is characterized by increased bone turnover (Swenson and Reece, 1993). Osteocalcin also may be used as a marker for metastatic bone disease in humans in which malignant cells secrete paracrine factors that stimulate osteoclasts to resorb bone resulting in weakened areas of the skeleton (Coleman et al., 1988). The previous study found that bone GLA protein was above the normal range (1.4 - 3.8 ng/ml) in 50% of the men and women with bone metastasis and these values were higher ($p<.0001$) than those of the control group. However, there is some debate on whether osteocalcin is a marker of bone formation or an indicator of bone matrix metabolism or turnover. When the formation and resorption processes are considered together, osteocalcin may prove to be an excellent indicator of bone turnover. However, when these two processes are uncoupled, osteocalcin is considered a marker of bone formation. Duda et al. (1988) evaluated osteocalcin and alkaline phosphatase (an indicator of global bone formation activity [B-ALP]) in several patients with metabolic disorders and in normal controls. Results from this study concluded that osteocalcin and B-ALP acted differently in patients with glucocorticoid excess, Paget's disease, chronic renal failure and osteolytic metastasis. This difference suggests that these markers are indicative of different types of changes in osteoblastic

function in which osteocalcin may reflect overall bone turnover rather than bone formation exclusively.

A commercially available radioimmunoassay kit for measurement of human osteocalcin (Osteocalcin Radioimmunoassay Kit, Incstar Co, Stillwater, MN.) has been validated for use in horses (Hope et al., 1993). Recovery of bovine osteocalcin standard added to equine serum was consistent over several trials, linear over dilutions of 1:2 to 1:32, and consistent with cross-reactivity data previously reported by Patterson-Allen et al. (1982) in which horse serum showed dose-response curves parallel to the bovine protein standard. The serum osteocalcin concentrations in these horses also were similar to previously reported values (2-20 ng/ml) (Lepage et al., 1990 and Lepage et al., 1991). Serum levels of osteocalcin have been found to exhibit a biphasic circadian pattern in which levels remained fairly constant during the day and fluctuated significantly at night (Lepage et al., 1991). Similar results were reported in humans which found that osteocalcin follows a circadian rhythm in which levels fall during the morning, rise in the afternoon and reach a peak at night (Gundberg et al., 1985). In contrast, horses housed under continuous lighting experienced no significant changes in serum BGP concentration over a 24 hour period (Hope et al., 1993). Osteocalcin levels also vary with age. Delmas et al. (1986) demonstrated that serum osteocalcin levels paralleled growth velocity in children aged 2-19 years. A significant inverse correlation (r^2=.75, $p<.01$) has been found between age and serum BGP in Standardbred horses (Lepage et al., 1990). Horses less than one year and 1.5 to 2.5 years old had higher mean osteocalcin concentrations (47.3 and 35.7 ng/ml, respectively) than those that were older than 3.5 years (6.7 ng/ml).

Similar results were reported previously in humans in which osteocalcin levels declined to normal ranges in children once puberty was completed (Kruse and Kracht, 1986). These data suggest that bone formation rate may be reduced as an animal reaches adulthood.

The physiological processes involved in bone metabolism and the hormones controlling bone formation and turnover rate are extremely intricate in their functions and require continued research in order to fully understand these biological mechanisms. Osteocalcin, which has not yet been evaluated in response to changes in dietary cation-anion difference, may prove to be a valuable index of the dynamic changes bone undergoes during growth and dietary-induced metabolic alterations. The objectives of this study were to evaluate the effects of DCAD on bone metabolism by monitoring changes in serum osteocalcin concentration and correlating this information with skeletal growth data.

CHAPTER III

EVALUATION OF BONE MINERAL CONTENT IN EQUINE CADAVERS AND PREGNANT MARES

INTRODUCTION

Due to its dynamic nature, bone tissue is capable of exchanging certain ions, such as calcium and phosphorus, in order to maintain homeostatic circulating blood levels. Because of this biological regulation, metabolic and nutritionally induced bone disorders could be of concern, especially in the young growing horse. Bone composition and the mechanism of controlling mineral deposition and resorption have long been studied in other species (Duckworth and Hill, 1953; Field et al., 1974; Taylor et al., 1960; Weidman and Rogers, 1950; Ellinger et al., 1952). However, few reports have been published which evaluate bone mineral content (BMC) or quantify specific minerals in equine bone at various sites. Researchers have employed the use of photon absorptiometry in order to measure BMC in both equine and bovine species (Jeffcott et al., 1986; Zetterholm and Dalen, 1978). More recently, biopsy techniques have been used for determination of BMC in other species (Bobilya et al., 1991; Breur et al., 1988; Combs et al., 1991). Misheff et al. (1992) described a procedure in which unicortical and transcortical biopsy

specimens were taken from the rib of standing horses for histologic and histomorphometric evaluation. This technique proved to have excellent success and could have potential benefits in diagnosing metabolic and nutritional bone disease. Therefore, the purpose of this study was to determine the bone composition of the twelfth rib and compare the BMC between the rib and third metacarpal of the horse.

MATERIALS AND METHODS

Experiment I

Twenty fresh equine cadavers of Quarter Horse breeding were used to evaluate bone mineral status in the 12th rib and third metacarpal. These animals were being kept at the Oklahoma Animal Disease and Diagnostic Laboratory for postmortem evaluation following death by natural causes. Bone biopsy procedures were performed approximately 12 h postmortem in three stallions, seven geldings and ten mares ranging in age from 1 to 20 years old. Unicortical samples were taken on the left side at the midpoint of the diaphysis of the third metacarpal (cannon bone) and the twelfth rib. Biopsy samples were obtained using a 12mm internal diameter Galt stainless steel trephine which was attached to a power drill. Specimens were immediately weighed, placed in freezer bags, and stored at -20°C until chemical analysis could be performed.

Upon thawing, samples were dried to a constant weight at 100°C. Fat was removed from each biopsy sample by washing with petroleum ether. Extracted samples

were then returned to the oven and redried. The weight of the dry, fat-free bone was taken and the sample was ashed at 500°C for 12 hours and allowed to cool. Weight of the cooled, ashed sample was determined after which ash weight as a percentage of wet weight (AWW%) and dry, fat-free ash percentage (FFA%; ash weight / extracted weight) were calculated. Bone samples were ground to the consistency of flour using a mortar and pestle to achieve homogeneity. An aliquot of ash weighing .1g was then dissolved in concentrated HCL and diluted with distilled deionized water prior to analysis of calcium (Ca) and phosphorus (P). Calcium was determined using flame atomic absorption spectroscopy (Oklahoma Disease and Diagnostic Laboratory) and phosphorus was measured using a colorimetric test (Kodak Ektachem Clinical Chemistry Slides PHOS, OSU Veterinary Medicine Clinical Pathology Laboratory). Least squares means were calculated and data were analyzed using the general linear model procedure of SAS (1985). The one-way analysis of variance used to evaluate ash, calcium and phosphorus percentages is given in Table I.

Experiment II

Ten pregnant Quarter Horse mares, which were being used to study the influence of restricted movement on physical fitness, were simultaneously utilized to evaluate bone mineral status. Mares were blocked by age and expected foaling date and then randomly allotted to either the control or the treatment group (5 mares/group). Treatment mares were housed in 4' x 8' tie stalls and allowed no exercise. Control mares were kept on native prairiegrass pasture at a stocking rate of 6 acres/mare. All mares were fed a 15%

CP pelleted ration on a body weight basis (Table III). Mares on pasture were fed free choice prairiegrass hay while mares in the stalls were fed hay at 1% of their body weight. Bone biopsies were taken from the twelfth rib on day 0 and day 90 from the left and right sides, respectively. Mares were restrained in standing stocks and sedated with xylazine (300 mg intravenously). An area over the twelfth rib, approximately 20 x 20 cm^2, was clipped and scrubbed for surgery. Lidocaine was administered (30 ml subcutaneously) both anterior and dorsal to the incision site to form an inverted "L" block. A skin incision was made over the rib in the center of the area which had been aseptically prepared. The subcutis and cutaneous trunci muscles were retracted to expose the rib after which the periosteum was scraped away. A 12mm Galt trephine was centered on the rib and a unicortical biopsy was taken by boring through the lateral cortex and into the medullary cavity. Following extraction, the samples were immediately weighed, placed in freezer bags, and stored at -20°C until chemical analysis could be performed. Biopsy samples were prepared and analyzed as described in Experiment I. Following the surgical procedure, mares were treated with 30ml of Penicillin-G twice daily and administered 2g of phenylbutazone orally once a day for five days. Analysis of variance appropriate for a split-plot design was used to evaluate the percentages of calcium, phosphorus and ash (Table II). Least squares means were calculated and tested for significance using the pdiff procedure of SAS (1985).

TABLE I. ANALYSIS OF VARIANCE TABLE USED TO TEST THE MAIN EFFECTS OF SITE AND SEX ON ASH, CALCIUM AND PHOSPHORUS PERCENTAGES IN CADAVERS.

Source	Degrees of Freedom
Site	1
Sex	1
Site x Sex	1
Residual	36

TABLE II. ANALYSIS OF VARIANCE TABLE USED TO TEST THE MAIN EFFECTS OF HORSE, TREATMENT AND TIME ON ASH, CALCIUM AND PHOSPHORUS PERCENTAGES IN PREGNANT MARES.

Source	Degrees of Freedom
Treatment	1
Horse (Treatment)	8
Time	1
Treatment x Time	1
Residual	8

RESULTS AND DISCUSSION

Experiment I

Mean biopsy ash weight as a percentage of wet weight (AWW%) and dry, fat-free ash percentage (DFF%, ash weight/fat free weight) for the rib and cannon bone are given in Table IV. The dry, fat-free ash ranged from 59-70%, which is similar to values from metacarpal bones (50-65%) in horses ranging in age from 1-30 years (El Shorafa et al., 1979). These values are also comparable to adult bone on a dry weight basis (Swenson and Reece, 1993), rib bone in cattle (Little, 1972) and whole bovine, porcine and ovine bones removed from animals of different ages (Field et al., 1974).

Both AWW% and DFF% were higher in the third metacarpal than in the rib ($P<.01$). Field et al. (1974) indicated that dry, fat-free ash percentages were significantly higher in the femur versus the rib in cattle, sheep and pigs. Cox and Balloun (1971) further demonstrated that the DFF% varied considerably among several individual bones in two lines of laying hens. Mean percentage ash values in the previous study ranged from 52 for the tarsus bones to 64 for the humerus, with the rib and femur measuring approximately 57.

Percent calcium was similar between biopsy sites while phosphorus levels were significantly higher in the cannon compared to the rib (Table IV). Baker et al. (1946) found no notable difference in the calcium content of the human femur and ribs. Correspondingly, calcium in bone ash was shown not to differ ($P>.05$) between the vertebrae, rib and femur in other species (Field et al., 1974). In laying hens, calcium levels

were similar among fifteen different bones including the metacarpal, rib and femur (Taylor et al., 1960).

The difference in percent ash and phosphorus between the metacarpal and the rib may be due to the composition of the individual bones. The diaphysis of the long bones is comprised of compact cortical bone surrounding the medullary cavity which contains the spongy or cancellous bone. The epiphyseal and metaphyseal regions of long bones are composed primarily of cancellous tissue made up of small particles of bone called trabeculae. The flat bones consist mainly of spongy bone surrounded by a cortex of compact bone. Hill et al. (1962) determined that bones containing a higher ratio of cancellous tissue to compact bone are more readily resorbed, therefore resulting in a lower percentage of ash in ewes. In lactating ewes, it has been suggested that the spongy bones are more sensitive to resorption than the rest of the skeleton and that compact bone is extensively resorbed only during severe calcium deficiency (Benzie et al., 1955). The previous study found that ewes receiving a daily calcium supplement had a significantly higher percent ash in the ribs and vertebrae than those which did not receive the supplement. However, no variation in the percentage of ash was discovered in the metacarpal mid-shaft between the groups. These findings would indicate that the ewes receiving the calcium deficient diet are primarily resorbing mineral from the bones containing a large proportion of cancellous tissue. Further research has suggested that the rib bones and vertebrae are the most responsive to dietary changes in calcium and are more desirable indicators of calcium status than the more compact bones (Hendrikson, 1968; Walker et al., 1993). In lactating ewes fed a diet low in P, vertebral bones were the

most readily affected by resorption as well as being the first to be repaired (Benzie et al., 1959). Benzie et al. (1959) confirmed that the cervical vertebrae lost 50% of the original ash, which was recovered shortly thereafter. However, the shafts of the metacarpals lost about 15% and made no recovery by the end of the experiment.

Resorption and repair of the skeleton may also vary greatly along the length of the long bones. When radioactive calcium was studied, its uptake per gram of ash was the highest in regions of spongy bone and lowest in the compact tissue (Armstrong and Barnum, 1948). Over time, ^{45}Ca was redistributed equally throughout the bone so that the epiphyseal regions and vertebrae contained the same amounts as the compact bone. In gestating and lactating rats fed varying levels of dietary calcium, there was only a slight decrease in percent ash in the shafts of the femur and tibia (compact bone) while the head and metaphysis (cancellous bone) exhibited marked depressions (Ellinger et al., 1952). Likewise, femoral bones from the cat, rabbit and rat yielded lower ($P<.01$) Ca and P levels in the cancellous bone versus the cortical tissue. These findings are further substantiated through the use of radio-active phosphorus (^{32}P). The uptake of ^{32}P is much greater in bone surrounding the Haversian canals than in the more compact bone in both adult humans and animals (Duckworth and Hill, 1953).

Experiment II

The restriction of exercise appeared to have no effect on rib bone mineral content as the percentage of ash, calcium and phosphorus was similar ($P>.05$) between the two groups (Table V). Research has shown that laying hens housed in cages often develop brittle and weak bones more often than hens maintained in floor pens (Rowland and

Harms, 1970; Rowland et al., 1968). Furthermore, layers kept on litter floors had higher tibia and humerus breaking strength and percent bone ash than birds maintained in cages (Meyer and Sunde, 1974). In contrast, broilers and laying hens reared in cages and floor pens exhibited no significant difference in percent ash of the tibia (Bond et al., 1991; Rowland et al., 1972).

The role of exercise in preventing bone loss in humans has long been an area of speculation among researchers. There is some support however, for the concept that disuse results in bone mass loss and that sedentary individuals have less bone mass than those that exercise. Chestnut et al. (1993) compiled several studies demonstrating that exercising women had higher lumbar bone mineral density (BMD) than sedentary individuals. Others have detected no difference between exercising and sedentary women in lumbar spine BMD (Kirk et al., 1989) and no significant increase in spinal BMD in exercising women (Nelson et al., 1991). Furthermore, Sidney et al. (1977) determined that exercise did not increase bone mineral content as calcium showed no change with training.

Concerning animals, prolonged exercise in the rat had positive effects on mineral density in the femur but not the rib in which bone loss was induced by metabolic acidosis (Myburgh et al., 1989). In older female rats (25 mo) and mature mice, bone mass and mineral content was higher in the exercised versus the sedentary animals (Bell et al., 1980; McDonald et al., 1986). McDonald et al. (1986) further demonstrated that exercising female rats (7,14 and 19 mo age groups) had a significantly higher concentration of calcium and greater ^{45}Ca uptake in the femur and humerus as compared with their

respective controls. It is also interesting to note that exercise in the younger rats (7 and 14 mo) increased mineralization of only the weight bearing bones (femur and humerus) while the older animals (19 mo) experienced an additional increase in bone mineral content of the rib in response to exercise. These results differ from Barengolts et al. (1993) who noted that treadmill exercise had no effect on ash weight of the femur, tibia or vertebra in rats. Raab et al. (1991) further supported these findings as no differences were detected in bone mineral content of the femur between exercising and sedentary sows.

SUMMARY AND CONCLUSIONS

In conclusion, the biopsy technique described in experiment II may be helpful in diagnosing metabolic and nutritionally induced bone disorders. Results from these two experiments also indicate that bone biopsies taken from the rib could prove to be a useful diagnostic tool in evaluating the calcium status in the horse as calcium levels were similar between cancellous and compact tissue. Further research is needed however, to determine the correlation between mineral levels in the rib and cannon bone in exercising and sedentary horses. This information coupled with a simple biopsy technique could prove useful in allowing researchers to draw conclusions concerning mineral metabolism without sacrificing the animal.

TABLE III. DIET COMPOSITION

Ingredient	**% Diet DM**	**Nutrient**	**% Diet DM**
Corn	66.95	CP%[a]	15.00
Soybean Meal	15.00	DE[b]	3.40
Cottonseed Hulls	16.05	Ca%	.52
TM Salt	.49	P%	.45
Limestone	.79	Mg%	.18
Dical Phosphate	.72	K%	.65
		S%	.16
		Na%	.22
		Cl%	.36

[a] CP= Crude Protein
[b] DE= Digestible Energy (Mcal/kg)

TABLE IV. ASH, CALCIUM AND PHOSPHORUS PERCENTAGES OF THE TWELFTH RIB AND THIRD METACARPAL IN EQUINE CADAVERS[a]

	Biopsy Site		
Item	**Metacarpal**	**Rib**	**S.E.M.**
AWW[b]	65.12[d]	52.73[e]	.64
DFF[c]	69.54[d]	63.05[e]	.40
Calcium	42.40	42.00	1.09
Phosphorus	14.70[d]	13.14[e]	.12

[a] Values are least squares means.

[b] AWW= Ash as a percent of wet weight.

[c] DFF= Dry, fat-free ash percentage.

[d,e] Means within rows with different superscripts differ ($P<.01$).

TABLE V. ASH, CALCIUM AND PHOSPHORUS PERCENTAGES OF THE TWELFTH RIB IN MARES[a]

Item[b]	Control	Treatment	S.EM.
Ash	38.6	40.9	2.6
Calcium	39.7	36.2	1.8
Phosphorus	20.6	20.0	1.2

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

CHAPTER IV

EFFECTS OF DIETARY CATION-ANION DIFFERENCE ON MINERAL BALANCE, SERUM OSTEOCALCIN AND GROWTH IN WEANLING HORSES

INTRODUCTION

In recent years, the effects of dietary cation-anion difference on acid-base status, growth performance, mineral balance and bone formation have been evaluated in horses, swine, poultry and dairy cattle. Baker et al. (1992) demonstrated that horses consuming highly anionic diets underwent a nutritionally-induced metabolic acidosis as witnessed by a decrease in blood pH, P_{CO2} and HCO_3^-. Furthermore, research has indicated that horses consuming diets low in DCAD may be in a negative calcium balance due to an increased urinary excretion of calcium (Wall et al., 1991; Baker et al., 1993; 1997). These findings suggest that this condition, if prolonged, could lead to an osteoporotic weakening of the skeletal system as seen in poultry and swine (Austice, 1984; Edwards, 1984; Halley et al., 1987; Patience et al., 1987). This increase in calcium excretion could in turn raise the parathyroid hormone (PTH) activity resulting in an increase in calcium absorption from the intestine and resorption from the bone. Goff et al. (1991) found that osteoclastic bone resorption was more responsive to PTH as plasma hydroxyproline

concentration (an index of calcium resorption activity) was higher in dairy cows fed a low DCAD diet as compared to those fed a diet high in DCAD. However, the use of PTH and hydroxyproline measurements to assess skeletal mineralization in the horse is limited since an assay has not yet been validated in the horse to accurately quantify these parameters. An alternative biological marker that has been used as an index of bone formation is osteocalcin. This is a bone specific protein which has been validated for use in horses (Hope et al., 1993). Serum osteocalcin concentration may indicate the status of osteoid production and has been shown to be greater in diseases characterized by increased bone turnover (Swenson and Reece, 1993).

Growing horses have a high degree of stress placed on the skeletal system especially, young racehorses which often begin training early in their two-year old year. Therefore, there is an enormous potential for skeletal injury, and in today's competitive horse industry, proper nutrition is imperative to optimize the full genetic potential of the animal. If manipulating the DCAD could be shown to improve calcium balance or retention, then skeletal demineralization may be minimized using this dietary approach. However, a recent study in weanlings has found that horses consuming diets low in DCAD actually had higher calcium balances than those fed diets high in DCAD despite an increased urinary excretion of calcium (Wall et al., 1997). It appears that these horses were able to compensate for the increased urinary excretion by enhancing intestinal calcium absorption. The major difference between this study and the others previously mentioned is that these weanlings were fed over a period of six months where the others were evaluated only in the short term. Furthermore, Wall et al. (1997) found no

difference between treatments in body weight gain or any of the skeletal growth parameters measured. These results suggest that animal performance and mineral metabolism may not be adversely affected by DCAD. Therefore, the objectives of the present were to evaluate the long term effects of DCAD on growth, mineral metabolism and serum osteocalcin in an attempt to verify previous findings and quantify changes in bone mineralization.

MATERIALS AND METHODS

Experimental Design and Treatments

Sixteen Quarter Horse weanlings were used in a split plot design experiment to determine the effects of dietary cation-anion difference (DCAD) on mineral metabolism, dry matter digestibility, urine pH, serum osteocalcin levels and growth. Horses were blocked by age and sex and then randomly allotted to the two treatment groups (High or Low) with four fillies and four colts per treatment. The weanlings were pair weaned at 120 days of age, broke to lead and then housed in individual 12' x 12' box stalls. During the 30 days between weaning and 150 days of age, the weanlings were halter broke, brushed, and fitted with urine harnesses so that they could be handled with minimal stress during the trial. The 25 week trial consisted of three 72 h collection periods at 150, 240 and 330 days of age and weekly growth data was recorded on hip, hock, knee, shoulder, and wither height, heart girth circumference and body weight. All horses received a minimum of 3 h of free exercise per day in a 60'x 60' round pen. Horses were

fed approximately 2.0% of their body weight per day in total ration, which was divided between two feedings (7 a.m. and 5 p.m.), and allowed free access to water. Daily feed intake was monitored by measuring refusals after both feedings. Prior to and throughout the trial, horses were immunized, dewormed and given routine health care.

The diets consisted of a pelleted concentrate of corn, soybean meal and cottonseed hulls produced at the Oklahoma State University Feedmill. The concentrate was fed in a 70:30 ratio with native prairiegrass hay. Experimental diets with a DCAD of +325 (High, H) and -52 (Low, L) were formed by supplementing diet H with sodium bicarbonate and diet L with calcium chloride (Table VI). The DCAD of the diets was calculated as meq (Na + K) - (Cl + S)/ kg of diet DM. Rations were formulated to contain equivalent amounts of digestible energy (DE=2.9 Mcal/kg DM) and crude protein (CP=14.5%) across treatments . Diets were analyzed and found to contain approximately equal amounts of DE, CP, calcium, phosphorus, magnesium and sulfur (Table VII).

Growth Measurements

I. Wither height. The vertical distance from the ground to the highest protruding thoracic vertebra in centimeters (cm).

II. Hip height. The vertical distance from the coronary band on the posterior side of the hoof to the furthest protruding point of the buttocks in cm.

III. Hock height. The vertical distance from the coronary band on the posterior side of the hoof to the point of the hock (Tubercalcis) in cm.

TABLE VI. COMPOSITION OF TREATMENT DIETS, AS FED BASIS

	Treatment			
Ingredient (%)	High	High Cr_2O_3	Low	Low Cr_2O_3
Ground Corn	40.15	40.00	40.35	40.20
Soybean Meal	16.55	16.55	17.05	17.05
Cottonseed Hulls	10.00	10.00	10.70	10.70
TM Salt	.50	.50	.50	.50
Limestone	.80	.80	---	---
Dicalcium Phosphate	.50	.50	.30	.30
Sodium Bicarbonate	1.50	1.50	---	---
Calcium Chloride	---	---	1.10	1.10
Chromic Oxide	---	.15	---	.15
Prairie Grass Hay	30.00	30.00	30.00	30.00
Total	100.00	100.00	100.00	100.00
DCAD	**+325**		**-52**	

TABLE VII. DIET ANALYSIS (DRY MATTER BASIS)

	Treatment	
Item	**High**	**Low**
DE, Mcal/kg	2.77	2.81
CP%	15.22	15.71
Ca%	.74	.76
P%	.40	.43
Mg%	.18	.20
Na%	.69	.24
K%	.97	1.01
Cl%	.46	1.14
S%	.15	.15

IV. Shoulder height. The vertical distance from the coronary band on the anterior side of the hoof to the point of the shoulder in cm.

V. Knee height. The vertical distance from the coronary band on the anterior side of the hoof to the end of the distal radius in cm.

VI. Body weight. Weight determined at a single weighing, 6 hours before the morning feeding, and recorded to the nearest pound.

VII. Heart girth. The circumference of the thorax immediately posterior to the front leg in cm.

Urine and Fecal Collection

Total urine production was collected via urine harnesses from the fillies (Figure 1) and the colts (Figure 2) every 4 hours for 72 hours post-feeding. A 50 ml sample was immediately analyzed for pH using a Fisher Accumet Model 950 pH meter which was standardized prior to each four hour collection. A representative sample of 4% of the total volume was composited over time for each horse and time period. These composite samples were frozen until mineral analysis could be performed.

Multiple fecal grab samples were taken for 72 hours to represent every 2 hours post-feeding during each collection period. Chromic oxide (Cr_2O_3) was added at 2% of the diet as an indigestible marker for the determination of fecal volume. Grab samples were immediately frozen for later mineral analysis.

Urinary Mineral and pH Analysis

For the analysis of calcium, phosphorus and magnesium, 30 ml aliquots were taken from the composite samples and acidified with .5 ml of HCL. These samples were

Figure 1. Weanling filly fitted with a urine collection harness.

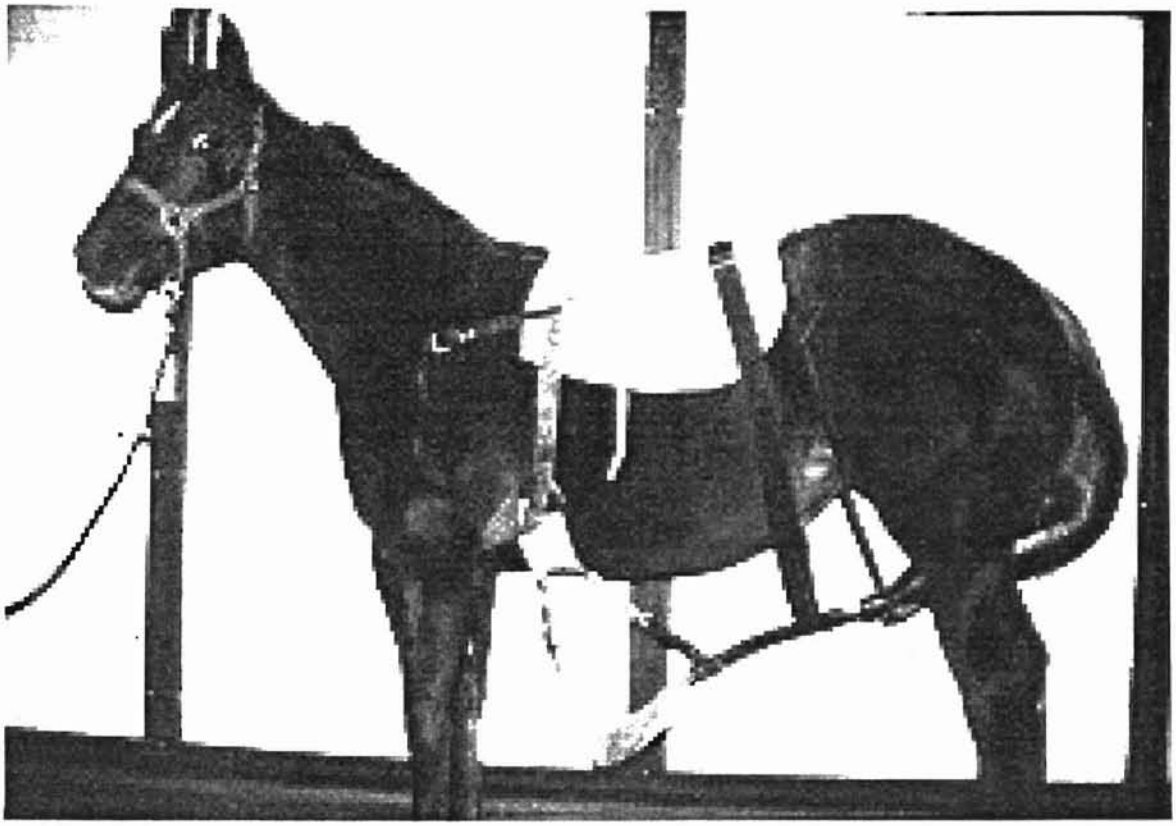
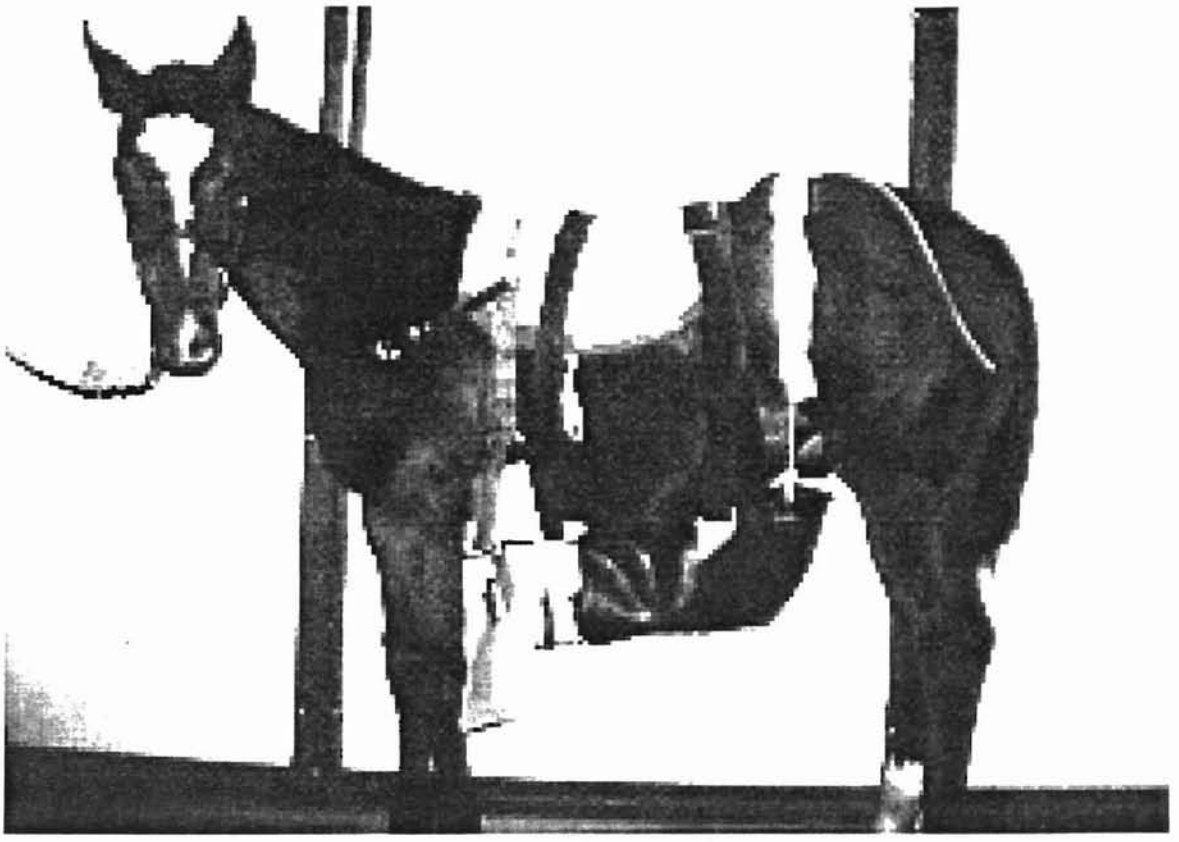


Figure 2. Weanling colt fitted with a urine collection harness.



then analyzed using an Ektachem 700 analyzer (Kodak Ektachem, Model 700, Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY.) and read at 680 nm. Urine sodium, potassium and chloride concentration was determined by analyzing 30 ml aliquots from the composite samples on a Beckman System E4A Electrolyte Analyzer (Beckman Institute, Brea, CA 92621). Urine pH was analyzed using a Fischer Accumet Model 950 pH meter.

Feed and Fecal Mineral Analysis

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

Chromium Analysis

For the analysis of fecal and feed chromium, 1 gram of sample was weighed out into pre-dried beakers, dried at 60° C for 24 hours and then weighed again to determine a final dry weight. Samples were ashed in a muffle furnace at 500° C for 6 hours then

weighed again to determine ash weight. To the ashed sample, 3 ml of solution A (30 ml $MnSO_4$ + 1 liter Phosphoric acid 85%).and 4 ml of $KBrO_3$ were added and then digested on a plate until the solution turned purple in color. The mixture was transferred to a 100 ml volumetric flask and 12.5 ml of $CaCl_2$ were added. The solution was allowed to set for 1 hour in order for the solutes to settle to the bottom of the flask. A 1 ml sample was pipetted off the top of the flask and diluted 1:6 in water. Chromium concentration was determined using an atomic absorption spectrophotometer (Model 4000, Perkin-Elmer Corp., Norwalk, CT).

Blood Collection and Analysis

Venous blood samples were drawn the day before, and the first day of each collection period at 8:00 a.m. using plain glass Vacutainers®. Samples were allowed to clot at room temperature and then centrifuged for 20 minutes at 2,500 rpm after which the serum was removed and frozen for subsequent analysis of osteocalcin. Serum osteocalcin concentration was determined using a commercially available radioimmunoassay kit for measurement of human osteocalcin (INCstar, Stillwater, MN). This kit was previously validated for use in horses (Hope et al., 1993) in which assay sensitivity was .16 ng/ml and recovery of bovine osteocalcin standard was linear. Hope et al. 1993 also documented dilutional parallelism by assaying pooled equine serum at 4 dilutions and correcting the mean result for dilution. Following preparation, all samples were read on a Cobra II Auto-Gamma Counter (Packard Instrument Co., Meriden, CT 06450). Each sample was run in duplicate and any duplicates with a coefficient of

variation over 15% were re-analyzed. In order to determine if there were circadian variations in serum osteocalcin levels, two 24 hour collections were conducted with 3 of the weanlings at 5 months of age in July and 5 of the weanlings at 11 months of age in March. Venous blood samples were taken under natural lighting conditions every 3 hours for 24 hours via 14 gauge indwelling jugular catheters starting at 7 a.m. Approximately 3ml of Lidocain were administered subcutaneously at the puncture site prior to placement of the catheters. On average, the sunrise and sunset during July was 6:00 a.m. and 9:00 p.m., respectively. During March, sunrise was at 6:30 a.m. and sunset at 6:30 p.m. Blood was prepared and analyzed as previously mentioned above. Mean intra-assay and inter-assay coefficients of variation (CV) across the four kits used in this study were 2.32% and 4.53%, respectively.

Statistical Analysis

All data were analyzed by analysis of variance appropriate for a split plot design experiment according to the SAS User's Guide (1985). A minimum significance level was declared at $P<.05$. Data for urine pH, growth parameters and osteocalcin concentration were analyzed using a general linear models procedure for repeated measures with time as the repeated variable (Table VIII). Least squares means over time were then calculated within a period and orthogonal contrasts were used to detect differences between treatment means. Data for mineral balances, dry matter digestibilities, and urine volume were also analyzed using the general linear models procedure and least squares means were calculated for each parameter within a period (Table IX). Orthogonal contrasts were then used to test for differences between treatment means. Polynomial regression

analysis was performed on all growth and osteocalcin data in order to determine best fit models over time for each parameter measured (Table X). Indicator variables (dummy variables) were used to determine differences in intercepts and slopes between treatments (Table XI). The response function for the fitted regression model is given by the following equations:

$$Y= \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \varepsilon$$

X_1= Age

X_2= 1 if High Diet
0 if Low Diet

Low Diet⇒ $Y= \beta_0 + \beta_1 X_1$

High Diet⇒ $Y= (\beta_0 + \beta_2) + (\beta_1 + \beta_3) X_1$

β_2⇒ Indicates how much greater or smaller the Y-intercept of the response function is for the treatment coded 1 than the treatment coded 0.

β_3⇒ Indicates how much greater or smaller the slope of the response function is for the treatment coded 1 than the treatment coded 0.

TABLE VIII. ANALYSIS OF VARIANCE TABLE USED TO TEST THE MAIN EFFECTS OF HORSE, TREATMENT, SEX AND PERIOD ON GROWTH PARAMETERS, URINE PH AND OSTEOCALCIN CONCENTRATION OVER TIME.

Source	Degrees of Freedom
Treatment	1
Sex	1
Treatment x Sex	1
Horse x Treatment x Sex	12
Time	1
Treatment x Time	1
Time x Sex	1
Period	2
Treatment x Period	2
Time x Period	2
Sex x Period	2
Treatment x Time x Period	2
Residual	63

TABLE IX. ANALYSIS OF VARIANCE TABLE USED TO TEST THE MAIN EFFECTS OF HORSE, TREATMENT AND PERIOD ON FECAL AND URINARY PARAMETERS.

Source	Degrees of Freedom
Treatment	1
Sex	1
Treatment x Sex	1
Horse x Treatment x Sex	12
Period	2
Period x Sex	2
Treatment x Period	2
Residual	24

TABLE X. POLYNOMIAL REGRESSION ANALYSIS TABLE USED TO DETERMINE BEST FIT MODELS OVER TIME FOR THE PARAMETERS OF GROWTH.

Source	Degrees of Freedom
Treatment	1
Horse (Treatment)	14
Age (Treatment)	2
Age x Age (Treatment)	2
Residual	373

TABLE XI. REGRESSION ANALYSIS TABLE USED TO DETECT DIFFERENCES IN INTERCEPT AND SLOPE BETWEEN TREATMENTS FOR THE PARAMETERS OF GROWTH.

Source	Degrees of Freedom
Age	1
Dummy	1
Age x Dummy	1
Residual	46

RESULTS AND DISCUSSION

DM Intake, Digestibility and Fecal Output

The effect of DCAD on dry matter digestibility, dry matter intake (DMI) and daily fecal output is shown in Table XII. Values for dry matter digestibility ranged from 46.18 to 50.51% on the low diet and 48.24 to 52.97% on the high diet across all periods. This data is similar to that of Baker et al. (1997) who reported DM digestibility values of 47.69% and 50.08% for horses fed low and high DCAD diets, respectively. At 150, 240 and 330 days of age, no difference ($P>.05$) was detected in dry matter intake in weanlings consuming diets H and L. Fecal output was higher ($P<.05$) in horses on diet L (2029 g/d) than those on diet H (1928 g/d) at 150 days of age. This increased output resulted in a decreased DM digestibility for diet L (50.51%) compared to diet H (52.97%). These responses were similar at 240 days of age in which horses consuming diet L had a higher ($P<.05$) fecal output (3268 g/d vs. 3143 g/d) and lower DM digestibility (46.18% vs. 48.23%) than those on diet H. At 330 days of age, no difference in fecal output or DM digestibility was found between treatments H and L. The changes in fecal output and digestibility observed at 150 and 240 days of age agree with Wall et al. (1993) who reported an increase ($P<.05$) in fecal output and a subsequent decrease in DM digestibility in exercising horses consuming a low DCAD diet versus those fed a diet high in DCAD. These findings are also consistent with Haydon and West (1990) who found that an increase in DCAD from -50 to 400 meq/kg diet DM resulted in a linear increase in apparent dry matter digestibility in growing pigs. In contrast, Baker et al. (1997)

observed no significant differences in dry matter digestibility or fecal output between horses consuming diets high (+409) and low (0) in DCAD. Wall et al. (1997) further demonstrated that growing horses fed diets low (-35) and high (+350) in DCAD had similar ($P>.05$) fecal outputs and dry matter digestibilities at 240 and 360 days of age. Differences found in fecal output and DM digestibility may be due to the acidic nature of the diet low in DCAD which resulted in a lower intestinal pH. The activity of many digestive enzymes in the intestine has an optimal pH range of 6.5 to 7.5 (Wall et al., 1993) while protease enzyme activity in sheep is not optimized until pH is >7.5 (Ben-Ghedalia et al., 1974). As digesta enters the duodenum, its pH slowly increases as it moves through the intestine. Church (1988) has reported that many sources of supplemental protein have an isoelectric point (pH of greatest protein solubility) near or above 7.0 and that plant source proteins are affected much more by pH than animal source proteins. Furthermore, a decrease in intestinal pH may inhibit the microbial population (cellulolytic bacteria) and reduce protozoa numbers in the hindgut which would decrease degradation of fiber. Acidity can also decrease the availability of nutrients for microbes (i.e. Cellulolytic bacteria require bicarbonate) and can alter the ionic state of other nutrients thereby changing their availability to microbes (Church, 1988). Additionally, a prolonged exposure to low pH may result in ulceration of the gastric mucosa. If feeding a diet low in DCAD decreases the pH of the digestive tract, then digestion and absorption of feedstuffs could be reduced which would increase fecal output and decrease digestibility.

TABLE XII. EFFECT OF DCAD ON DAILY DM INTAKE, FECAL OUTPUT AND DRY MATTER DIGESTIBILITY[a]

	Treatment		
Item	**High**	**Low**	**S.E.M.**
150 DAYS OF AGE			
DM Intake g/d	4193.18	4005.90	140.65
Fecal Output g/d	1928.04[b]	2029.20[c]	27.24
DM Digestibility %	52.97[b]	50.51[c]	.48
240 DAYS OF AGE			
DM Intake g/d	6177.91	5963.78	140.65
Fecal Output g/d	3143.65[b]	3268.44[c]	27.24
DM Digestibility %	48.24[b]	46.18[c]	.48
330 DAYS OF AGE			
DM Intake g/d	6321.73	6439.24	156.46
Fecal Output g/d	3198.88	3273.11	30.30
DM Digestibility %	50.15	48.87	.53

[a] Values are least squares means ± SE.

[b,c] Means within a row with different superscripts differ (P<.05).

Urine Volume and pH

Data on daily urine output and mean urine pH are given in Table XIII. Urine volume was similar ($P>.05$) between treatments at 150, 240 and 330 days of age. This agrees with results in mature exercising horses in which no difference in urinary output was observed between diets high and low in dietary electrolyte balance (Topliff et al., 1989). At 150 days of age, mean urine pH was lower ($P<.01$) in weanlings on diet L (5.71) than on diet H (8.39). This result was consistent at 240 and 330 days of age as mean values for urine pH remained significantly reduced in horses fed the low diet versus those receiving the high. Related studies have also shown that mean urine pH was lower ($P<.05$) in horses (Popplewell et al., 1993, Wall et al., 1992) and cows (Escabosa et al., 1984) fed rations low in DCAD as opposed to those consuming diets high in DCAD. Similarly, urine pH in horses (Baker et al., 1992) and dairy cattle (Tucker et al., 1988) has been shown to increase linearly with increasing DCAD. The increase in urine pH noted in the present study as well as the previously mentioned studies is probably due to increased bicarbonate excretion from the addition of $NaHCO_3$ to the high DCAD diets. The decrease in urinary pH may be attributed to excretion by the kidney of excess chloride ions. In a state of chronic metabolic acidosis, chloride is filtered from the blood and combines with hydrogen ions in the tubule lumen. This HCl then binds with ammonia from the breakdown of glutamic acid in the tubule cell and is excreted as ammonium chloride (NH_4Cl). The simultaneous excretion of hydrogen with chloride results in a decrease in urinary pH.

TABLE XIII. EFFECT OF DCAD ON URINE VOLUME AND pH[a]

	Treatment		
Item	**High**	**Low**	**S.E.M.**
150 DAYS OF AGE			
Volume, ml/d	4768.25	3781.88	249.09
pH	8.39[b]	5.71[c]	.21
240 DAYS OF AGE			
Volume, ml/d	4732.25	4724.75	249.09
pH	8.63[b]	7.04[c]	.21
330 DAYS OF AGE			
Volume, ml/d	4754.00	4927.73	277.08
pH	8.69[b]	7.08[c]	.24

[a] Values are least squares means ± SE.

[b,c] Means within a row with different superscripts differ (P<.01).

Mineral Balance

The effect of DCAD on daily urinary mineral intake, excretion and balance is shown in Tables XIV, XV and XVI. At 150 days of age, the increase ($P<.05$) in urinary sodium excretion from 10.26 g/d for diet L to 17.87 g/d for diet H corresponded to higher ($P<.05$) sodium intakes of horses consuming diet H (28.63 g/d) versus diet L (9.61). No difference in fecal sodium excretion was observed between treatments during any period. Those horses consuming diet H retained more ($P<.05$) sodium (7.52 g/d) than those consuming diet L (- 2.52) as the increased urinary excretion did not fully compensate for the increased intake. These results were consistent at 240 and 330 days of age where sodium intake, urinary excretion and balance were higher ($P<.05$) in weanlings fed diet H as opposed to diet L. In a related study, Wall et al. (1997) found that higher sodium intakes in weanlings consuming diets high in DCAD (+ 353) resulted in increased ($P<.05$) urine and fecal sodium excretion compared to diets low in DCAD (-35). However, the increased urine and fecal excretion did not offset increased intake as those horses consuming the high DCAD diet had a higher ($P<.05$) sodium balance than those on the low DCAD diet. Similarly, Wall et al. (1993) noted that exercising horses consuming a diet with supplemental sodium experienced an increased ($P<.05$) urinary sodium excretion (14.03) and balance (8.86) compared to the non-supplemented diet (8.57 and 3.47, respectively). Urinary excretion of sodium is directly related to intake. When intake increases, then the plasma concentration of sodium also increases and this elevates the amount of sodium filtered through the glomerulus per unit of time. However, sodium

reabsorption from the tubule does not increase proportionally and thus more sodium is excreted in the urine.

The intake of potassium was similar across treatments at 150, 240 and 330 days of age as both diets contained the same concentration of potassium and because DMI did not differ between diets across all periods. Consequently, no difference in urinary and fecal excretion or potassium balance was detected. This agrees with data in horses (Wall et al., 1997) and dairy cattle (Delaquis and Block, 1995; Tucker et al., 1991) in which potassium metabolism was not affected by feeding diets high and low in dietary cation-anion difference.

Due to the addition of chloride to the low DCAD diet, increased ($P<.05$) intakes of chloride were noted in horses consuming diet L versus diet H across all sampling periods. Despite this increased intake, no differences in fecal chloride excretion were detected at any day of age. At 150 days of age, urinary chloride excretion was higher ($P<.05$) in weanlings fed diet L (34.65 g/d) than those on diet H (12.66 g/d). It appears that this increased chloride excretion did atone for the increase in intake as chloride balance was not different ($P>.05$) between treatments H (4.17 g/d) and L (9.02 g/d). At 240 days of age, the higher intakes also resulted in an increase ($P<.05$) in urinary chloride excretion for diet L (48.91 g/d) versus diet H (18.25 g/d). The kidney is the main route of chloride excretion; however, this increase in renal excretion did not counter the higher intake for there was a significant increase in chloride balance on diet L (15.12 g/d) compared to diet H (6.61 g/d). The response at 330 days of age was similar to the previous period in that a higher ($P<.05$) urinary chloride excretion (53.03 and 21.61 g/d)

and chloride balance (15.87 and 4.60 g/d) was observed for horses consuming diet L versus diet H, respectively. These data agree with others (Baker et al., 1993; Tucker et al., 1988b; Wall et al., 1993; Wall et al., 1997) who have reported significant increases in urinary chloride excretion in response to increased intakes of chloride on low DCAD diets. Wall et al., (1997) further demonstrated a higher ($P<.05$) chloride balance in weanlings consuming a low DCAD diet compared to a diet high in DCAD at 240, 300 and 360 days of age.

At 150, 240 and 330 days of age, no difference in magnesium intake was observed for diets H and L. Also intestinal absorption was not altered as fecal magnesium excretion was similar ($P>.05$) between treatments at all ages. At 150 days of age, urinary magnesium excretion was higher ($P<.05$) in horses consuming diet L (2.16 g/d) than those on diet H (1.14 g/d). This increase in excretion resulted in a lower ($P<.05$) magnesium balance for diet L (-1.59 g/d) compared to diet H (.35 g/d). A similar response in urinary excretion and magnesium balance was observed at 240 days of age in horses fed diet L (3.50 and -2.18 g/d) versus diet H (2.05 and -.47 g/d), respectively. These results were consistent at 330 days of age as horses consuming diet L had a higher ($P<.05$) urinary magnesium excretion (3.27 vs. 1.71 g/d) and lower ($P<.05$) magnesium balance (-1.48 vs. .48 g/d) than those fed diet H. These data correspond with results in dairy calves (Jackson et al., 1992) where urinary magnesium excretion decreased linearly with increasing DCAD. Roy et al. (1982) reported that acute metabolic alkalosis enhanced magnesium reabsorption in the renal tubule which resulted in a significant decrease in urinary excretion in rats. A later study revealed that average daily urinary excretion of

magnesium was higher and daily magnesium balance was lower in cats fed acidified diets as compared to control diets (Ching et al., 1989). These results do not agree with studies in growing and exercising horses (Wall et al., 1993; Wall et al., 1997) as no differences in urinary excretion and magnesium balance were observed between diets high and low in DCAD. The effect of dietary acidity on the renal excretion and reabsorption of magnesium is uncertain as few studies have analyzed the effects of chronic dietary acidity on magnesium metabolism. The increase in urinary excretion of magnesium in the present study may have been a secondary result to the elevated urinary calcium excretion observed across all periods. While the renal handling of magnesium varies considerably between species, magnesium excretion generally parallels calcium excretion which would help explain the increased loss of magnesium in the urine.

Phosphorus intake did not differ significantly between diets H and L across day of age. At 150 days of age, urinary and fecal phosphorus excretion and balance were not different ($P>.05$) between treatments. In contrast, urinary phosphorus excretion at 240 days of age was higher ($P<.05$) in horses consuming diet H (.84 g/d) as opposed to those on diet L (.18 g/d). At 330 days of age, horses on diet H ($P<.05$) excreted more urinary phosphorus (1.13 g/d) than those on diet L (.34 g/d). The reason for this increase in excretion remains unexplained as the concentration in the diets and the phosphorus intakes were similar among treatments. Despite this finding, no difference in phosphorus balance was observed among diets H and L during either period. Furthermore, intestinal absorption of phosphorus was unaltered across all ages as fecal excretion was not different ($P>.05$) between diets. Fecal excretion did however account for the majority of

the phosphorus lost from the body. The elevated urinary phosphorus levels found on diet H at 240 and 330 days of age concur with that of Wall et al. (1997) and Jackson et al. (1992) in which urinary excretion rates of phosphorus increased ($P<.05$) as the DCAD of the diet increased. Wall et al. (1997) further showed that phosphorus balance in growing horses at 8 and 12 months of age was not significantly different between diets high and low in DCAD. These results do not agree with other studies (Baker et al., 1993; Baker et al., 1997) in which urinary phosphorus excretion in sedentary horses remained unchanged across diets with varying dietary cation-anion differences. In contrast, Lemann et al. (1967) demonstrated that an induced metabolic acidosis increased ($P<.05$) urinary phosphorus excretion in normal human subjects.

Calcium intakes were similar between diets across all periods. At 150 days of age, horses consuming diet L excreted more ($P<.05$) calcium in the urine (5.00 g/d) than those fed diet H (1.31 g/d). These horses also had lower ($P<.05$) fecal calcium excretion (12.52 g/d) compared to diet H (17.35 g/d). Due to this decrease in excretion of fecal calcium, horses on diet L were able to compensate for the increased urinary calcium excretion as no difference ($P>.05$) in calcium retention was detected between diets L (12.53 g/d) and H (12.78 g/d). At 240 days of age, urinary calcium excretion was higher ($P<.05$) for diet L (9.80 g/d) than for diet H (7.15 g/d). Fecal calcium excretion was also lower ($P<.05$) for horses on the low diet (21.25 g/d) versus those on the high (13.66 g/d). This significant decrease in fecal calcium excretion resulted in an increased ($P<.05$) calcium balance for horses consuming diet L (13.66 g/d) compared to those on diet H (7.15 g/d). The response in calcium metabolism at 330 days of age was similar to the previous period.

Horses fed diet L had higher ($P<.05$) urinary calcium excretion (10.59 g/d) and lower ($P<.05$) fecal excretion of calcium (22.02 g/d) than those fed diet H (2.73 g/d and 39.12 g/d, respectively). Calcium balance was again dramatically increased ($P<.05$) in horses consuming diet L (15.68 g/d) versus those on diet H (5.57 g/d).

Previous studies in horses (Baker et al., 1993; Topliff et al., 1989; Wall et al., 1997) dairy cattle (Tucker et al., 1988b; Abu Damir et al., 1994) and sheep (Abu Damir et al., 1991; Takagi and Block, 1991) have shown that reducing the dietary cation-anion difference significantly increases urinary calcium excretion. Furthermore, Lemann et al. (1967) concluded in humans that chronic metabolic acidosis, induced by oral administration of NH_4Cl, decreases renal tubular calcium reabsorption resulting in increased urinary calcium excretion. It has also been suggested that low DCAD diets inhibit tubular reabsorption of calcium due to the induced metabolic acidosis (Beck and Webster, 1976).

During chronic metabolic acidosis, the body relies on additional buffer systems since serum bicarbonate will eventually stabilize despite continued acid retention. It has been suggested that bone mineral salts (calcium carbonate and calcium phosphate) could serve as an extra-renal buffer source which may be titrated in response to sustained acid loads (Lemann et al., 1972). Research has indicated that metabolic acidosis is associated with increased bone calcium loss and that resorption of bone mineral may provide additional quantities of buffer (Lemann et al., 1965; Lemann et al., 1966; Barzel, 1969b; Abu Damir et al., 1994). The addition of NH_4Cl to the diets of lambs resulted in a significant increase in plasma parathyroid hormone (PTH) indicating an increase in bone

turnover (Abu Damir et al., 1991). Rib samples from these lambs also showed higher tartrate-resistant acid phosphatase and lower alkaline phosphatase verifying an increase in resorption and a decrease in bone formation, respectively. Further evidence of enhanced calcium mobilization has been reported in dairy cattle (Block et al. 1984; Goff et al., 1991) in which the feeding of highly anionic diets resulted in increased hydroxyproline concentrations indicating enhanced bone calcium resorption activity.

The lower fecal calcium excretion observed in weanlings consuming the low DCAD diet is in agreement with data from other studies. Wall et al. (1997) reported that growing horses fed highly anionic diets experienced a decrease in fecal calcium excretion when compared to those fed a highly cationic diet at 240, 300 and 360 days of age. A decrease in excretion of fecal calcium was also noted in anaerobically exercised horses consuming a low DCAD (+130) diet compared to those fed a high DCAD (+354) diet (Wall et al., 1993). The enhanced intestinal absorption of calcium may be related to the hormone, 1,25 dihydroxyvitamin D (1,25 -$(OH)_2D_3$), which regulates the active transport of calcium across the intestine. During metabolic acidosis, PTH is activated and stimulates the conversion of 25-$(OH)D_3$ to 1,25-$(OH)_2$ D_3 (active form) in the kidney. Abu Damir et al. (1994) found that cows consuming an acidic diet had significantly higher plasma 1,25-$(OH)_2D_3$ concentrations and a higher estimated fractional absorption of calcium than those fed an alkali diet. Furthermore, Beck and Webster (1976) suggested that metabolic acidosis inhibits renal tubular reabsorption of calcium and enhances the ability of PTH to mobilize bone calcium.

Despite the increased urinary calcium excretion observed in the horses consuming the low DCAD diet, calcium balance was increased over those on the high diet due to the enhanced intestinal absorption of calcium. This finding agrees with data from Wall et al. (1997) in which a decreased fecal calcium excretion resulted in increased calcium balance in weanlings consuming a low DCAD diet. Ching et al. (1989) further demonstrated that cats fed an acidic diet experienced a decreased fecal calcium excretion which allowed for a partial to complete compensation in calcium balance despite an increased urinary calcium excretion. However, these results do not agree with other studies in horses (Wall et al., 1993; Baker et al., 1997) and humans (Lemann et al., 1966) where chronic metabolic acidosis led to reduced or negative calcium balances. Thus, it appears from the present study that growing horses can respond to chronic metabolic acidosis by increasing intestinal calcium absorption in order to compensate for the increased urinary calcium excretion.

Calcium and Phosphorus Metabolism

Concerning calcium and phosphorus metabolism, there were significant alterations in the intake, amount absorbed and apparent digestibilities over time and between diets during this trial (Tables XVII and XVIII). Absolute intake of calcium and phosphorus per day increased significantly between day 150 and 240 but was similar between day 240 and 330. Weanlings were fed as a percentage of their body weight and the increase in body weight between 150 and 240 days of age (71 kg) was greater than the increase between 240 and 330 (42 kg) as shown in Table XVII. This would help explain the observed increase between days 150 and 240 and the lack of difference between 240 and

TABLE XIV. EFFECT OF DCAD ON MINERAL BALANCE AT 150 DAYS OF AGE[a]

	Treatment		
Item	**High**	**Low**	**S.E.M.**
Sodium			
Intake g/d	28.63[b]	9.61[c]	.66
Urine g/d	17.87[b]	10.26[c]	1.52
Fecal g/d	3.24	1.86	1.02
Balance g/d	7.52[b]	-2.52[c]	1.53
Potassium			
Intake g/d	41.51	39.66	1.39
Urine g/d	19.06	17.66	1.26
Fecal g/d	18.49	19.44	1.45
Balance g/d	3.97	2.56	2.68
Chloride			
Intake g/d	19.08[b]	45.67[c]	1.19
Urine g/d	12.66[b]	34.65[c]	2.08
Fecal g/d	2.23	1.98	.41
Balance g/d	4.17	9.02	1.90
Magnesium			
Intake g/d	7.97	7.61	.27
Urine g/d	1.14[b]	2.16[c]	.27
Fecal g/d	6.50	7.04	.32
Balance g/d	.35[b]	-1.59[c]	.40
Phosphorus			
Intake g/d	17.61	16.82	.59
Urine g/d	.92	.63	.19
Fecal g/d	10.27	9.73	1.04
Balance g/d	6.43	6.47	1.15
Calcium			
Intake g/d	31.45	30.05	1.06
Urine g/d	1.31[b]	5.00[c]	.51
Fecal g/d	17.35[b]	12.52[c]	1.38
Balance g/d	12.78	12.53	1.65

[a] Values are least squares means.

[b,c] Means within a row with different superscripts differ (P<.05).

TABLE XV. EFFECT OF DCAD ON MINERAL BALANCE AT 240 DAYS OF AGE[a]

	Treatment		
Item	**High**	**Low**	**S.E.M.**
Sodium			
Intake g/d	44.13[b]	14.3[c]	.66
Urine g/d	22.61[b]	9.54[c]	1.52
Fecal g/d	10.12	10.69	1.02
Balance g/d	11.39[b]	-5.92[c]	1.53
Potassium			
Intake g/d	61.16	59.04	1.39
Urine g/d	23.70	27.01	1.26
Fecal g/d	23.51	23.71	1.45
Balance g/d	13.95	8.33	2.68
Chloride			
Intake g/d	29.42[b]	67.99[c]	1.19
Urine g/d	18.25[b]	48.91[c]	2.08
Fecal g/d	4.55	3.97	.41
Balance g/d	6.61[b]	15.12[c]	1.90
Magnesium			
Intake g/d	11.74	11.33	.27
Urine g/d	2.05[b]	3.50[c]	.27
Fecal g/d	10.15	10.02	.32
Balance g/d	-.47[b]	-2.18[c]	.40
Phosphorus			
Intake g/d	25.95	25.05	.59
Urine g/d	.84[b]	.18[c]	.19
Fecal g/d	21.47	19.61	1.04
Balance g/d	3.64	5.27	1.15
Calcium			
Intake g/d	46.33	44.73	1.06
Urine g/d	2.99[b]	9.80[c]	.51
Fecal g/d	36.21[b]	21.25[c]	1.38
Balance g/d	7.15[b]	13.66[c]	1.65

[a] Values are least squares means.

[b,c] Means within a row with different superscripts differ (P<.05).

TABLE XVI. EFFECT OF DCAD ON MINERAL BALANCE AT 330 DAYS OF AGE[a]

	Treatment		
Item	**High**	**Low**	**S.E.M.**
Sodium			
Intake g/d	45.76[b]	15.45[c]	.74
Urine g/d	24.84[b]	10.70[c]	1.69
Fecal g/d	11.17	11.36	1.15
Balance g/d	9.78[b]	-6.62[c]	1.70
Potassium			
Intake g/d	62.59	63.75	1.55
Urine g/d	23.69	27.76	1.41
Fecal g/d	26.51	28.76	1.62
Balance g/d	12.39	7.22	2.98
Chloride			
Intake g/d	30.51[b]	73.41[c]	1.33
Urine g/d	21.61[b]	53.03[c]	2.32
Fecal g/d	4.29	4.51	.46
Balance g/d	4.60[b]	15.87[c]	2.11
Magnesium			
Intake g/d	12.01	12.24	.30
Urine g/d	1.71[b]	3.27[c]	.31
Fecal g/d	9.83	10.43	.36
Balance g/d	.48[b]	-1.48[c]	.44
Phosphorus			
Intake g/d	26.55	27.05	.66
Urine g/d	1.13[b]	.34[c]	.21
Fecal g/d	23.92	22.46	1.16
Balance g/d	1.50	4.24 ± 1.40	1.28
Calcium			
Intake g/d	47.41	48.29	1.18
Urine g/d	2.73[b]	10.59[c]	.57
Fecal g/d	39.12[b]	22.02[c]	1.54
Balance g/d	5.57[b]	15.68[c]	1.84

[a] Values are least squares means.

[b,c] Means within a row with different superscripts differ (P<.05).

330 in absolute intake over time. Furthermore, absolute intake of both minerals was the same (P>.05) between diets H and L. Intakes of both calcium and phosphorus, expressed as mg/kg BW, were also similar between diets however, intake rose dramatically (P<.05) between 150 and 240 days of age and was lowered (P<.05) between day 240 and 330. This decline in intake between period 2 and 3 can be explained by the fact that as body weight continued to increase significantly (Table XVII) the absolute intake of calcium remained similar between the two periods.

Calcium digestibility on the High diet (Table XVIII) was greater (P<.05) at day 150 (44.63%) versus days 240 (20.82%) and 330 (16.82%). Consequently, the amount of calcium absorbed and retained in horses consuming diet H was significantly lower at 240 and 330 days of age compared to 150. No difference in digestibility of calcium across day of age was detected in horses consuming diet L with digestibilities ranging from approximately 51 to 57%. These values agree with the estimated absorption efficiency of dietary calcium (50%) set forth by the NRC (1989). Similarly, the amount of calcium absorbed and retained was not different over time in these horses. Between diets H and L, the digestibility and the amount of calcium absorbed were higher (P<.05) in horses on diet L than those on diet H at all measured intervals. Retention of calcium did not differ between diets at 150 days of age but was enhanced (P<.05) in weanlings consuming the low diet versus the high. An increased urinary excretion of calcium in diet L was the reason for similar calcium retentions between diets during the first period despite the higher digestibility. These data agree with that of Wall et al. (1997) in which horses fed a diet low in DCAD had higher mean calcium digestibilities (32%, 41% and 31%) compared

to those on the high diet (14%, 0% and 4%) at 240, 300 and 360 days of age, respectively.

Absorption of calcium involves an active transport mechanism which takes place mainly in the upper small intestine (particularly in the duodenum). This process is primarily dependent upon source, intestinal pH, dietary levels of other minerals and vitamin D which will all be discussed in an attempt to explain the differences detected in calcium metabolism between diets. Vitamin D_3 (1,25-$(OH)_2D_3$) enters the cell membrane of the intestinal absorptive cell where it binds to a high affinity receptor in the cytoplasm. This hormone-receptor complex then moves into the nucleus and activates specific genes resulting in transcription of mRNA and subsequent protein synthesis (Best, 1985). These proteins include calcium binding protein, calcium activated ATPase and alkaline phosphatase. Vitamin D_3 promotes calcium absorption by increasing calcium permeability which enhances calcium flux into the intestinal absorption cell. The permeability of the brush border membrane is altered by 1,25-$(OH)_2D_3$, possibly through a change in membrane lipid structure. Calcium is then transported out of the absorptive cell across the basolateral membrane which may be an active transport mechanism involving calcium-activated ATPase (Best 1985). Inadequate levels of 1,25-$(OH)_2D_3$ could therefor contribute to decreased absorption rates resulting in lower digestibilities. Goff et al. (1991) found that the amount of 1,25-$(OH)_2D_3$ was greatly diminished in cows fed highly cationic diets and that reducing the alkalinity by addition of anions to the diet increased the ability of the animal to produce 1,25-$(OH)_2D_3$. Abu Damir et al. (1994) further demonstrated that acidic diets fed to dairy cows increased plasma concentrations

of 1,25-$(OH)_2D_3$ which may have stimulated both intestinal calcium absorption and bone resorption thereby preventing milk fever.

The acidity of the diet may have also altered calcium absorption through changes in intestinal pH. A low intestinal pH facilitates calcium uptake through enhanced solubility (Swenson and Reece, 1993). This may help explain the increased amounts of calcium absorbed and the higher digestibilities observed in horses consuming diet L versus diet H. Furthermore, the source of calcium on the low DCAD diet may have contributed to the increased absorption rates. The primary source of calcium on the high diet was limestone while calcium on the low diet was provided mainly by calcium chloride which is much more available to the animal. The relative biological value (RBA) of calcium sources relates the biological availability of calcium in that source to that of a standard source (calcium carbonate=100). In ruminants, the RBA of calcium chloride is 120-130 while limestone is 90 (Peeler, 1972; Beeson et al., 1976). This means that calcium chloride is 20 to 30% more available than the standard source and 30 to 40% more available than limestone. Due to this difference in bioavailability, the higher calcium absorption seen in horses consuming diet L may have simply been due to the source of calcium utilized in the diets.

The lowered absorption rates of calcium witnessed across periods may also be due to interactions with other minerals. Sodium intake on the high diet was significantly greater than that on the low and may have interfered with calcium absorption. Calcium transport across the intestinal wall can be inhibited by high concentrations of sodium in the lumen (Harrison and Harrison, 1963). Hurwitz et al. (1967) also found that active

transport in the ileum of chicks only took place when sodium concentrations were low. This negative effect of sodium on the calcium pump may be due to competition of sodium and calcium ions for a common carrier.

Phosphorus digestibility, absorption and retention was not different ($P>.05$) between treatments during any period however, both diets experienced significant reductions in these parameters with increasing age. Digestibility of phosphorus and the amount absorbed and retained was higher ($P<.05$) at day 150 compared to days 240 and 330 for diets H and L. These findings are in partial agreement with those of Wall et al. (1997) in which growing horses sampled at 240, 300 and 360 days of age were found to have extremely low phosphorus digestibilities (7 to 16%) while consuming diets high and low in DCAD. Unlike calcium absorption, phosphorus is absorbed primarily in the large intestine particularly the large and small colon (Schryver et al., 1972). It appears from these results that growing horses will only absorb a certain amount of phosphorus despite the continued increase in intake and growth. This data would suggest that a homeostatic mechanism is involved in the regulation of phosphorus metabolism and that these weanlings are only absorbing the amount of phosphorus they require from the diet.

According to NRC recommendations, weanlings consuming diet H would require 42.24 g/d and 28.48 g/d of calcium in the diet at 240 and 330 days of age, respectively. This is assuming a 50% absorption efficiency of calcium which would mean that 21.12 and 14.24 grams of calcium are absorbed per day. The digestibilities of calcium for diet H were 20.82 and 16.82% during these periods which led to absorption rates of only 9.64 and 7.97 g/d at 240 and 330 days of age, respectively. Despite these lowered absorption

TABLE XVII. EFFECT OF DCAD ON THE ABSOLUTE INTAKE OF CALCIUM AND PHOSPHORUS[a].

	Day of age			
Item	**150**	**240**	**330**	**S.E.M.**
Calcium				
Intake, g/d				
Diet H	31.45[b]	46.33[c]	47.41[c]	1.06
Diet L	30.05[b]	44.73[c]	48.97[c]	1.18
Phosphorus				
Intake, g/d				
Diet H	17.61[b]	25.95[c]	26.55[c]	.59
Diet L	16.82[b]	25.05[c]	27.42[c]	.66

[a] Values are least squares means.

[b,c] Means within a row with different superscripts differ (P<.05).

TABLE XVIII. EFFECT OF DCAD ON CALCIUM AND PHOSPHORUS METABOLISM

	Day of age			
Item	**150**	**240**	**330**	**S.E.M.**
Calcium				
Intake, mg/kg BW				
Diet H	151.21[b]	165.50[c]	146.98[b]	4.06
Diet L	150.05[b]	165.20[c]	151.85[b]	4.52
Digestibility, %				
Diet H	44.63[bd]	20.82[cd]	16.82[cd]	3.32
Diet L	57.53[e]	51.58[e]	53.85[e]	3.70
Absorbed, mg/kg BW				
Diet H	67.29[bd]	34.56[cd]	24.40[cd]	6.34
Diet L	86.85[e]	85.24[e]	82.12[e]	7.05
Retention, mg/kg BW				
Diet H	61.09[b]	24.01[cd]	16.03[cd]	5.96
Diet L	62.18	49.25[e]	48.91[e]	6.63
Phosphorus				
Intake, mg/kg BW				
Diet H	84.68[b]	92.68[c]	82.31[b]	2.27
Diet L	84.03[b]	92.51[c]	85.04[b]	2.53
Digestibility, %				
Diet H	41.31[b]	16.56[c]	9.62[c]	4.85
Diet L	40.80[b]	20.92[c]	15.83[c]	5.39
Absorbed, mg/kg BW				
Diet H	35.42[b]	15.47[c]	7.79[c]	4.56
Diet L	34.27[b]	19.36[c]	14.17[c]	5.08
Retention, mg/kg BW				
Diet H	31.02[b]	12.43[c]	4.24[c]	4.37
Diet L	31.52[b]	18.76[c]	13.17[c]	4.86

[a] Values are least squares means.

[b,c] Means within a row with different superscripts differ (P<.05).

[d,e] Means within a column with different superscripts differ (P<.05) between treatments.

values, no difference was observed between diets H and L in any skeletal growth parameters measured during this trial (Table XIX). According to the NRC, phosphorus requirements for these weanlings at 240 and 330 days of age are 19.43 and 16.17 g/d in the diet assuming a 45% absorption efficiency. This results in 8.74 and 7.28 g/d of phosphorus that would be absorbed. Weanlings in this study however, only absorbed 4.77 and 3.43 g/d of phosphorus at 240 and 330 days of age, respectively. These data demonstrate that horses in this trial are absorbing only half the amount of phosphorus recommended for growth by the NRC.

Growth Measurements

Mean values for all parameters of growth (body weight, heartgirth circumference, hip, shoulder, knee, hock and wither height) did not differ significantly between males and females on this study. This agrees with Cunningham and Fowler (1961) who found that both males and females tended to grow uniformly to 18 months of age. After this point, the rate of growth in males compared to the females increased progressively up to five years of age.

Average daily gains (ADG) of weanlings were not significantly altered by dietary treatment (Table XIX). This agrees with Jackson et al. (1992) in which no difference ($P>.05$) was detected in ADG among dairy calves fed anionic (0 DCAD) and cationic (+520 DCAD) diets. In contrast, Beighle et al. (1988) reported that calves fed an anionic (+120 DCAD) diet had higher feed intakes and average daily gains than calves fed a cationic (+550 DCAD) diet. Average daily gain in growing steers was found to increase quadratically with increasing dietary electrolyte balance (DEB) as steers fed diets

TABLE XIX. EFFECT OF DCAD ON GROWTH PARAMETERS[ab]

	Treatment		
Parameter	**High**	**Low**	**S.E.M.**
ADG, kg	.65	.67	.03
BW, kg	265.84	267.33	11.23
Wither height, cm	129.73	128.28	1.01
Hip Height, cm	130.96	129.78	.93
Hock height, cm	55.93	55.28	.52
Shoulder height, cm	93.75	92.80	.80
Knee height, cm	39.12	38.90	.40
Heart girth, cm	144.50	142.32	2.13

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

TABLE XX. EFFECT OF DCAD ON MEAN BODY WEIGHT AND AVERAGE DAILY GAIN OVER TIME[a].

	Day of age			
Item	**150**	**240**	**330**	**S.E.M.**
BW, kg				
Diet H	208.92[bc]	280.11[ce]	322.67[d]	2.53
Diet L	200.57[bf]	270.74[cf]	321.98[d]	2.82
ADG, kg				
Diet H	.54[g]	.97[h]	.54[g]	.28
Diet L	.47	.53	.48	.32

[a] Values are least squares means ± SE.

[b,c,d] Means within a row with different superscripts differ (P<.05).

[e,f] Means within a column with different superscripts differ (P<.05) between treatments.

[g,h] Means within a row with different superscripts differ (P<.10).

containing a DEB of 150 and 300 meq/kg of DM had higher ADG than those fed 0 or 450 meq/kg of DM (Ross et al., 1994).

Body weight (BW) was similar (P>.05) between treatments (Table XIX) and increased quadratically (P<.01) in response to increasing age (Figure 3). These results are similar to the response reported by Boren et al. (1986) who indicated that weight gains in weanling Quarter Horses were best described by a second degree polynomial. Furthermore, the NRC (1978) suggests that weight gain to any mature weight should exhibit a quadratic response over time. The intercepts and the slopes for the regression of age on body weight were significantly different between treatments. This difference in the slope of the two lines would indicate that weanlings gained weight at a dissimilar rate. Mean body weight was higher in horses consuming diet H versus diet L at 150 and 240 days of age however, no difference in body weight was detected at the end of the study (Table XX). One reason for the observed difference in slope may be because the weanlings in the High group began the trial at a significantly higher mean weight and appear to be reaching their mature weight at an earlier age than those in the Low group. This is further demonstrated in horses on diet H as ADG increased (P<.05) between day 150 and 240 but declined (P<.05) between 240 and 330 days of age (Table XX). Average daily gains given in Table XX are calculated over a seven day period and are not an accurate reflection of overall gain. These means were listed to illustrate growth rate during the collection periods.

Mean heart girth circumference was not affected (P>.05) by dietary treatment (Table XIX). Heart girth increased quadratically (P<.01) with increasing age in both

Figure 3. Response of body weight to dietary cation-anion difference in growing horses.

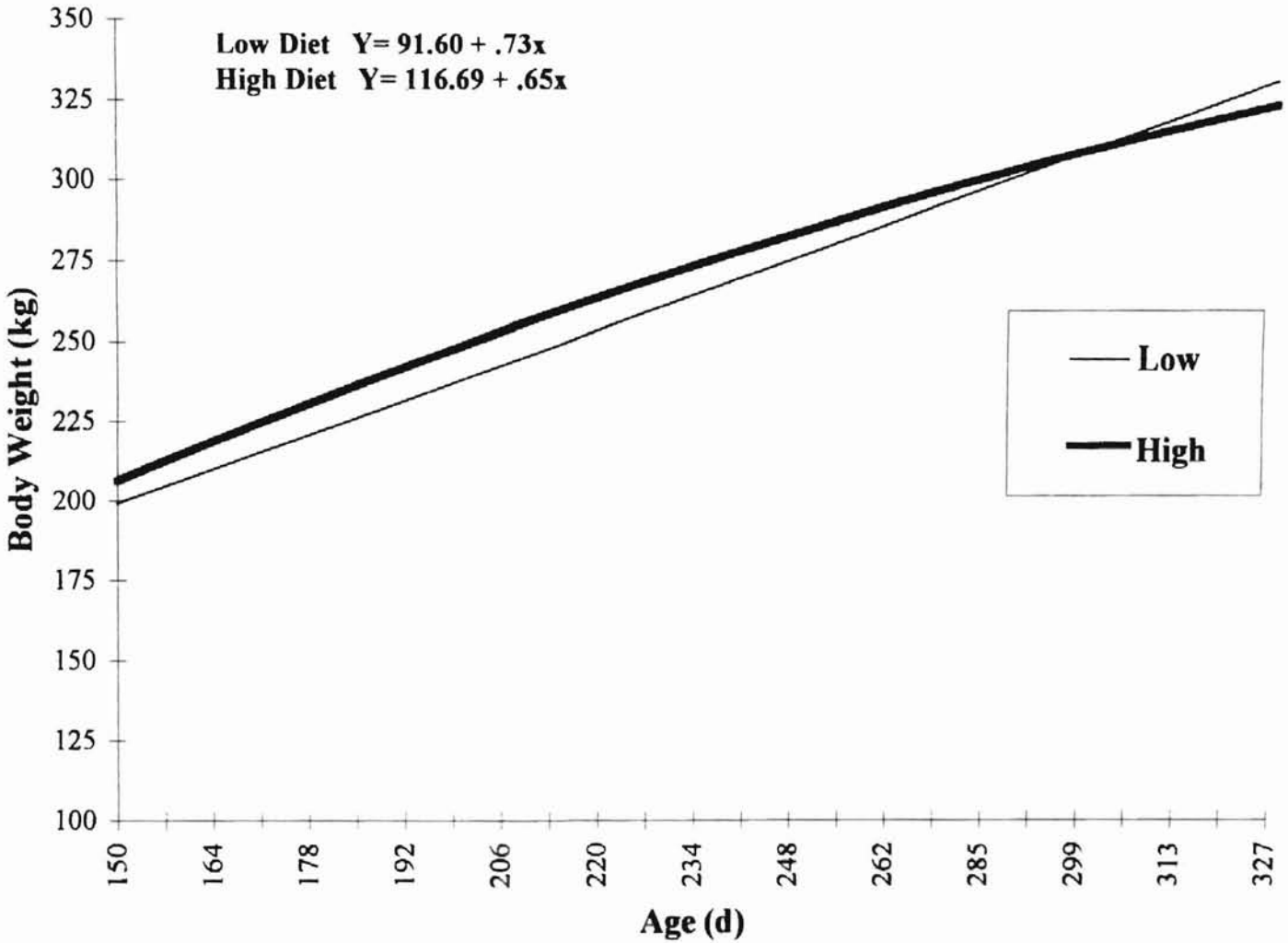
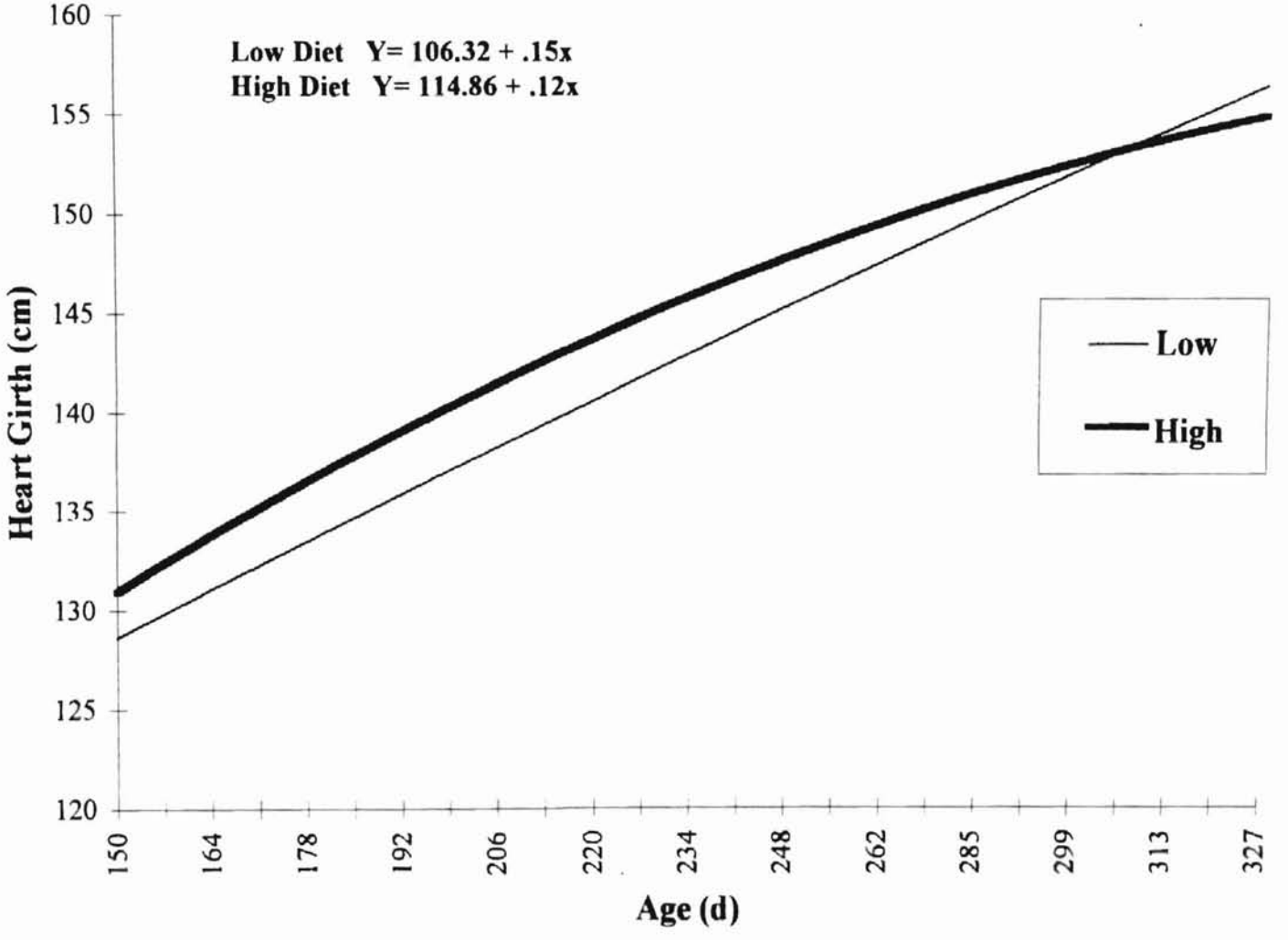


Figure 4. Response of heart girth to dietary cation-anion difference in growing horses.



treatments (Figure 4). Initial heart girth circumference differed significantly between treatments as indicated by the difference in intercepts. Further, the slopes for the regression of age on heart girth were different ($P<.01$). The response over time exhibited by heart girth paralleled changes in body weight. This finding may be explained by the fact that body weight and heart girth were highly correlated ($r=.96$, $p<.001$) in these weanlings. A previous study in horses (Henneke et al., 1983) has also shown that BW and heart girth are significantly related ($r=.90$).

Mean values for height at the withers (Table XIX), which are similar to those reported by Boren et al. (1986), did not differ ($P>.05$) between diets H and L. Wither height demonstrated a significant quadratic response with increasing age (Figure 5). Likewise, Boren et al., (1986) found that height at the withers increased quadratically over time in growing horses. Initial wither height was significantly higher for weanlings consuming diet H than for those consuming diet L. However, the repines of wither height to increasing age was similar between the two groups as no difference ($P>.05$) in slope was detected. This data indicates that weanling growth rate, as measured by height at the withers, is similar over time.

There were no significant differences in mean hip height or hock height between treatments (Table XIX). Height at the hip and hock increased quadratically ($P<.01$) over time as shown in Figures 6 and 7, respectively. In contrast, Boren et al. (1986) reported that both hip and hock height were best described by a third degree (cubic) polynomial. The intercepts for the regression of age on hip and hock height were significantly higher

Figure 5. Response of wither height to dietary cation-anion difference in growing horses.

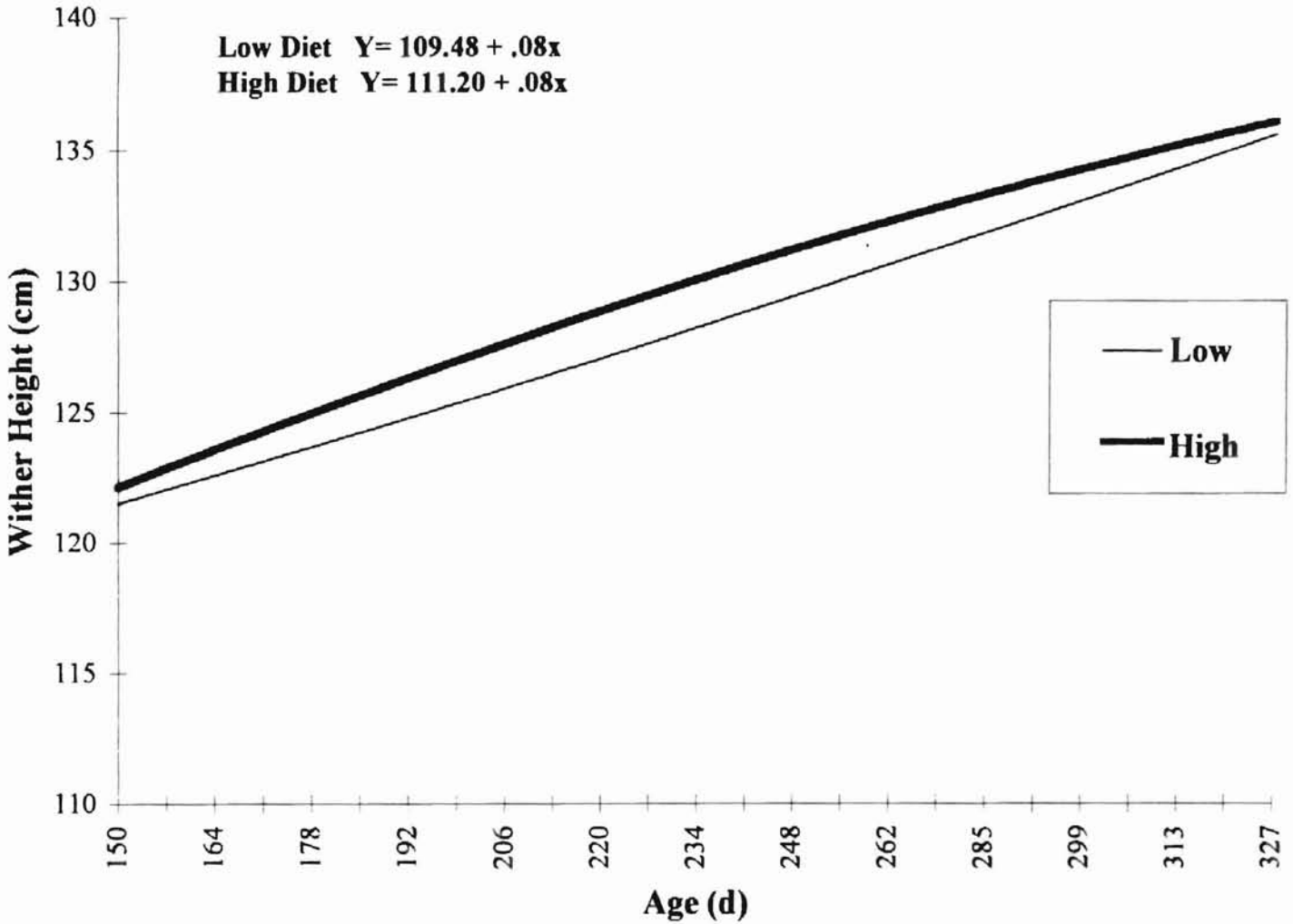


Figure 6. Response of hip height to dietary cation-anion difference in growing horses.

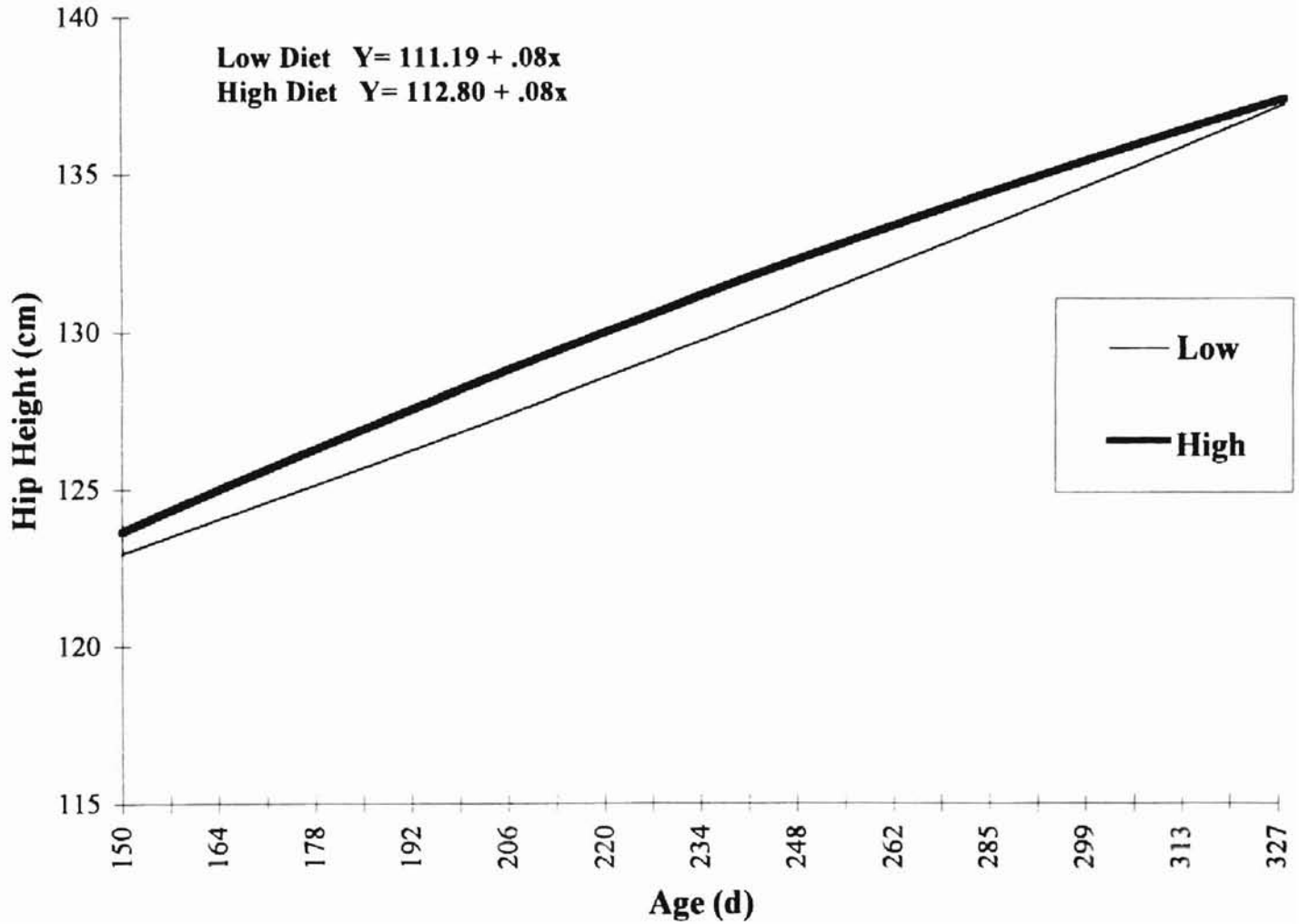
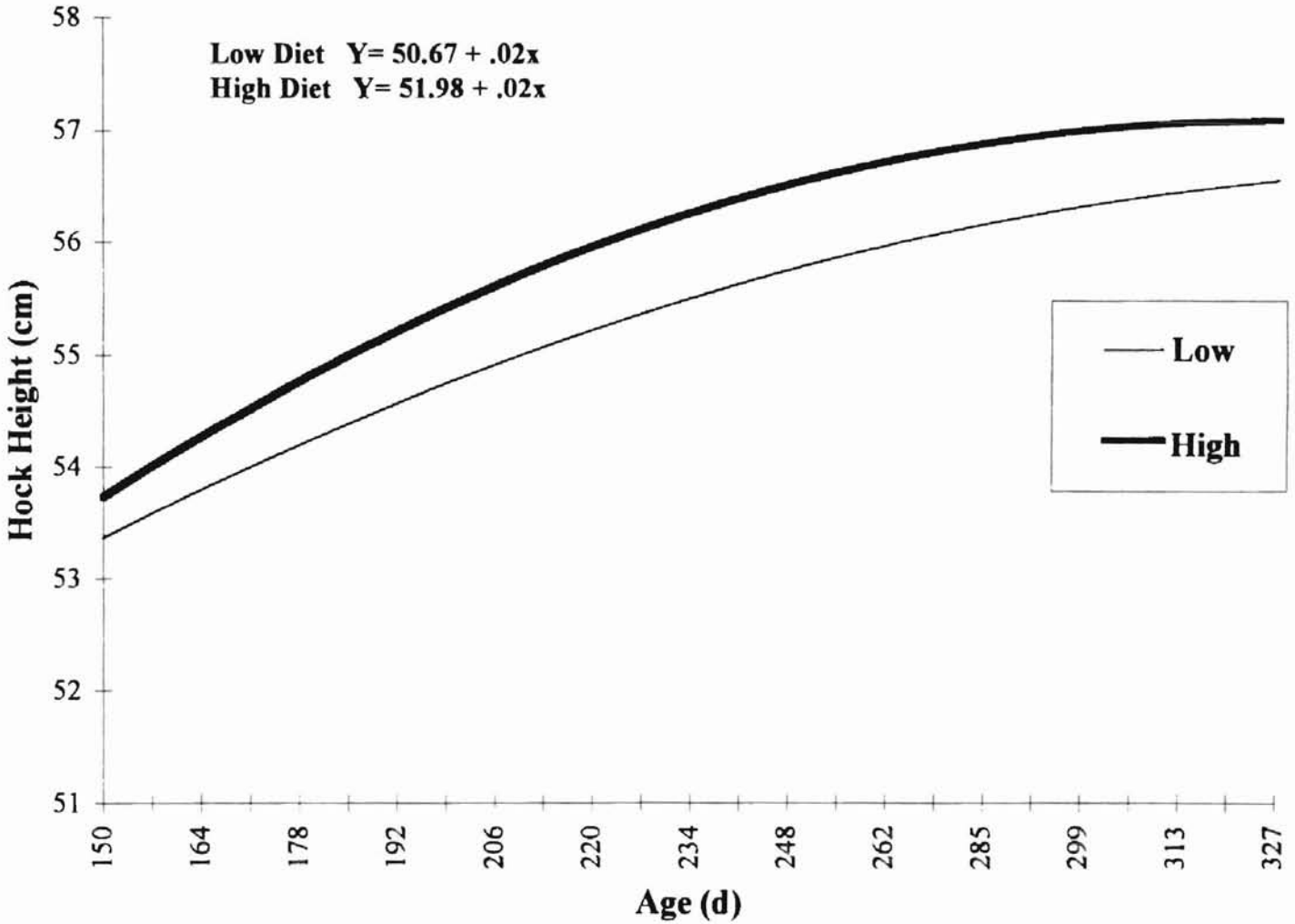


Figure 7. Response of hock height to dietary cation-anion difference in growing horses.



(P<.05) for diet H than diet L but the slopes were similar (P>.05) in their response to increasing age.

Treatment did not significantly alter shoulder height as groups H and L had mean values of 93.75 and 92.80 cm, respectively (Table XIX). These values are similar to that of Boren et al. (1986) who found an average shoulder height of 93.95 cm in weanlings measured from 120 to 300 days of age. Regression analysis showed a significant quadratic effect of shoulder height over time (Figure 8). This differs from Boren et al. (1986) who noted that height at the shoulder increased cubically with increasing age. One reason for this difference may due to the fact that growth measurements in the previously mentioned study began at 120 days of age and the response curves indicate that shoulder height increases most rapidly from birth to about 150 days of age. Treatment means for initial shoulder height were the same (P>.05) between groups H and L. Further, the slopes for the regression of age on shoulder height were similar (P>.05) between treatments.

Mean height at the knee did not differ significantly between weanlings consuming diets H and L (Table XIX). Knee height remained unchanged in both treatments throughout the trial as initial and final values for all weanlings were 38.88 and 39.14 cm, respectively. This data would indicate that there is limited metacarpal growth after 5 months of age. Similarly, Cunningham and Fowler (1961) discovered in Quarter Horses that 82 per cent of the length from the knee to the ground was present at birth and that knee height reached maturity at six months of age. No significant linear or quadratic effect was noted in either treatment (Figure 9). Boren et al. (1986) reported that weekly

Figure 8. Response of shoulder height to dietary cation-anion difference in growing horses.

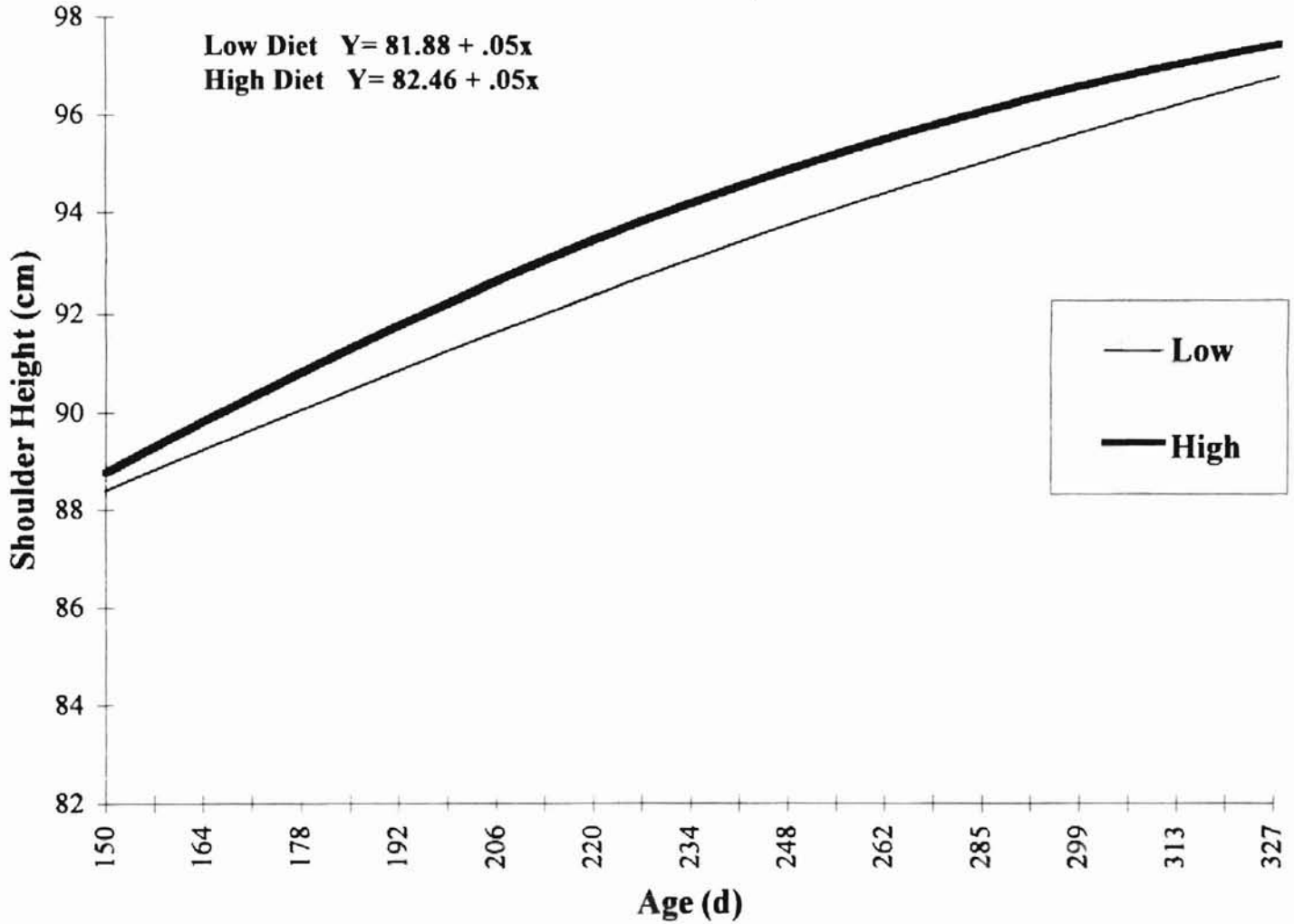
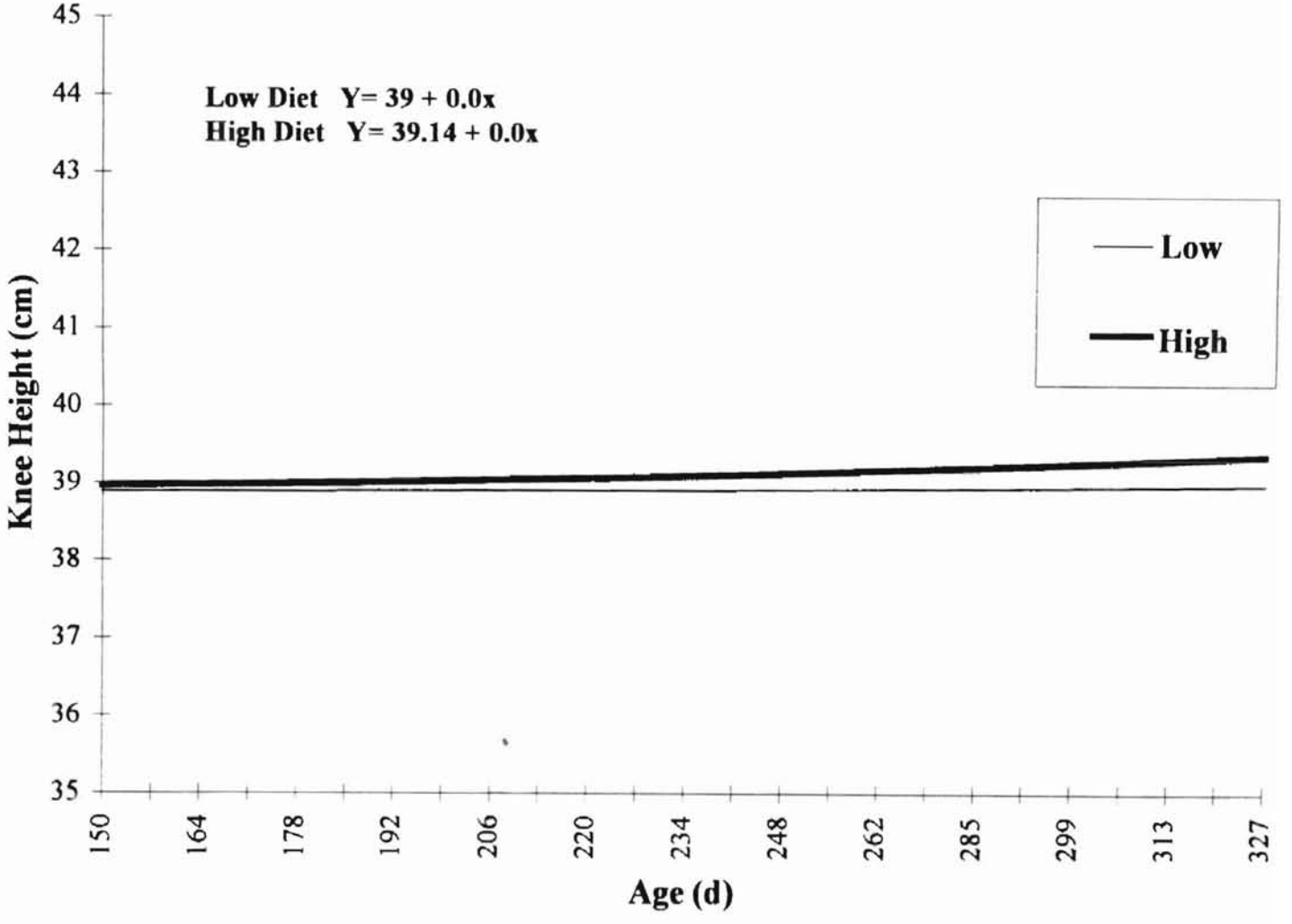


Figure 9. Response of knee height to dietary cation-anion difference in growing horses.



measurements for height at the knee were best described by a fourth degree (quartic) polynomial and that knee height increased from 41.06 cm at 4 months of age to 44.12 cm at 10 months of age. It is interesting to note however, that the response curve in the previously mentioned study shows that knee height increases at a decreasing rate over time after approximately 170 days of age. Regression of age on knee height demonstrated that initial height at the knee was not significantly different between treatments H and L and that the weanlings response over time was similar in that no difference (P>.05) in slope was found between the two groups.

Osteocalcin

The 24 hour osteocalcin profiles for weanlings at 4 and 11 months of age have been evaluated in horses (Fletcher, 1998). The response of osteocalcin over time in these horses was similar (P>.05) between the two age groups as the age x time interaction was not significant. Furthermore, osteocalcin levels did not fluctuate significantly during the light or dark period. Results from this trial indicate that sampling time does not have to be strictly regulated as there is no significant diurnal variation. These results agree with Hope et al. (1993) who reported no significant differences in osteocalcin values at any sample time over a 24 hour period. All blood samples in the present study were taken during the daylight hours and at the same time of day during the three sampling periods.

Sex did not influence serum osteocalcin levels as mean concentrations were similar between females and males at 150 (13.02 vs. 14.52 ng/ml), 240 (30.31 vs. 31.83 ng/ml) and 330 (39.43 vs. 36.36 ng/ml) days of age, respectively. These results agree with Lepage et al. (1992) who found no significant effect of sex in Standardbred horses at ages

of less than six months and 6-18 months. Other studies in adult humans (Galli and Caniggia, 1985 and Carrasco et al., 1988) have found no difference in osteocalcin between males and females. In contrast, Epstein et al. (1984) has shown that mean serum osteocalcin concentrations were higher in adult women versus men. Difference in osteocalcin may be due to hormonal relationships as these women experienced a marked increase in osteocalcin after the age of 40 which suggests an effect of decreased estrogen levels.

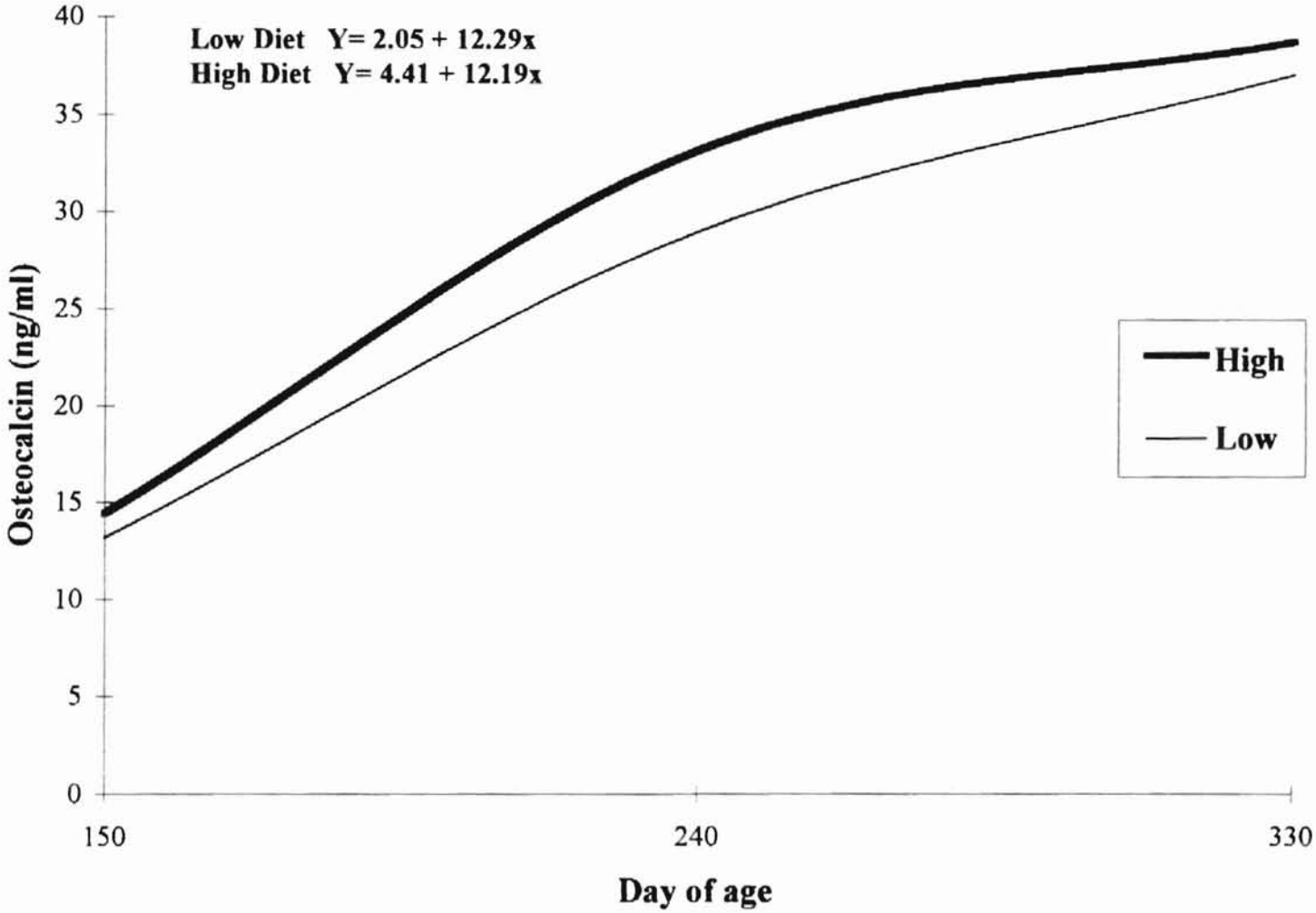
Regression analysis showed that serum osteocalcin levels increased quadratically ($P<.05$) with increasing age in both treatments (Figure 10). This finding is similar to the response of the skeletal growth parameters (hip, hock, wither and shoulder height) in which a significant quadratic effect was observed over time. Furthermore, there was a significant correlation ($P<.01$) between osteocalcin and wither ($r = .81$), hip ($r = .82$), hock ($r = .65$) and shoulder ($r = .72$) height (Table XXI) . Knee height and osteocalcin were not highly correlated ($r = .05$, $p>.10$) as cannon bone length did not change over time. These results suggest that as the weanlings approach puberty and skeletal growth occurs, there is an increase in total osteoblastic activity and bone formation indicated by the increase in osteocalcin concentration over time. In support of this observation, Sorva et al. (1997) demonstrated that peak osteocalcin levels were achieved at the time of maximal bone matrix formation and mineralization of bone in boys at puberty. However, the levels of osteocalcin in this study are increasing quadratically and they may start to decline as the horses mature. Lepage et al. (1990) reported mean osteocalcin concentrations of 47.3, 35.7 and 6.7 ng/ml for horses less than 1 year of age, between 2.5

TABLE XXI. CORRELATION COEFFICIENTS FOR OSTEOCALCIN AND PARAMETERS OF SKELETAL GROWTH

Parameter	**Osteocalcin**
Wither height	.81[a]
Hip height	.82[a]
Hock height	.65[a]
Shoulder height	.72[a]
Knee height	.05[b]

[a] Correlation coefficient was significant at P<.01.
[b] Correlation coefficient was not significant P>.10.

Figure 10. Effect of DCAD on serum osteocalcin levels over time.



and 3.5 years and older than 3.5 years, respectively. This demonstrates a negative correlation between osteocalcin and age which denotes a reduction in bone formation as the animal grows older.

Mean osteocalcin concentrations for weanlings at 150, 240 and 330 days of age are given in Table XXII. Osteocalcin levels were similar ($P>.05$) among treatments H and L at 150 and 330 days of age. The response of osteocalcin to DCAD at 240 days of age differed from previous periods in that horses consuming diet L had a lower ($P<.05$) osteocalcin concentration (28.97 ng/ml) than those on diet H (33.18 ng/ml). Now whether this decrease in osteocalcin is due to diet or a difference in growth during this period is unclear. Colle et al. (1988) confirmed in humans that children with a short stature had lower levels of osteocalcin than an age-matched control group. These results would indicate that skeletal size has an effect on osteocalcin concentration. In the present study, weanlings in the high group began the trial at a higher ($P<.05$) hip and wither height and were taller at 240 days of age. This difference in skeletal maturity may have resulted in the lower osteocalcin levels observed on diet L. Furthermore, horses consuming diet H had a higher ADG (.97kg) during this period than those on diet L (.53kg). On the other hand, Carter et al. (1996) demonstrated in young growing pigs that increasing dietary calcium and phosphorus intakes led to increased bone mineralization and decreased serum osteocalcin and 1,25-$(OH)_2D_3$ concentrations. Levels of serum osteocalcin and 1,25-$(OH)_2D_3$ in these pigs were also negatively correlated ($P<.01$) with end measures of bone mineralization such as bone breaking strength and bone ash. These findings may be explained by the relation of osteocalcin production to 1,25-$(OH)_2D_3$.

TABLE XXII. EFFECT OF DCAD ON SERUM OSTEOCALCIN LEVELS[a]

	Treatment		
Item	**High**	**Low**	**S.E.M.**
150 DAYS OF AGE			
Osteocalcin, ng/ml	14.39	13.15	.66
240 DAYS OF AGE			
Osteocalcin, ng/ml	33.18[b]	28.97[c]	1.53
330 DAYS OF AGE			
Osteocalcin, ng/ml	38.75	37.48	2.48

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

Price and Bakoul (1980; 1981) found that osteocalcin production is enhanced by and parallels increases in $1,25(OH)_2D_3$ in both *in vitro* and *in vivo* studies, respectively. Thus, as calcium and phosphorus intake increases, the need for $1,25\text{-}(OH)_2D_3$ to enhance intestinal calcium and phosphorus absorption decreases which may result in a lowered osteocalcin level with the higher levels of intake. Further research has also proposed that osteocalcin may function in the regulation of osteoclastic recruitment which would increase resorption of calcium and phosphorus from the bone (Glowacki et al., 1991; Colombo et al., 1994; Carter et al., 1996). This mechanism would therefore suggest that osteocalcin is a predictor of overall bone turnover which is comprised of both bone formation and resorption. Due to the conflicting data presented above, it is difficult to draw conclusions about the increased osteocalcin levels over time and the lowered osteocalcin levels observed in horses on diet L during the second period. However, the results from the present study indicate that osteocalcin is positively correlated with indices of skeletal growth and that concentration increases with increasing age. These findings support the evidence that osteocalcin is a measure of osteoblastic activity which reflects the rate of bone formation. Still, further research is needed to quantify changes in bone metabolism in response to DCAD and correlate this data with serum osteocalcin concentrations.

SUMMARY AND CONCLUSIONS

In summary, previous research (Tucker et al., 1988; Topliff et al., 1989; Baker et al., 1993, 1997) has demonstrated an increase in urinary calcium excretion in animals consuming diets low in DCAD. Present data suggests that, if this condition were prolonged, these animals could be predisposed to an osteoporotic weakening of the skeletal system due to an increased loss of calcium from the bone. Results from the present study indicate that horses consuming highly anionic diets can compensate for the increased urinary calcium excretion by enhancing intestinal absorption of calcium. Consequently, this decrease in fecal calcium excretion results in a large positive calcium balance. Horses consuming diets high in DCAD however, may experience a decline in calcium digestibility and absorption possibly due to an interaction between sodium and calcium that inhibits calcium transport across the intestine. Furthermore, phosphorus digestibility decreased on both diets with increasing intake and age. This suggests that NRC requirements for phosphorus in growing horses may be overestimated. It appears from this trial that horses have a homeostatic mechanism which regulates phosphorus metabolism. This mechanism apparently allows absorption of only the amount of phosphorus required for adequate growth regardless of dietary intake. Despite the decreased calcium digestibility in horses consuming diet H and the lowered phosphorus digestibilities observed on both diets, growth performance was not significantly effected by DCAD. Horses consuming diets H and L demonstrated normal growth responses over time and no difference between treatments was detected. The large positive

correlation between osteocalcin and indices of skeletal growth indicates increased bone formation and osteoblastic activity with increasing age. However, further research is needed in the horse to establish the relationship between osteocalcin and bone mineralization. Overall results from this study illustrate the animals enormous capacity to maintain metabolic equilibrium despite the ratio of cations to anions in the diet. These findings further suggest that growing horses can tolerate a wide variation in DCAD without experiencing adverse effects in skeletal growth.

LITERATURE CITED

Abu Damir, H., D. Scott J.K. Thomson, J.H. Topps, W. Buchan and K. Pennie. 1990. The effect of a change in blood acid-base status on body composition and mineral retention in growing lambs. Anim. Prod. 51: 527.

Abu Damir, H., D. Scott, N. Loveridge, W. Buchan and J. Milne. 1991. The effects of feeding diets containing either $NaHCO_3$ or NH_4Cl on indices of bone formation and resorption and on mineral balance in the lamb. Exp. Physiol. 76:725.

Abu Damir, H., M. Phillippo, B.H. Thorp, J.S. Milne, L.Dick and I.M. Nevison. 1994. Effects of dietary acidity on calcium balance and mobilization, bone morphology and 1,25 dihyroxyvitamin D in prepartal dairy cows. Res. Vet. Sci. 56:310.

Armstrong, W.D. and C.P. Barnum. 1948. Concurrent use of radioisotopes of calcium and phosphorus in the study of the metabolism of calcified tissues. J. Biol. Chem. 172:199.

Austice, R.E. 1984. Excess dietary chloride depresses egg shell quality. Poul. Sci. 63:1773.

Baker, L.A., D.R. Topliff, D.W. Freeman, R.G. Teeter and J.E. Breazile. 1992. Effect of dietary cation-anion balance on acid-base status in horses. J. Equine Vet. Sci. 12: 160.

Baker, L.A., D.R. Topliff, D.W. Freeman, R.G. Teeter and J.E. Breazile. 1993. Effect of dietary cation-anion balance on urinary mineral excretion in horses. Proc. 13th Equine Nutr. Physiol. Symp. Gainesville, FL. p. 44.

Baker, L.A., D.R. Topliff, D.W. Freeman, R.G. Teeter and B. Stoecker. 1997. The comparison of two forms of sodium in the dietary cation-anion difference equation: Effects on mineral balance in sedentary horses. Proc. 15th Equine Nutr. Physiol. Symp. Ft. Worth, TX. p. 145.

Baker, S.L., E.C. Butterworth and F.A Langley. 1946. The calcium and nitrogen content of human bone tissue cleaned by micro-dissection. Biochem. J. 40:391.

Barengolts, E.I., D.J. Curry, M.S. Bapna and S.C. Kukreja. 1993. Effects of endurance exercise on bone mass and mechanical properties in intact and ovariectomized rats. J. Bone Min. Res. 8:937.

Barzel, U.S. 1969a. The effects of chronic acid and alkali administration on bone turnover in adult rats. Clin. Sci. 36: 517.

Barzel, U.S. 1969b. The effect of excessive acid feeding on bone. Calc. Tiss. 4:94.

Beck, N. and S.K. Webster. 1976. Effects of acute metabolic acidosis on parathyroid hormone action and calcium mobilization. Amer. J. Physiol. 230:127.

Beeson, W.M., T.W. Perry, N.L. Jacobson and K.D. Wiggers. 1976. Calcium in beef and dairy nutrition. NFIA, West Des Moines, Iowa.

Beighle, D.E., W.B. Tucker and R.W. Hemken. 1988. Intercations of dietary cation-anion balance and phosphorus: Effects on growth and serum inorganic phosphorus in dairy calves. J. Dairy Sci. 71:3362.

Bell, R.R., D.Y. Tzeng and H.H. Draper. 1980. Long-term effects of calcium, phosphorus and forced exercise on the bones of mature mice. J. Nutr. 110:1161.

Ben-Ghedalia, D., H. Tagari, A. Bondi and A. Tadmor. 1974. Protein digestion in the intestine of sheep. Brit. J. Nutr. 31:125.

Benzie, D., A.W. Boyne, A.C. Dalgarno, J. Duckworth and R. Hill. 1959. Studies of the skeleton of the sheep. III. The relationship between phosphorus intake and resorption and repair of the skeleton in pregnancy and lactation. J. Agric. Sci. 52:1.

Benzie, D., A.W. Boyne, A.C. Dalgarno, J. Duckworth, R. Hill and D.M. Walker. 1955. Studies of the skeleton of the sheep. I. The effect of different levels of dietary calcium during pregnancy and lactation on individual bones. J. Agric. Sci. 46:425.

Best, J.B. 1985. Best and Taylor's Physiological Basis of Medical Practice. Eleventh Edition. Williams and Wilkins.

Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J. Dairy Sci. 67:2939.

Bobilya, D.J., M.G Maurizi, T.L. Veum and W. C. Allen. 1991. A bone biopsy procedure for neonatal pigs. Lab. Anim. 25:222.

Bond, P.L., T.W. Sullivan, J.H. Douglas and L.G. Robeson. 1991. Influence of age, sex, and method of rearing on tibia length and mineral deposition in broilers. Poul. Sci. 70:1936.

Boren, S.R. 1984. The effect of energy and protein density on growth of weanling Quarter Horses. M.S. Thesis. Oklahoma State Univ., Stillwater, OK.

Breur, G.J., R.F. Slocombe, T.D. Braden. 1988. Percutaneous biopsy of the proximal humerus growth plate in dogs. Am. J. Vet. Res. 49:1529.

Carrasco, M.G., M. Gruson, M.C. de Vernejoul, M.A. Denne and L. Miravet. 1988. Osteocalcin and bone morphometric parameters in adults without bone disease. Calcif. Tissue Int. 42:13.

Chestnut, C.H. 1993. Bone mass and exercise. Amer. J. Med. 95(Suppl 5A):34S.

Ching, S.V., M.J. Fetteman, D.W. Hamar, L.A. Nagode and K.R. Smith. 1989. The effect of chronic dietary acidification using ammonium chloride on acid-base and mineral metabolism in the adult cat. J. Nutr. 119:902.

Church, D.C. 1988. The Ruminant Animal: Digestive Physiology and Nutrition. Prospect Heights, IL: Waveland Press Inc.

Coleman, R.E., G. Mashiter, I. Fogelman, K.D. Whitaker, M. Caleffi, D.W. Moss and R.D. Rubens. 1988. Osteocalcin: A potential marker of metastatic bone disease and response to treatment. Eur. J. Cancer Clin. Oncol. 24:1211.

Colombo, G., P.Fanti, C. Yao and H.H. Malluche. 1993. Isolation and complete amino acid sequence of osteocalcin from canine bone. J. Bone Miner. Res. 8:733.

Combs, N.R., E.T. Komegay, M.D. Lindemann, D.R. Notter and F.H. Welker. 1991. Evaluation of a bone biopsy technique for determining the calcium and phosphorus status of swine from weaning to market. J. Anim. Sci. 69:664.

Cox, A. and S. L. Balloun. 1971. Prediction of total skeleton mineralization from individual bone data. J. Poul. Sci. 50:186.

Delaquis, A.M. and E. Block. 1995. Acid-base status, renal function, water, and macromineral metabolism of dry cows fed diets differing in cation-anion difference. J. Dairy Sci. 78:604.

Delmas, P.D. P. Chatelain, L. Malaval and G. Bonne. 1986. Serum bone Gla-protein in growth hormone deficient children. J. Bone Min. Res. 1:333.

Duckworth, J. and R. Hill. 1953. The storage of elements in the skeleton. Nutr. Abstr. Rev. 23:1.

Duda, R.J., J.F. Obrien, J.A. Katzman, J.M. Peterson K.G. Mann and B.L. Riggs. 1988. Concurrent assays of circulating bone Gla-protein and bone alkaline phosphatase: Effects of sex, age and metabolic bone disease. J. Clin. Endocrin. Metab. 66:951.

Edwards, H.M. Jr. 1984. Studies on the etiology of tibial dyschondroplasia in chickens. J. Nutr. 114:1001.

Ellinger, G.M., J. Duckworth, A.C. Dalgarno and M.H. Quenouille. 1952. Skeletal changes during pregnancy and lactation in the rat: Effect of different levels of dietary calcium. Brit. J. Nutr. 6:235.

El Shorafa, W.M., J.P. Feaster and E.A Ott. 1979. Horse metacarpal bone: Age ash content, cortical area and failure stress interrelationships. J. Anim. Sci. 49:979.

Epstein, S., R. McClintock, G. Bryce, J. Poser, C.C. Johnston and S. Hui. 1984. Differences in serum GLA protein with age and sex. Lancet. i:307.

Escabosa, A., C.E. Coppock, L.D. Rowe, W.L. Jenkins and C.E. Gates. 1984. Effects of dietary sodium bicarbonate and calcium chloride on physiological responses of lactating dairy cows in hot weather. J. Dairy Sci. 67: 574.

Field, R.A., M.L. Riley, F.C. Mello, M.H. Corbridge and A.W. Kotula. 1974. Bone composition in cattle, pigs, sheep and poultry. J. Anim. Sci. 39:493.

Fetteman, M.J., J.M. Coble, D.W. Hamar, R.W. Norrdin, H.B. Seim, R.D. Kealy, Q.R. Rogers, K. McCrea and K. Moffat. 1992. Effect of dietary phosphoric acid supplementation on acid-base balance and mineral and bone metabolism in adult cats. Am. J. Vet. Res. 53:2125.

Fletcher, K.L. 1998. The effects of age and exercise on serum osteocalcin concentration in horses. M.S. Thesis. Oklahoma State Univ., Stillwater, OK.

Freeden, A.H., E.J. DePeters and R.L. Baldwin. 1988a. Characterization of acid-base disturbances and effects on calcium and phosphorus balances of dietary fixed ions in pregnant and lactating does. J. Anim. Sci. 66: 159.

Freeden, A.H., E.J. DePeters and R.L. Baldwin. 1988b. Effects of acid-base disturbances caused by differences in dietary fixed ion balance on kinetics of calcium metabolism in ruminants with high calcium demand. J. Anim. Sci. 66: 174.

Galli, M. and M. Caniggia. 1985. Osteocalcin in normal adult humans of different sex and age. Horm. Metab. Res. 17:165.

Gaynor, P.J., P.J. Mueller, J.K. Miller, N. Ramsey, J.P. Goff and R.L. Horst. 1989. Parturient hypocalcemia in Jersey cows fed alfalfa haylage-based diets with different cation to anion ratios. J. Dairy Sci. 72: 2525.

Glowacki, J., C. Rey, M.J. Glimcher, K.A. Cox and J. Lian. 1991. A role for osteocalcin in osteocalst differentiation. J. Cell. Biochem. 45:292.

Goff, J.P., R.L. Horst, F.J. Mueller, J.K. Miller, G.A. Kiess and H.H. Dowlen. 1991. Addition of chloride to a prepartal diet high in cations increases 1,25 dihydroxyvitamin D responses to hypocalcemia preventing milk fever. J. Dairy Sci. 74: 3863.

Gundberg, C.M., M.E. Markowitz, M. Mizruchi and J.F. Rosen. 1985. Osteocalcin in human serum: A circadian rhythm. Clin. Endocrinol. Metab. 60: 736.

Halley, J.T., T.S. Nelson, L.K. Kirby and Z.B. Johnson. 1987. Effect of altering dietary mineral balance on growth, leg abnormalities, and blood base excess in broiler chicks. Poultry Sci. 66: 1684.

Harrison, H.E. and H.C. Harrison. 1963. Sodium, potassium and intestinal transport of glucose, l-tyrosine, phosphate and calcium. Am J. Phsyiol. 205: 107.

Haydon, K.D. and J.W. West. 1990. Effect of dietary electrolyte balance on nutrient digestibility determined at the end of the small intestine and over the total digestive tract in growing pigs. J. Anim. Sci. 68:3687.

Hendrikson, P.A. 1968. Periodontal disease and calcium deficiency. Acta Odontol. Scand. 26:1.

Hill, R. 1962. The provision and metabolism of calcium and phosphorus in ruminants. Wld. Rev. Nutr. Diet. 3:129.

Hope, E., S.D Johnston, R.L. Hegstad, R.J. Geor and M.J. Murphy. 1993. Effects of sample collection and handling on concentration of osteocalcin in equine serum. Am. J. Vet. Res. 54:1017.

Hunt, J.N. 1956. The influence of dietary sulfur on the urinary output of acid in man. Clin. Sci. 15:119.

Hurwitz, S., H.C. Harrison and H.E. Harrison. 1967. Effect of vitamin D_3 on the in vitro transport of calcium by the chick intestine. J. Nutr. 91: 319.

Jackson, J.A., D.M. Hopkins, Z. Xin and R.W. Hemken. 1992. Influence of cation-anion balance on feed intake, body weight gain, and humoral response of dairy calves. J. Dairy Sci. 75: 1281.

Jeffcott, L.B., R.N. McCartney and V.C. Spears. 1986. Single photon absorptiometry for the measurment of bone mineral content in horses. 118:499.

Kass, E.H. 1957. Bacteriuria and the diagnosis of infections of the urinary tract; with observations on the use of methionine as a urinary antiseptic. Arch. Intern. Med. 100:709.

Kilmer, L.H., L.D. Muller and T.J. Snyder. 1981. Addition of sodium bicarbonate to rations of postpartum dairy cows: Physiological and metabolic effects. J. Dairy Sci. 64:2357.

Kirk, S., C.F. Sharp, N. Elbaum, D.B. Endres, S.M. Simons, J.G. Mohler and R.K. Rude. 1989. Effect of long-distance running on bone mass in women. J. Bone Min. Res. 4:515.

Kraut, J.A., D.R. Mishler, F.R. Singer and W.G. Goodman. 1986. The effects of metabolic acidosis on bone formation and bone resorption in the rat. Kidney Int. 30:694.

Kruse, K. and U. Kracht. 1986. Evaluation of serum osteocalcin as an index of altered bone metabolism. Eur. J. Pediatr. 145:27.

Kunkel, M.E., Z.K. Roughhead, E.A. Nichter and J.M. Navia. 1986. The effects of dietary acid stress on bone metabolism in young ovariectomized rats. Brit. J. Nutr. 55:79.

Lemann, J. E.J. Lennon. 1972. Role of diet, gatsrointestinal tract and bone in acid-base homeostasis. Kidney Int. 1:275.

Lemann, J. E.J. Lennon, A.D. Goodman, J.R. Litzow and A.S. Relman. 1965. Net balance of acid loads in subjects given large loads of acid or alkali. J. Clin. Inv. 44:507.

Lemann, J., J.R. Litzow and E.J. Lennon. 1966. The effects of chronic acid loads in normal man: Further evidence for participation of bone mineral defense against chronic meatabolic acidosis. J. Clin. Inv. 45:1608.

Lemann, J., J.R. Litzow, E.J. Lennon and O.A. Kelley. 1967. Studies of the mechanism by which chronic metabolic acidosis augments urinary mineral excretion in man. J. Clin. Inv. 46: 1318.

Lemann, J. and A.S. Relman. 1959. The relation of sulfur metabolism to acid-base balance and electrolyte excretion: The effects of DL-methionine in normal man. J. Clin. Invest. 38:2215.

Lepage, O.M., L. DesCoteaux, M. Marcoux and A. Tremblay. 1991. Circadian Rhythms of osteocalcin in equine serum. Correlation with alkaline phosphatase, calcium, phosphate and total protein levels. Can. J. Vet. Res. 55:5.

Lepage, O.M., M. Marcoux, A. Tremblay and G. Dumas. 1992. Sex does not influence serum osteocalcin levels on Standardbred horses of different ages. Can J. Vet. Res. 56:379.

Lepage, O.M., S. Laverty, M. Marcoux and A. Tremblay. 1990. Serum osteocalcin or bone Gla-protein, a biochemical marker for bone metabolism in horses: Differences in serum levels with age. Can J. Vet. Res. 54:223.

Little, D.A. 1972. Bone biopsy in cattle and sheep for studies of phosphorus status. Australian Vet. J. 48:668.

Malluche, H.H., M.C. Faugere, P. Fanti and P.A. Price. 1984. Plasma levels of Gla protein reflect bone formation in patients on chronic maintenance dialysis. Kidney Int. 26:869.

McDonald, R., J. Hegenauer and P. Saltman. 1986. Age-related differences in the bone mineralization pattern of rats following exercise. J. Gerontol. 41:445.

Meyer, W.A. and M.L. Sunde. 1974. Bone breakage as affected by type housing or an exercise machine for layers. Poul. Sci. 53:878.

Misheff, M.M., S.M. Stover and R.R. Pool. 1992. Corticocancellous bone biopsy from the 12th rib of standing horses. Vet. Surg. 21:133.

Mongin, P. 1980. Electrolytes in nutrition: A review of basic principles and practical application in poultry and swine. Proc. Third Annual Int. Min. Conf. Orlando, FL. p. 1.

Mongin, P. 1981. Recent advances in dietary anion-cation balance: Applications in poultry. Proc. Nutr. Soc. 40: 285.

Myburgh, K.H., T.D. Noakes, M. Roodt and F.S. Hough. 1989. Effect of exercise on the development of osteoporosis in adult rats. J. Appl. Physiol. 66:14.

National Research Council, 1978. Nutrient Requirements of Horses. 4th rev. ed. National Academy Press, Washington, DC p. 3.

National Research Council, 1989. Nutrient Requirements of Horses. 5th rev. ed. National Academy Press, Washington, DC p. 10.

Nelson, M.E., E.C. Fisher, F.A. Dilmaninan,G.E. Dallal and W.J. Evans. 1991. A one-year walking program and increased calcium in postmenopausal women: Effects on bone. Am. J. Clin. Nutr. 53:1304.

Patience, J.F., R.E. Austice and D.R. Boyd. 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64: 457.

Patience, J.F. and M.S. Wolynetz. 1989. Influence of dietary undetermined anion on acid-base status and performance in pigs. J. Nutr. 120:579.

Peeler, H.T. 1972. Biological availability of nutrients in feeds: Availability of major mineral ions. J. Anim. Sci. 35: 695.

Petito, S.L. and J.L. Evans. 1984. Calcium status of the growing rat as affected by diet acidity from ammonium chloride, phosphate and protein. J. Nut. 14: 1049.

Popplewell, J.C., D.R. Topliff, D.W. Freeman and J.E. Breazile. 1993. Effects of dietary cation-anion balance on acid-base balance and blood parameters in anaerobically exercised horses. Proc. Thirteenth Equine Nutr. Physiol. Symp. Gainesville, FL. p. 191.

Price, P.A. and S.A. Bakoul. 1980. 1,25-Dihydroxyvitamin D_3 increases synthesis of the vitamin K-dependent bone protein by osteosarcoma cells. J. Biol. Chem. 255:11660.

Price, P.A. and S.A. Bakoul. 1981. 1,25-Dihydroxyvitamin D_3 increases serum levels of the vitamin K-dependent bone protein. Biochem. Biophys. Res. Commun. 99:928.

Raab, D.M., T.D. Crenshaw, D.B. Kimmel and E.L. Smith. 1991. A histomorphometric study of cortical bone activity during increased weight bearing exercise. J. Bone Min. Res. 6:741.

Raab, D.M., E.L. Smith, T.D. Crenshaw and D.P. Thomas. 1990. Bone mechanical properties after exercise training in young and old rats. J. Appl. Physiol. 68:130.

Ralston, S.L. 1994. The effect of diet on acid-base status and mineral excretion in horses. Equine Practice. 16:10.

Ralston, S.L., C. Puzio and D. Cuddeford. 1993. Dietary carbohydrate, acid-base status and urinary calcium and phosphorus excretion in horses. Proc. Thirteenth Equine Nutr. Physiol. Symp. Gainsville, FL. p. 42.

Robins, S.P. 1994. Biochemical markers for assessing skeletal growth. Eur. J. Clin. Nutr. 48:S199.

Roby, K.A., W. Chalupa, J.A. Orsini, A.H. Elser and D.S. Kronfeld. 1987. Acid-base and electrolyte balance in dairy heifers fed forage and concentrate diets: Effect of sodium bicarbonate. Amer. J. Vet. Res. 48:1012.

Rose, B.D. 1984. Clinical physiology of acid-base and electrolyte disorders. Second Edition. MacGraw Hill Book Co., New York, NY.

Ross, J.G., J.W. Spears and J.D. Garlich. 1994. Dietary electrolyte balance on performance and metabolic characteristics in growing steers. J. Anim. Sci. 72:1842.

Rowland, L.O., J.L. Fry, R.B. Christmas, A.W. O'Steen and R.H. Harms. 1972. Differences in tibia strength and bone ash among strains of layers. Poul. Sci. 51:1612.

Rowland, L.O. and R.H. Harms. 1970. The effect of wire pens, floor pens and cages on bone characteristics of laying hens. Poul. Sci. 49:1223.

Rowland, L.O., H.R. Wilson, J.L. Fry and R.H. Harms. 1968. A comparison of bone strength of caged and floor layers and roosters. Poul. Sci. 47:2013.

Roy, D.R., K.L. Blouch and R.L. Jamison. 1982. Effects of acute acid-base disturbances on renal tubule reabsorption of magnesium in the rat. Am. J. Physiol. 243 (Renal Fluid Electrolyte Physiol. 12): F197.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.

Sauveur, S. and P. Mongin. 1978. Tibial dyschondroplasia, a cartilage abnormality in poultry. Ann. Biol. Anim. Biochem. Biophys. 18: 87.

Schryver, H.F., H.F. Hintz, P.H. Craig, D.E. Hogue and J.E. Lowe. 1972. Site of phosphorus absorption from the intestine of the horse. J.Nutr. 102:143.

Scott, D. 1976. Changes in mineral, water, and acid-base balance associated with feeding and diet digestion and metabolism in the ruminant. Proc. IVth International Symposium on Ruminant Physiology. p. 205.

Scott, D. and G.H. McIntosh. 1975. Changes in blood composition and urinary mineral excretion in the pig in response to acute acid-base disturbance. Quart. J. Exper. Physiol. 60: 131.

Scott, D. and W. Buchan. 1981. Changes in blood composition and urinary mineral excretion in the sheep in response to acute acid-base disturbance. Res. Vet. Sci. 31: 43.

Sorva, R., R. Anttila, M.A. Siimes, A. Sorva, R. Tahtela and M. Turpeinen. 1997. Serum markers of collagen metabolsim and serum osteocalcin in relation to pubertal developement in 57 boys at 14 years of age. Pediatr. Res. 42:528.

Spears, J.W., J.C. Burns and P.A. Hatch. 1985. Sulfur fertilization of cool season grasses and effect on utilization of minerals, nitrogen, and fiber by steers. J. Dairy Sci. 68:347.

Sidney, K.H., R.J. Shephard and J.E. Harrison. 1977. Endurance training and body composition of the elderly. Amer. J. Clin. Nutr. 30:326.

Stewart, P. 1981. How to Understand Acid-Base: A quantitative acid-base primer for biology and medicine. Elsevier, New York.

Swenson, M.J. and W.O. Reece. 1993. Duke's Physiology of Domestic Animals. Eleventh Edition. Ithica, New York: Cornell University Press.

Takagi, H. and E. Block. 1991. Effects of manipulating dietary cation-anion balance on macromineral balance in sheep. J. Dairy Sci. 74:4202.

Taylor, T.G., J.H. Moore and F. Hertelendy. 1960. Variations in the mineral composition of individual bones of the skeleton of the domestic fowl. Brit. J. Nutr. 14:49.

Topliff, D.R., M.A. Kennerly, D.W. Freeman, R.G. Teeter and D.G. Wagner. 1989. Changes in urinary and serum calcium and chloride concentrations in exercising horses fed varying cation-anion balances. Proc. Eleventh Equine Nutr. Physiol. Symp. Stillwater, OK: Oklahoma State University. p. 1.

Tucker, W.B., G.A. Harrison and R.W. Hemken. 1988a. Influence of dietary cation-anion balance on milk, blood, urine and rumen fluid in lactating dairy cattle. J. Dairy Sci. 71: 346.

Tucker, W.B., Z. Xin and R.W. Hemken. 1988b. Influence of dietary calcium chloride on adaptive changes in acid-base status and mineral metabolism in lactating dairy cows fed a diet of sodium bicarbonate. J. Dairy Sci. 71: 1587.

Tucker, W.B. and J.F Hogue. 1990. Influence of sodium chloride or potassium chloride on systemic acid-base status, milk yield, and mineral metabolism in lactating dairy cows. J. Dairy Sci. 73: 3485.

Tucker, W.B., J.F. Hogue, D.F. Waterman, R.S. Swenson, Z. Xin, R.W. Hemken, J.A. Jackson, G.D. Adams and L.J. Spicer. 1991. Role of sulfur and chloride in the dietary cation-anion balance equation for lactating dairy cattle. J. Anim. Sci. 69: 1205.

Wachman, A. and D.S. Bernstein. 1970. Parathyroid hormone in metabolic acidosis: Its role in pH homeostasis. Clin. Orthoped. Related Res. 69: 252.

Walker, G.L., D.M. Danielson, E.R. Peo and R.F. Mumm. 1993. Effect of calcium source, dietary calcium concentration and gestation phase on various bone characteristics in gestating gilts. J. Anim. Sci. 71:3003.

Wall, D.L., D.R. Topliff, D.W. Freeman, D.G. Wagner and J.E. Breazile. 1992 Effects of dietary cation-anion balance on urinary mineral excretion in exercised horses. J. Equine Vet. Sci. 12:168.

Wall, D.L., D.R. Topliff, D.W. Freeman, D.G. Wagner, J.W. Breazile and W.A. Stutz. 1993. Effect of dietary cation-anion balance on mineral balance in the anaerobically exercised horse. Proc. 13th Equine Nutr. Physiol. Symp. Gainesville, FL. p. 50.

Wall, D.L., D.R. Topliff, D.W. Freeman, J.W. Breazile and R.G. Teeter. 1997. The effect dietary cation-anion balance in growing horses. Proc. 15th Equine Nutr. Physiol. Symp. Ft. Worth, TX. p. 145.

Weidman, S.M. and H.J. Rogers. 1950. Studies on the skeletal tissues: The degree of calcification of selected mammalian bones. Biochem. J. 47:493.

West, J.B. 1985. Best and Taylor's Physiological Basis of Medical Practice. Eleventh edition. Baltimore, MD: Williams and Wilkins.

Zetterholm, R. and N. Dalen. 1978. Bone mineral determination in coccygeal vertebrae by dichromatic photon absorptiometry. Acta Vet. Scand. 19:1

Vita

Steven Robert Cooper

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE EFFECT OF DIETARY CATION-ANION DIFFERENCE ON GROWTH AND MINERAL BALANCE IN GROWING HORSES

Major Field: Animal Science

Biographical:

Personal Data: Born in Lubbock, Texas, March, 1970, the son of Mickey and Nancy Cooper

Education: Graduated from New Deal High School, New Deal, Texas, in May 1988; received Bachelor of Science Degree in Animal Production from Texas Tech University in Lubbock in May, 1992; received Master of Science Degree from University of Illinois, Urbana, Illinois in August, 1995; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in December, 1998.

Professional Organizations: Member of Equine Nutrition & Physiology Society and American Registry of Professional Animal Scientists.

Professional Experience: Teaching and Research Assistant, Department of Animal Science, Equine Section, Oklahoma State University, January, 1995 to present; Assistant Coach, OSU Horse Judging Team, January, 1995-1997.