THE COMPARISON OF TWO FORMS OF SODIUM AND POTASSIUM AND CHLORIDE VERSUS SULFUR IN THE DIETARY CATION-ANION BALANCE EQUATION AND SUBSEQUENT EFFECTS ON ACID-BASE STATUS AND MINERAL BALANCE IN SEDENTARY HORSES

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CHAPTER I

INTRODUCTION

In today's competitive horse industry, proper nutrition is imperative to gaining the full genetic potential of the horse, regardless of the events taken part in, or the production state of the animal. Numerous studies dealing with the the impact of various nutrients on equine physiology and performance have demonstrated that proper nutrition can and does improve such factors as growth, skeletal soundness, reproductive efficiency and exercise performance. One specific area of study that is relatively new to equine researchers is dietary cation-anion balance (DCAB). This equation refers to the ratio of the strong inorganic cations sodium (Na^+) and potassium (K^+) to the strong inorganic anion (Cl^-) in the diet, and is defined as: meq (Na + K) - Cl / kg diet dry matter. Sodium, potassium and chloride are critical for the maintenance of osmotic balance as well as the acid-base status of the animal. Recently, dairy researchers have included the anion sulfur (S^-) in the DCAB equation (meq (Na + K) - (Cl + S) / kg diet dry matter), as it has been observed to have similar anionic effects on the acid-base status of the animal. This ratio of cations to anions in the diet has also been referred to as Dietary Electrolyte Balance, Dietary Cation-Anion Difference and the Strong Ion Difference by researchers in other species. Altering this ratio by

increasing (excess sodium or potassium in relation to chloride or sulfur) or decreasing (excess chloride or sulfur in relation to sodium or potassium) has been shown to have substantive effects on the acid-base status of the animal, as well as such production parameters as growth (swine), structural soundness and eggshell quality (poultry), and prevention of milk fever in dairy cows.

At the present time, the National Research Council's Nutrient Requirements for Horses (NRC, 1989) has no specific recommendations regarding the DCAB for any class of horses, or state of production. Recent research at Oklahoma State University has shown that as the DCAB increases, (addition of Na^+, K^+), the acid-base status is increased, and apparent daily calcium balance is increased as well. Furthermore, increasing the DCAB has been shown to have a beneficial effect on equine exercise performance. On the other hand, decreasing the DCAB, (addition of Cl^-) has been shown to have a deleterious effect on the acid-base status of the animal as well as on daily calcium balance in both sedentary and exercising horses. However, there has been some debate as to the exact cause of the increase in performance noted when the DCAB is increased, i.e. whether the beneficial effects were due to the form of cation fed (e.g. $NaHCO_3$) or to the cation itself. Furthermore, it is not currently known whether the addition of sulfur has the same anionic effect in the equine as does the addition of chloride.

This study was undertaken to quantify and compare the effects of two different forms of sodium and potassium (forms of $NaHCO_3$, $KHCO_3$, Na-citrate and K-citrate). and that of sulfur versus chloride, on the acid-base status and mineral balance in mature, sedentary horses.

CHAPTER II

LITERATURE REVIEW

HISTORY OF DIETARY CATION-ANION BALANCE

In the past, much attention has been paid to the requirements for maintenance and production of the major nutrients, i.e. protein, carbohydrate and recently, fat, as well as vitamins and minerals to a lesser extent. While the other nutrients have been subdivided into subclasses (e.g., carbohydrates as structural and nonstructural; vitamins as fat or water soluble), minerals present quite a different picture. This nutrient class contains many individual components required individually by the animal. However, minerals have only been divided into two subclasses, macro and micro, and these classifications are based not on any biological significance or function, but on the concentration of the mineral found in the body. However, minerals rival any of the other nutrients as far as necessity to the animal as they are integral components of many biological functions including expression and regulation of genes, enzyme systems that regulate cellular function, osmotic balance, detoxification, acid-base balance and in structural roles (e.g. bone).

The dietary fixed cations, sodium (Na^+), and potassium (K^+), the fixed anion chloride (Cl^-), and the acidogenic anion sulfur (S^-) have recently received a great deal of attention concerning their role in the dietary cation-anion balance

(DCAB, also referred to as dietary electrolyte balance or strong ion difference). Sodium and chloride have traditionally been supplied in the diet in the form of common salt (NaCl) added to the concentrate portion of rations. Furthermore, most livestock rations today contain an adequate amount of potassium as most moderate to high quality hays contain more than adequate amounts to meet the needs of the animal. More important, however, is the amount of these minerals in relation to others in ration ingredients and supplements. It has become apparent to dairy cattle, poultry and swine, and recently equine nutritionists as well, that the ratio between these cations and anions in the diet has a major impact on animal nutrition.

The concept of balancing rations for cations and anions is not new in animal nutrition and was first applied to chickens. One of the earliest researchers to propose an equation which would define a balance of the nonmetabolizable strong ions sodium, potassium and chloride in the diet was Mongin (1980). This equation took into account only these monovalent elements as they seemed to have the most metabolic impact on acid-base physiology, and they appear to be the ones most readily available via absorption from the gut (Austic, 1988). The equation is defined as follows: meq $(Na^+ + K^+) - Cl^-$/100g diet dry matter, and uses the units milliequivalents (meq), as opposed to millimoles, as these elements produce their physiological effects on the body according to their valence rather than their gram molecular weight. Since then, dairy cattle, swine, poultry, beef cattle and equine researchers have conducted numerous experiments dealing with the effect of DCAB on acid-base status, calcium balance, growth, dry matter digestibility and structural soundness.

There are some misconceptions regarding the DCAB equation, however. Block (1994) stated that no reaction occurs when Na^+ or K^+ form alkalis, but these ions (as well as Cl^- and SO_4^-) indirectly affect the H^+ concentration in the

body via buffer systems, kidney function and cellular respiration. Therefore, the DCAB of a diet does not affect the acidogenic or alkalogenic properties of the feeds, but affects metabolic processes of the body after absorption.

According to Stewart (1981), the H^+ and HCO_3^- concentrations are dependent, not independent variables, and are dependent upon strong ions, Na^+, K^+, Cl^-, SO_4^-, the partial pressure of CO_2 (pCO_2), and weak acids such as albumin. This author refers to the strong ion difference (SID) calculated as (Sum of all strong base cation concentrations) - (Sum of all strong acid anion concentrations), and that this value in body fluids is almost always positive and is approximately +40 meq/l.

DCAB Effect on Other Species

Rabbit

The cation-anion balance of the diet was first reported to have an effect on physiological factors by Morgen and Berger (1915). These researchers demonstrated that sodium carbonate was more effective than sodium chloride in attempting to increase the mineral content in rabbit bones. These authors suggested that the carbonate salt acted to increase the alkaline reserve. Thacker (1959) inferred from this hypothesis that calcium, potassium, sodium and magnesium deficiencies could be caused by the manipulation of the individual level, and by the cation-anion balance of the ration. Thacker (1959) demonstrated that rabbits fed a ration based on timothy hay grown in heavily fertilized soil which previously had not supported proper growth, hemoglobin or bone ash levels (Keener and Thacker, 1958), was rendered adequate by the addition of a salt of sodium, potassium, magnesium or calcium carrying an anion

capable of being oxidized to CO_2 and H_2O in the animal. It was also suggested that the mineral imbalance of the diet induced a calcium and potassium deficiency in the unsupplemented animals even though the diet contained adequate levels of these elements.

Rat

Studies concerning the influence of DCAB in the rat have concentrated on the effects on bone physiology. Barzel and Jowsey (1969) demonstrated that rats consuming ammonium chloride for a long period of time had increased bone resorption. However, this loss of bone tissue was prevented by the ingestion of sodium and potassium carbonate, apparently by the stimulation of bone formation. This physiological response of the bone was attributed to changes in systemic acid-base balance, and it was suggested that the intracellular mechanism controlling calcium deposition and resorption in the bone was sensitive to systemic pH.

Newell and Beauchene (1975) investigated the effects of acid stress and age on renal, serum and bone responses in 13 and 25 month old rats fed ammonium chloride at 2% of the diet for nine months. The acid stressed animals showed significant decreases in urinary pH, and also significant increases in urinary calcium, phosphorus and total H^+ excretion. However, they observed no effect on calcium content of the bone due to the diet. Petito and Evans (1984) evaluated the effects of ingestion of ammonium chloride, phosphates and protein on calcium status in growing rats. Ammonium chloride was fed to the treatment group of animals at 1.0% of the diet. Treatment rats had decreased blood pH and increased urinary cAMP and calcium concentrations. Furthermore, treatment rats had a two-fold increase in fecal calcium, and had lower specific gravity of the femur. Beck and Webster (1976)

suggested that metabolic acidosis inhibits the tubular reabsorption of calcium in the nephron, and that this inhibition, coupled with the ingestion of ammonium chloride may explain how cAMP and calcium could both be excreted in the urine at higher levels. Goulding and Campbell (1984) demonstrated that rats fed salt supplements excreted more calcium in the urine and had less calcium in the bone as compared to control.

Poultry

Poultry nutritionists were the first livestock nutritionists to recognize and study the effects of dietary cation-anion balance on production traits. Early research in this area concentrated on the effects the elements sodium, potassium and chloride had on growth and food consumption through their roles in the maintenance of osmotic pressure and acid-base status of the animal.

Neishiem and coworkers (1964) demonstrated that chicks suffered decreases in growth rate when fed excesses of dietary chloride or sulfate supplied as glutamic acid hydrochloride, calcium chloride or calcium sulfate. However, this decreased growth rate was alleviated by supplying equimolar amounts of potassium or sodium supplied as salts of glutamate or carbonate. These researchers also demonstrated that chicks suffered decreased growth rate when fed excess sodium supplied as sodium glutamate. This depression in growth was alleviated when equivalent amounts of chloride were fed. Melliere and Forbes (1966) performed a similar study and demonstrated that food consumption and growth were maximized when chicks were fed a cation-anion ratio of 1.2 to 1.8. A ratio of 0.6 cation to anion almost completely inhibited growth. These authors also reported that sodium chloride or potassium chloride did not reduce food consumption or weight gain when added to the diet at levels equal to the highest amount of hydrochloride. Feeding excess calcium did not

alleviate the depression in growth demonstrated by feeding excess chloride. However, excess magnesium intake partially alleviated this depression in growth.

During the 1960's, a group of researchers began to study the effect of acid-base balance on egg shell calcification in the hen (Frank and Beger, 1965; Howes, 1967; Anderson, 1967; Mongin, 1968). All of these studies demonstrated that the calcification process of the egg shell could be altered by manipulating the acid-base status of the laying hen. Feeding diets with a low DCAB resulted in decreased calcification of the egg shell.

Cohen, Hurwitz and Bar (1972) studied the effects of dietary sodium and chloride on blood pH, pCO_2, HCO_3^-, Cl^-, Na^+ and egg shell formation in laying hens. These authors hypothesized that dietary sodium and chloride were the alkalogenic and acidogenic agents, respectively, and that the acid-base response would depend on the ratio between these two components. It was demonstrated that excess dietary sodium fed with a constant level of dietary chloride produced an alkalosis, and excess dietary chloride fed with a constant level of sodium produced an acidosis. When sodium and chloride were added to the total diet in equal amounts, no differences in the acid-base balance of the animal were detected. Therefore, these researchers determined that the acid-base status of the body, as measured by blood pH, pCO_2 and HCO_3^-, was a function of the ratio of sodium to chloride, and not the absolute amount of either. These researchers also stated that the actual pH of the diet was irrelevant in producing a metabolic alkalosis or acidosis. Feeding pH neutral calcium chloride caused an acidosis, whereas feeding the acid salt sodium monophosphate caused an alkalosis.

Cohen and Hurwitz (1974) studied the response of blood parameters to dietary sodium, potassium and chloride in laying hens. These authors

demonstrated that supplemental sodium or potassium in the diet resulted in an increase in blood pH and HCO_3^-, while the supplementation of chloride resulted in a decrease in these same parameters. These findings suggested that the responses of sodium and potassium are additive in offsetting the metabolic acidosis caused by excess dietary chloride. These findings agreed with those of Neshiem et al. (1964) who demonstrated that growth retardation caused by excess dietary chloride could be alleviated with the addition of sodium and potassium salts, devoid of chloride, to the diet.

As stated previously, Mongin (1980) was the first to propose a cation-anion balance equation using the elements sodium, potassium and chloride. This equation $((Na^+ + K^+) - Cl^-))$/100g diet DM could be used to quantify the acid or base generating power of the diet. The author's defense of this equation was based on the results of two experiments. The first, performed by Mongin and Saveur (1973) demonstrated that animals fed diets with a range of -20 to +40 meq/100 g diet DM had plasma bicarbonate levels linearly related to that sum. The second experiment was performed by Hurwitz et al. (1973) who demonstrated that animals fed diets containing equivalent amounts of sodium and potassium had a blood pH markedly dependent on dietary chloride.

Hamilton and Thompson (1980) demonstrated a decrease in blood pH, bicarbonate level and eggshell strength in hens when the chloride level in the diet was increased from .11 to 2.13%. These findings agreed with those of Hall and Helbacka (1959), Hunt and Aitken (1962) and Saveur and Mongin (1971) who reported that eggshell calcification was depressed in hens fed excessive levels of acid chlorides. Furthermore, it has been demonstrated that egg shell strength was increased when hens were fed a diet with increased cations (Frank and Burger, 1965; Howes, 1967; Mongin, 1968). Austic (1984) also observed a

decrease in strength and thickness of the eggshell from hens consuming diets with excess dietary chloride.

The dietary cation-anion balance has also been associated with developmental bone abnormalities in fowl, particularly tibial dyschondroplasia (TD). Leach and Neshium (1965) described this disorder in young chicks and later discovered that this condition could be affected by the cation-anion balance of the diet (Leach and Neshium, 1972). Saveur and Mongin (1978) reported an increase in the incidence of TD resulting from metabolic acidosis caused by excessive dietary chloride. Halley et al. (1987) studied the effect of dietary mineral balance on growth, leg abnormalities and blood base excess in chicks. It was observed that base excess was negatively correlated with 3-week body weights and the incidence of TD. These findings agreed with later work that demonstrated the relationship between the anionic content of the diet and a subsequent alteration in acid base status and higher incidence of TD (Edwards, 1984; Hamilton and Thompson, 1980; Hurwitz et al., 1973; Mongin, 1981).

Riley and Austic (1983) studied the effects of dietary electrolytes on digestive tract pH and acid-base status of chicks. The cation-anion balance of the diet was altered by the addition of potassium bicarbonate or calcium chloride. It was observed that chicks consuming a diet with excess chloride had decreased plasma HCO_3^-, base excess of the blood and pCO_2. The pH of the crop was also depressed by dietary chloride, however, the pH of the proventriculus, duodenum, or middle and distal portions of the small intestine were not affected.

Swine

In the early 1980's, swine researchers took note of the effect of cation-anion balance in the diet. Yen et al. (1981) studied the effect of calcium chloride

as a regulator of feed intake and weight gain in pigs. It was demonstrated that crossbred barrows fed a basal diet with 4% $CaCl_2$ had lower daily feed intake, weight gain and gain/feed ratios as compared to those fed the basal diet alone. Those pigs fed $CaCl_2$ also had lower blood pH, HCO_3^-, tCO_2 (total CO_2 in the blood) and base excess. These parameters were restored to normal levels in pigs fed a diet containing calcium chloride and sodium bicarbonate.

Patience et al. (1987) fed 8 - 12 week old pigs five rations with electrolyte balances (defined in this study as meq (Na + K) - Cl/kg diet DM) ranging from -85 to +341. It was observed that growth and feed intakes were maximized in those pigs fed diets with an electrolyte balance between 0 and +341, while values for these variables decreased in those pigs consuming the lowest electrolyte balance (-85 meq/kg diet DM). Furthermore, as the electrolyte balance in the diet dropped below a base level of +175 meq/kg diet DM, blood pH and HCO_3^- levels dropped, which is evidence of a metabolic acidosis. Golz and Crenshaw (1984) studied the effects of sodium, potassium and chloride on growth in young swine. These authors suggested that dietary potassium and chloride levels have an interactive effect on gain when the sodium level is held constant. Optimum growth occurred when the K to Cl ratio was approximately 2:1 (.57% K and .27% Cl) and the sodium level in the diet was held between .03 and .60%.

Haydon and West (1990) examined the effects of dietary cation-anion balance on nutrient digestibility in growing pigs. Apparent nutrient digestibilities were determined by fitting the animals with ileal T-cannulas. Experimental diets consisted of a corn-soybean meal base, and electrolyte balance was altered by substituting $CaCl_2$ for $CaCO_3$, or $NaHCO_3^-$ for corn and soybean meal, resulting in four experimental diets with cation-anion balances of -50, +100, +250 and +400 meq/kg diet dry matter. Apparent preileal digestibility increased linearly for

N, energy, dry matter and all amino acids, except alanine and methionine, as the electrolyte balance of the diet increased. Furthermore, blood pH, tCO_2, HCO_3^- and base excess concentrations increased with increasing dietary cation-anion balance.

Dairy Cattle

Coppock (1986) reviewed the current literature on the effect of DCAB on production parameters in livestock. At that time, there was very little interest in this area by dairy cattle researchers. Coppock evaluated and calculated the DCAB in various beef and dairy experiments that had been conducted. It was suggested that the ruminant could more easily withstand a higher DCAB than could poultry. Escobosa et al. (1984) demonstrated that cows consuming a diet with a negative cation-anion balance exhibited decreased feed intake. Since then, much progress has been made studying the effects of DCAB on production traits in dairy cattle.

Block (1984) studied the effects of DCAB on the incidence of parturient paresis (milk fever) in dairy cows. Previous research had indicated a relationship between dietary anions and an increased calcium availability for lactation (Ender et al., 1971; Dishington, 1975; Lomba et al., 1978). Block (1984) demonstrated that cows fed a highly anionic diet (-128 meq/kg diet DM) during the dry period had significantly decreased incidences of parturient pariesis during lactation. Tucker et al. (1988) studied the effects of DCAB on milk, blood, urine and rumen fluid in lactating dairy cattle. It was observed that increasing the DCAB from -100 to +200 meq/kg diet DM resulted in a linear increase in blood pH and HCO_3^-, while actual milk yield was increased 8.6%. These researchers also noted that the responses observed, except for blood

bicarbonate, could be attributed to the DCAB itself, and not the effects of a single ion.

Because maintaining the blood pH at a constant level is critical for normal body function, several mechanisms in the body exist to maintain the concentration of HCO_3^- to pCO_2 in the blood at a constant ratio. This control is accomplished by a respiratory response by adjusting the respiration rate to control the blood levels of pCO_2, and a renal response by adjusting the excretion of bicarbonate to control blood bicarbonate concentrations (Tucker et al., 1988).

Beighle et al. (1990) reported that dairy calves fed diets with a low cation to anion ratio had higher concentrations of phosphorus in the blood and feces versus those calves fed diets with a higher cation-anion ratio. Those calves fed the low DCAB also showed lower concentrations of phosphorus in the bone. These researchers noted that when a low phosphorus diet was fed along with the low DCAB, these effects were amplified, indicating an interaction between DCAB and dietary phosphorus on the changes seen in blood, bone and fecal phosphorus concentrations.

Tucker et al. (1992) studied the influence of dietary sodium bicarbonate on potassium metabolism in young calves. Feed intake was not affected by supplemental potassium chloride or sodium bicarbonate, however, average daily gain increased with increased potassium and decreased with increased sodium bicarbonate. Urinary calcium excretion also declined with increased sodium bicarbonate while urine pH increased. Tucker et al. (1991a) also studied the influence of calcium chloride on systemic acid-base balance and calcium metabolism in dairy heifers. It was observed that urinary calcium excretion and blood free proton concentration (H^+) increased with increasing dietary $CaCl_2$, while blood HCO_3^- and urine pH decreased. These authors suggested that the increased Ca excretion in the urine was due to either an increased bone

mobilization or increased intestinal absorption of calcium. It was also noted that increasing the dietary level of chloride caused a subsequent increase in both plasma chloride and excretion of chloride in the urine.

Goff et al. (1991) studied the effects of the addition of chloride to a prepartal diet fed to dairy cows which was also high in cations. It was observed in this study that cows fed highly anionic diets, parathyroid hormone (PTH) had a greater effect on renal production of 1,25 dihydroxyvitamin D, resulting in increased intestinal absorption of calcium. Furthermore, osteoclastic bone resorption was more responsive to PTH as plasma hydroxyproline concentration was higher in cows fed the highly anionic diet. These researchers stated that the addition of anions to the diet was thought to induce a metabolic acidosis in the cow, which facilitated bone calcium resorption (Block, 1984). This is in agreement with Beck and Webster (1970) who indicated that bone, and perhaps renal tissue in rats is refractory to the effects of PTH in the alkaline state and the stimulatory effects of PTH are enhanced during metabolic acidosis. It has been observed in dairy cattle and poultry (Tucker, 1988; Austic, 1984) that this increased PTH activity is a possible cause for increased levels of ionized or free calcium in the blood and subsequent increased urinary calcium excretion.

Oetzal et al. (1991) studied the effects of six different anionic salts, $MgCl_2$, $MgSO_4$, $CaCl_2$, $CaSO_4$, NH_4Cl and $(NH_4)_2SO_4$, on palatability, acid-base status and urinary mineral excretion in nonlactating, nonpregnant cows. None of the anionic salts (DCAB of -170 meq/kg diet DM) decreased dry matter intake or blood pH, but did decrease blood HCO_3 and base excess. Urinary pH was also decreased, and fractional urinary excretion of Ca was increased by all treatments. These diets were only fed for one week periods, however, which might explain the unaltered blood pH.

Gaynor et al. (1989) fed preparturient dairy cows three diets with a DCAB of +220, +600 and +1260 meq/kg diet DM. The cows consuming the lowest DCAB excreted significantly more Ca and Mg in the urine compared to the other two diets and had higher concentrations of 1,25 dihydroxyVitamin D at three days prepartuition as well. These cows also had lower dry matter intakes as compared to those consuming the two diets with higher DCAB. Wang and Beede (1992) fed diets with added NH_4Cl and S to dry Jersey cows. Those cows fed the treatment diets had lower blood pH, higher concentrations of ionized Ca in the blood, higher urinary excretion of Ca and lowered urine pH.

Tucker et al. (1991b) studied the significance of S or Cl in the DCAB equation in lactating dairy cows. It was observed that Cl or S had similar acidogenic effects, as cows consuming diets with either anion had decreased blood HCO_3^- and urine pH, and increased urinary and plasma Ca. In addition, the plasma cation-anion balance was decreased by the addition of either anion. This author stated that it may be necessary to include a modifying coefficient for S in the DCAB equation, as the dietary source of S may have an effect on its absorption, and the variety of organic and inorganic forms in which S may be absorbed and used by the body adds to the variability of its effect on the acid-base status of the animal.

Jackson and Hemken (1984) studied the effects of dietary cation-anion balance on body weight gain and humoral response in dairy calves. These authors observed no difference in feed intake due to altered DCAB. Calves fed the highest DCAB (+130 meq/kg) did gain more, however. Blood pH, pCO_2 and HCO_3 were all lower, and urine pH and urinary Ca and Cl excretion were higher in calves fed the lowest DCAB (-180meq/kg). Furthermore, it was observed that the breaking strengths for the 7th and 9th ribs were higher for calves consuming the highest DCAB.

Sheep

Takagi and Block (1991a) studied the effect of DCAB on macromineral balance in sheep. Wethers were fed two levels of Ca (high and low) with three DCAB's (High Ca +284, +61, -27, Low Ca +343, +218 and +63 meq/kg). Wethers consuming the low DCAB had a reduced Ca retention due to a significant increase in urinary Ca excretion, however apparent absorption of Ca was similar between treatments. Takagi and Block (1991b) studied Ca kinetics in wethers, simulating the Ca loss during lactation by the infusion of ethalene glycol tetraacetate. The animals responded to this induced Ca loss by increasing both the true intestinal absorption of Ca and the amount of bone resorption. Treatments also produced an increase in ionized plasma Ca. These authors concluded that the size of the total exchangeable Ca pool did not differ between treatments. However, the reduced DCAB's increased the amount of Ca flux through the pool, particularly when Ca demand by the body was increased.

Beef Cattle

Ross et al. (1994) studied the effects of DCAB on performance in finishing beef steers. Steers were fed high concentrate, low roughage diets with a DCAB of 0, +150, +300 or +450 meq/kg diet DM. Average daily intake increased linearly with increasing DCAB the first 28 days, then quadratically the remainder of the study. During the entire feeding period, daily gain and carcass marbling scores tended to increase quadratically with increased DCAB. furthermore, gain and feed intake were maximized at a DCAB of +150 meq/kg.

Equine

Mineral Requirements

Sodium. The National Research Council (NRC), (1989) stated that in many cases, the sodium concentration of natural feedstuffs for horses is lower than 0.1%. Sodium is therefore commonly added to the diet in the form of sodium chloride (common salt or trace mineralized salt) at a rate of 0.5 to 1.0%. Sodium is often described as the major extracellular cation and has a major role in acid-base status and the osmotic regulation of body fluids. The optimal sodium concentration of the diet has been reported to be between 1.6 and 1.8g/kg diet dry matter for growth, maintenance and late gestation and 3.6 g/kg diet dry matter for moderate to heavy work (Jarrige and Martin-Rosset, 1981). Since there is limited data on specific requirements for sodium and the effect of physical activity and environment on the animals requirements, the NRC (1989) does not make specific recommendations for sodium intake. However, it is stated that the sodium concentration in the maintenance diet should be no lower than 0.1%.

Potassium. Potassium is the major intracellular ion involved with acid-base balance and the osmotic regulation of body fluid. The NRC (1989) lists the potassium concentration of forages and oilseed meals at 1 to 2% of dry matter, and of common cereal grains (corn, wheat and oats) at 0.3 to 0.4%. Hintz and Schryver (1976) estimated that mature horses required potassium at a level of .06 g/kg of body weight/day, or approximately 0.4% of the diet. Therefore, if forage constitutes a significant portion of the diet, than potassium requirements should easliy be met. Drepper and others (1982) estimated the potassium

requirements for a 600 kg horse to be 22 g/day for maintenance. Based on this research, the NRC (1989) estimated the potassium requirement for maintenance to be 0.05 g/kg of body weight or 1.52 g/Mcal of DE.

Chloride. Chloride is an important extracellular anion involved in acid-base balance, osmotic regulation, as a minor component of bile, and in the formation of hydrochloric acid which is an important component of gastric secretions necessary for proper digestion. However, chloride requirements of horses have not been established, and requirements are thought to be met when sodium requirements are met with salt (NaCl).

Magnesium. Magnesium is important as an activator of many enzymes, and has been observed to have interrelationships with other electrolytes, second messengers, hormone receptors, PTH secretion and action, Vitamin D metabolism and bone functions. The ever important Na - K 'pump' protein which maintains the electrical potential of cell membranes is also dependent on this mineral. The magnesium concentration of common feedstuffs has been listed at 0.1 to 0.3% (NRC, 1989). Researchers have estimated that the true absorption of magnesium from feeds is between 40 and 60% (Hintz and Schryver, 1972;, Meyer, 1979) and between 42 and 45% (McKenzie, 1981). Drepper et al. (1982) proposed a daily magnesium requirement of 12 g for maintenance in a 600 kg horse. Using the conservative value of 40% absorption efficiency, the NRC (1989) suggests a magnesium requirement of approximately 15 mg/kg body weight/day, or .46 g/Mcal DE.

Sulfur. The requirements for sulfur in the horse have received very little attention by researchers. Feeding adequate, high quality dietary protein will

usually provide a minimum of 0.15% organic sulfur. According to Jarrige and Martin-Rosset (1981) and the NRC (1989), that amount is adequate to meet the horses needs.

Calcium. The 1989 NRC estimates the true absorptive efficiency of calcium at approximately 70% in young horses and 50% in mature horses. For the purpose of estimating calcium requirements for all classes of horses, however, the NRC (1989) suggests a value of 50% absorptive efficiency be used, due to the possibility of calcium being bound to phytates in feed, rendering it unavailable to the animal. Using this value, the calcium requirement for maintenance is stated to be .04 g/kg of body weight/day or 1.22 g/Mcal of DE/day.

Phosphorus. The efficiency of phosphorus absorption in the horse is variable due to the age of the horse and the source and concentration of phosphorus in the diet. The NRC (1989) estimates that the efficiency of phosphorus absorption ranges between 30 and 55%. However, the NRC (1989) uses the more conservative figure of 35% for horses at maintenance, gestating mares and horses performing work as they all consume mainly plant sources of phosphorus. Using the above values, the NRC (1989) lists the phosphorus requirements for maintenance at 28.6 mg/kg of body weight/day or 0.87 g/Mcal of DE/day.

Equine Studies

In 1970, Schryver and others studied the effect of calcium intake on skeletal metabolism and the calcium homeostatic mechanisms of young, growing

ponies. Three levels of calcium were fed: low Ca (.15% of total diet), intermediate Ca (.80% of total diet) and high Ca (1.50% of total diet). Ca was supplied in the form of calcium carbonate at the expense of hay and corn in the diet. These researchers also used a kinetic analysis with a radioactive isotope of calcium so that more accurate determinations of the rate of exchange of calcium between body fluids and bone, and the rate of deposition and removal of calcium from the bone, could be measured. These researchers observed a large variation between groups for intake, excretion and retention of calcium in order to maintain calcium homeostasis, but there was no difference in the concentration of calcium in the plasma or on the size of the exchangeable pool. Ponies fed the low calcium diet had increased fractional absorption of calcium and had a decreased renal excretion rate. Furthermore, bone resorption was increased above the deposition rate resulting in a net transfer of calcium from the bone into the exchangeable pool. Despite these homeostatic mechanisms, these ponies experienced a net negative calcium balance. These researchers also observed opposite responses in ponies fed the high calcium diet. Unlike the rate of removal, however, the deposition rate of calcium was insensitive to the dietary level of calcium.

In a later study, Schryver and coworkers (1971a) studied the effect of high dietary phosphorus levels on calcium utilization and skeletal metabolism in growing Shetland ponies fed .4% calcium and either .2% or 1.2% phosphorus in the diet. Ponies fed the high phosphorus diet were observed to have increased phosphorus retention and plasma levels, while calcium absorption, renal excretion and retention all decreased, while total and endogenous fecal calcium excretion increased. Furthermore, Schryver et al. (1971b) showed that renal phosphorus excretion, total phosphorus absorption from the gut and phosphorus

retention were all dependent on phosphorus intake. The efficiency of phosphorus absorption averaged 45% across all diets.

In 1987, Schryver and others studied the effects of voluntary salt intake in mature, sedentary horses and its effect on mineral metabolism. Diets containing 1, 3 and 5% NaCl were fed, with a mean daily salt consumption ranging from 19 to 143 g with a mean of 53 g. These researchers observed that fecal excretion of calcium was higher in those horses consuming the 1% NaCl diet, and determined that calcium absorption and retention were greater at the higher levels of NaCl consumption. Furthermore they observed that phosphorus absorption and retention were greater at both the 3 and 5% levels of NaCl intake. These researchers stated that urinary sodium excretion was directly related to intake and that urinary excretion was the primary excretory path for sodium, as fecal excretion, intestinal absorption and retention of sodium were not affected by intake.

Young et al. (1989) evaluated the extent of mineral losses in feces, urine and sweat in miniature horses at rest and during exercise. During the exercise period, daily sodium intake increased and there was a trend for daily fecal excretion of sodium to increase. Also, urinary excretion of sodium decreased, possibly due to large amounts of sodium being lost in the sweat. Furthermore, both daily chloride intake and fecal concentration of chloride increased, contradictory to the work of Schryver et al. (1987). During the exercise period, daily intake of potassium and fecal excretion of potassium increased, while daily calcium intake and fecal excretion increased, resulting in an increase in daily calcium retention. Furthermore, both the daily intake and the daily fecal excretion of phosphorus increased, resulting in an increase in daily P retention.

Milne (1974) studied the effects of exercise on blood parameters, acid-base balance and electrolyte levels. This author proposed a linear relationship

between the changes in arterial and venous blood pH, pCO_2 and HCO_3^- in response to exercise, and suggested that arterial blood parameters could be predicted from venous blood values, with the exception of pO_2.

Numerous researchers have studied the effects of sodium bicarbonate infusion (dosing) on exercising horses. The impetus for these studies has come from empirical evidence from various race trainers that the intubation of sodium bicarbonate to horses before a race has a beneficial effect on the horses performance. Trainers use sodium bicarbonate because it has the potential to counteract the metabolic acidosis produced during a race (Lawrence et al., 1990).

Recently, many researchers have studied the effect of varying doses of sodium bicarbonate on acid-base status and serum mineral profiles in exercising and sedentary horses (Kelso et al., 1987; Lawrence et al., 1987, 1990; Roberts et al., 1991; Corn, et al., 1993; Kline et al., 1993; Hanson et al., 1993; Ferrante et al., 1993). In all of these studies, sodium bicarbonate was administered via nasogastric tube in doses ranging from 200 to 1000 mg/kg of body weight in an effort to determine the effects of dosage on blood HCO_3^- concentrations at the time of the exercise. In all instances, the infusion of sodium bicarbonate resulted in an increase in blood pH and HCO_3^-. However, the highest blood levels of these variables varied from 2 - 6 hr post intubation.

The increase in plasma HCO_3^- and decreased H^+ concentrations after infusion of sodium bicarbonate have been assumed to be directly attributable to the ingestion of HCO_3^- (Ferrante et al., 1993). However, as stated by Stewart (1981), and according to basic chemical principles, HCO_3^- concentration is a dependent variable. The most efficient buffer pair in the body is that of $H_2CO_3/NaHCO_3^-$, due to the high concentrations of HCO_3^- in the plasma relative to that of the other three major buffer pairs in the body. However, for HCO_3^- to

serve in that pair, it must be attached to a Na^+ ion (Breazile, 1990). Therefore, higher concentrations of Na (and possibly K) supplied in the diet would result in an increase in the amount of $NaHCO_3^-$ (and possibly $KHCO_3^-$) available in the plasma to facilitate the removal of H^+ out of the muscle during intense exercise. Recently, racetracks around the country have taken a hard stance against the use of $NaHCO_3^-$ dosing in horses (Snow, 1992). One reason is that when the infusion of $NaHCO_3^-$ is coupled with the use of furosemide, a diuretic, renal K excretion is exacerbated and the electrical potential of cardiac cells is altered. This alteration may lead to an arrhythmic heartbeat and possibe heart attack in intensely exercised horses.

DCAB Studies

Topliff et al. (1989) studied the effect of a low (+6.5 meq/kg) vs. a high (+150 meq/kg) DCAB on calcium and chloride metabolism in exercising mares. No change in serum calcium or chloride concentrations were observed. Horses consuming the low DCAB excreted more calcium in the urine (84.7 mg/dl) compared to those consuming the high DCAB (9.2 mg/dl). Those horses consuming the low DCAB also excreted more total calcium per day, as total urine output was not different. Furthermore, those horses consuming the low DCAB excreted greater amounts of chloride in the urine (176.1 meq/l) as opposed to those consuming the high DCAB (124.8 meq/l). This increased urinary excretion of calcium and chloride was attributed to the acid producing power of the diet.

Stutz et al. (1992) studied the effects of DCAB on blood variables in exercising horses. Four diets were fed with DCAB's of +5 (L), +107 (ML), +201 (MH) and +327 (H) meq/kg dry matter. Treatments were formed by the addition

of calcium chloride and ammonium chloride to diet L, calcium chloride to diet ML and sodium bicarbonate and potassium citrate to diet H. While at rest, those horses consuming diet L had lower venous blood pH, pCO_2 and HCO_3^- values as compared to those consuming the MH and H diets. However, no differences were observed in blood pH or acid-base parameters between treatments from 0 to 30 min post exercise.

Wall et al. (1991) evaluated the effects of DCAB on acid-base status and urinary mineral excretion in exercising horses. The diets fed were the same as in the trial performed by Stutz et al. (1992). Urine pH decreased significantly as the DCAB decreased. Furthermore, horses consuming the low DCAB excreted more calcium and chloride in the urine, compared to those consuming the medium high and high DCAB. Also, horses excreted more sodium in the urine when consuming the high DCAB compared to those consuming the other diets. The author stated that, depending on the calcium intake, exercising horses consuming a low DCAB could experience a negative daily calcium balance.

Baker et al. (1991) fed sedentary geldings diets with a DCAB of +21, +125, +231 and +350 meq/kg diet DM, and observed that urine pH decreased as the DCAB decreased. Also, arterial and venous blood pH, pCO_2 and HCO_3^- was decreased in horses consuming the lowest DCAB as compared to all other diets.

In 1993, Ralston and coworkers studied the effects of DCAB and high starch (DCAB 292 meq/kg diet DM) versus low starch (DCAB 200 meq/kg diet DM) diets on acid-base status and urinary Ca and P excretion. Blood pH was lower in horses consuming the high starch diet at 2 hr post feeding, and urine pH was lower at 8 hr post feeding as compared to those consuming the low starch diet. The diets were determined to have DCAB of +292 meq/kg DM (high starch) and +200 meq/kg DM (Low starch). However, no differences were detected in base excess of the blood, blood HCO_3^- or tCO_2.

Popplewell et al. (1993) studied the effects of DCAB on the acid-base status and blood parameters in the exercising horse. Experimental diets had cation-anion balances of +10, +95, +165 and +295 meq/kg diet DM. Urine pH was lower in horses consuming the low DCAB as compared to all other diets. Arterial and venous blood pH, HCO_3^-, pCO_2 and base excess increased as the DCAB increased pre-exercise, and all blood parameters were lower in horses consuming the low DCAB than all other diets at 60 min. post exercise. Blood lactate concentrations were higher, and heart rates were lower post exercise at all times in those consuming the high DCAB compared to all other diets. Times for the standard exercise test were also lower in those horses consuming the high DCAB compared to those consuming the low DCAB.

Baker et al. (1993) reported on the effects of DCAB on dry matter digestibility and mineral balance in both exercised and sedentary horses. In exercising horses, dry matter digestibility was lower for those consuming the lowest DCAB as compared to those consuming the high DCAB. No differences were detected in apparent Cl or Mg balance in sedentary horses, although apparent daily Cl balance was higher and apparent daily Mg balance was lower in exercising horses consuming the low DCAB as compared to exercising horses consuming all other diets. In sedentary horses, apparent Ca balance decreased significantly as the DCAB decreased, while in exercising horses, apparent Ca balance was higher in horses consuming the high DCAB as compared to those consuming the low DCAB.

In a review of dietary fixed ions and performance in dairy cows, Freeden (1993) hypothesizes on the effects of various salts of Na^+, K^+, Ca^{+2}, Mg^+, Cl^-, S^- (as SO_4^-) and P (as phosphoric acid). The author stated that both Na and K, if fed in the form of $NaHCO_3^-$ or $KHCO_3^-$, should be alkologenic, except in the case of $KHCO_3^-$ fed above the requirement in which case it could be acidogenic due

to a hypertonicity effect resulting in dilution of HCO_3^-. The author also stated that Cl, in the form of $CaCl_2$ and NH_4Cl would be acidogenic, while S^- in the form of SO_4, $CaSO_4$ and $MgSO_4$ would also be acidogenic, but less so when the accompanying cation is absorbed.

It is evident from experiments in swine, poultry, dairy cattle and other species that the cation-anion balance of the diet can have a significant effect on the acid-base status in the animal, as well as on various production parameters. It is also evident from mineral studies in other species and from those in the horse that DCAB can have a major effect not only on the acid-base status of the animal but also on mineral metabolism and apparent daily balance, particularly calcium. It was the purpose of the present study to compare the effects of two different sources of sodium and potassium, (Na Citrate, K Citrate, $NaHCO_3^-$, $KHCO_3^-$) and to compare the anionic acidogenic power of sulfur versus chloride on the DCAB equation, and the subsequent effects on the acid-base status and apparent mineral balance in sedentary horses.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

Six mature stock type geldings, four Quarter Horse type and two Paint horses, were used in a 6x6 Latin Square design experiment to compare the effects of two sources of sodium and potassium and that of chloride versus sulfur in the dietary cation-anion balance equation (DCAB) and the subsequent effects on the acid-base status and apparent mineral balance in sedentary horses. The 13 week trial consisted of six 12 day dietary adjustment periods followed by a 72 hour sample collection period.

Horses were individually stalled and were turned into an outdoor arena for free exercise for a minimum of 3 hours daily. Horses were fed at 8 AM and 8 PM daily. All horses were immunized and dewormed prior to, and received routine health care throughout, the trial.

Experimental Treatments

Experimental diets were produced at the Oklahoma State University Feedmill, and consisted of a pelleted base concentrate of corn, soybean meal and cottonseed hulls. The concentrate was fed in a 60:40 ratio with native

prairiegrass hay grown on the Oklahoma State University Animal Science Farm. The complete diet was fed in amounts to maintain a constant body weight throughout the trial. The six diets were formulated with the addition of anionic or cationic salts to achieve the desired cation-anion balance. Sulfur, as sulfate, was added at .65% to diet Low-Sulfur (L:S). Diet Low-Chloride (L:Cl) was supplemented with .65% ammonium chloride. The High Potassium Citrate diet (H:KC) was supplemented with 2.25% potassium citrate, while the High Potassium Bicarbonate diet (H:KB) was supplemented with 2.10% potassium bicarbonate. The High Sodium Citrate diet (H:NaC) was supplemented with 2.10% sodium citrate, while the High Sodium Bicarbonate diet (H:NaB) was supplemented with 1.75% sodium bicarbonate (Table I). Diets were formulated to contain 2.5 Mcal/kg DM and 9.5% crude protein across all treatments (Table II). Diets were analyzed and determined to contain approximately equivalent amounts of calcium, phosphorus and magnesium. Actual dry matter concentrations of the minerals in the total experimental diets are given in Table II. The varying concentration of these minerals resulted in dietary cation-anion balances of +0 (L:S), +53 (L:Cl), +405 (H: KC), +364 (H:KB), +360 (H:NaC) and +409 (H:NaB), on a dry matter basis.

Blood Collection

Venous blood samples were drawn on the first day of each collection period via 18 gauge catheter placed in the jugular vein approximately one hour prior to the first collection. Blood samples were then drawn just prior to the morning feeding, and hourly thereafter for 11 hours post feeding. Approximately 3 ml of blood was injected into a heparanized vacutainer blood collection tube and placed on ice until analyzed for blood

Table I. COMPOSITION OF DIETS, AS FED BASIS

Ingredient %	*L:S*	*L:Cl*	*H:KC*	*H:KB*	*H:NaC*	*H:NaB*
Ground Corn	35.00	33.00	33.00	33.00	33.00	33.00
Soybean Meal	5.70	6.00	6.00	6.00	6.00	6.00
Cottonseed Hulls	15.15	16.90	15.25	15.40	15.40	15.75
Limestone	.30	.30	.30	.30	.30	.30
Dical Phosphate	.50	.50	.50	.50	.50	.50
TMS	.50	.50	.50	.50	.50	.50
Chromic Oxide	.20	.20	.20	.20	.20	.20
Ammonium Chloride	---	0.65	---	---	---	---
Sulfur	0.65	---	---	---	---	---
Potassium Citrate	---	---	2.25	---	---	---
Potassium Bicarbonate	---	---	---	2.10	---	---
Sodium Citrate	---	---	---	---	2.10	---
Sodium Bicarbonate	---	---	---	---	---	1.75
Prairiegrass Hay	40.00	40.00	40.00	40.00	40.00	40.00
DCAB (meq/kg DM)	**+0**	**+53**	**+405**	**+364**	**+360**	**+409**

TABLE II. DIET ANALYSIS (DRY MATTER BASIS)

Constituent	*L:S*	*L:Cl*	*H:KC*	*H:KB*	*H:NaC*	*H:NaB*
DE, Mcal/kg	2.53	2.50	2.46	2.47	2.47	2.48
CP%	9.55	9.49	9.40	9.42	9.41	9.43
Calcium %	.56	.54	.57	.55	.55	.60
Phosphorus %	.37	.38	.37	.36	.36	.40
Magnesium %	.14	.16	.14	.12	.14	.14
Potassium %	.72	.79	2.07	1.95	.77	.79
Sodium %	.37	.36	.36	.35	1.11	1.30
Sulfur %	1.11	.12	.11	.12	.10	.13
Chloride %	.66	1.00	.66	.65	.64	.66
DCAB	**+0**	**+53**	**+405**	**+364**	**+360**	**+409**

gasses within one hour of collection. All blood samples were analyzed for pH, pCO_2, tCO_2, HCO_3^-, base excess, blood (BEb) and base excess, extracellular fluid (BEecf), using a blood gas analyzer (Instrumentation Laboratory Model 1304, Lexington, Ma.). An additional 10 ml of blood was allowed to clot for a minimum of 1.5 hr, centrifuged at 3600 rpm and resultant serum drawn and frozen for later mineral analysis. Venous serum samples were analyzed using a Roche^R Cobas Mira Automated Wet Chemistry Analyzer (Nutley, New Jersey).

Urine Collection

Total urine production was collected, beginning on the first day of each collection period, via urine harnesses, every 4 hr for 72 hr. The volume of urine produced was recorded for every four hour period. A 50 ml sample was analyzed for pH using a Fischer Accumet Model 950 pH meter with a standard glass body combination electrode which accounts for sample temperature. This pH meter was standardized prior to each four hour collection. After analysis of pH, these samples were then acidified with concentrated HCl at 3% of total volume and frozen for later mineral analysis. A separate 25 ml sample was taken at each interval and frozen for later analysis of chloride.

Fecal Collection

Fecal samples were obtained 6 times randomly over 72 hours of each collection period so that every 2 hours post feeding was represented. Chromic oxide was added at 2% of the total diet as an indigestible marker for the determination of fecal volume. Each sample was identified by horse, treatment

number and time and all samples were immediately frozen in freezer bags for later mineral analysis.

Laboratory Analyses

Urinary Mineral

Calcium, Sodium, Potassium and Magnesium

For analysis of calcium content of the urine, the composite samples were diluted with a .5% La + .1% K solution for a dilution rate of 1:2241.1 and analyzed on a Perkins-Elmer Model 4000 Atomic Absorption Spectrophotometer. For analysis of sodium, composite samples were diluted with distilled, deionized water for a final dilution rate of 1:10,000. Samples were analyzed using an Atomic Absorption Spectrophotometer using a 1 ppm standard. For the analysis of potassium, composite samples were diluted with a .1% La solution for a final dilution rate of 1:5102.04 or 1:15625.01. Samples were analyzed using an Atomic Absorption Spectrophotometer using a 2 ppm standard. For the analysis of magnesium, composite samples were diluted with a .1% La + .1% K solution for a final dilution rate of 1:5102.04. Magnesium concentration was determined using an Atomic Absorption Spectrophotometer, using a .400 ppm standard.

Phosphorus

For the analysis of phosphorus, 10 microliters of composited horse-trreatment-time urine sample was dispensed along with 1 ml of .4 mM ammonium molybdate in HSO_4 acid reagent (Sigma chemical #360-UV) into glass culture tubes. A 5 mg/dl P standard was used and samples were read on a Gilford Spectrophotometer at 340 nm.

Chloride

Urine chloride concentration was determined via potentiometric titration using a 100 meq Cl/l standard solution and were read on an HBI Digital Chloridometer (Haake Buchler Instruments, Inc.).

Sulfur

For the analysis of urinary sulfur, 2 ml of urine from each horse, period and trweatment combination was placed in a 50 ml erlenmyer flask. Five ml of a digestion mixture was then added to each flask. The digestion mixture contained 1.7 g ammonium metavanadate in 1050 ml concentrated nitric acid, 1200 ml of perchloric acid, and 7.5 g of potassium dichromate in 250 ml distilled, deionized water. A funnel was then placed in the neck of each flask, and flasks were heated on a hot plate at 80 degrees C for approximately 15 min until initial reaction was over. The digestion was then continued until the perchloric acid was fuming strongly and an orange-red precipitate appeared. Flasks were removed from heat and allowed to cool. Twenty five ml of an acid mixture, containing 50 ml glacial acetic acid, 20 ml HCl and 20 ml ortho-phosphoric acid in one L distilled, deionized water, was then added to the flasks. Flasks were then filled to volume with distilled, deionized water. A stock sulfur standard was made by dissolving 5.4341g of dried potassium sulfate into 1 l distilled deionized H_2O. Working standards were then made by pipetting 1, 2, 3, 4, 6, 8 and 10 ml of stock standard into 100 ml flasks. Two ml of a solution containing 15 mg potassium dichromate/ml, 5 ml of perchloric acid and 50 ml of acid mixture was then added to each flask, and flasks were filled to volume with distilled, deionized H_2O. Two ml of the digested samples and working standards were pipetted with 1 ml of a solution containing 100 g barium chloride and 50 ml

Tween 80 diluted to 1 L into glass culture tubes. Samples were read on a Gilford Spectrophotometer at 623 nm.

Fecal and Feed Analysis

Sodium, Potassium, Magnesium and Calcium

For the analysis of fecal Na, K, Mg and Ca, approximately 2 g of dried fecal sample was ashed for 5 hr at 500 degrees C. 10 ml of 20% HCl was then added to each beaker and covered with a watch glass. Samples were then digested on medium heat for approximately 10 min. Samples were then filtered into 100 ml flasks. Samples were then diluted with the appropriate diluent for each mineral, and read on a Perkins-Elmer Atomic Absorption Spectrophotometer. For the analysis of feed Na, K, Mg and Ca, 1 gram of composited dried sample was dried at 55 degrees C for 24 hours, weighed, and dried again for two hours until a final dry weight was confirmed. Samples were then ashed at 500 degrees C for four hours. Two ml of 1:1 HCl was then added and samples were then boiled on a hot plate at a temperature between 150 and 200 degrees F until evaporated to dryness. Twenty five ml of a blank solution containing 1.5 n HNO_3 and 0.5 n HCl was added and samples were then analyzed using Inductively Coupled Plasma Spectroscopy (Model ICAP61 Therno Jarrell-Ash).

Phosphorus

For the analysis of fecal concentration of phosphorus, 2 g of dried sample were ashed at 600 degrees C for 6 hr. Forty ml of 25% HCl and 2 ml of nitric acid was then added, and samples were digested by being brought to a boil on a hot plate. Samples were then transferred to 100 ml flasks and brought to volume

with distilled, deionized water. A 10 ml aliquot was then transferred into a 100 ml flask and 20 ml of Ammonium - Molybdovanadate reagent was added. Samples were then diluted to volume with distilled, deionized water, and read at 400 nm. For the analysis of feed P concentration, 1 gram of composited dried sample was dried at 55 degrees C for 24 hours, weighed, and dried again for two hours until a final dry weight was confirmed. Samples were then ashed at 500 degrees C for four hours. Two ml of 1:1 HCl was then added and samples were then boiled on a hot plate at a temperature between 150 and 200 degrees F until evaporated to dryness. Twenty five ml of a blank solution containing 1.5 n HNO_3 and 0.5 n HCl was added and samples were then analyzed using Inductively Coupled Plasma Spectroscopy (Model ICAP61 Therno Jarrell-Ash).

Chloride Analysis

For the analysis of fecal and feed chloride, 1 g of dried composited fecal sample and 1 g of each individual experimental diet was ashed for 4 hours at 500 degrees C. Before ashing, 20 ml of sodium carbonate was added to the dried sample to prevent the loss of chloride during ashing. After cooling, 20 ml of 20% nitric acid was added to the sample. Samples were then boiled on a hot plate on a setting of Low for 30 minutes. Samples were then transferred to 50 ml volumetric flasks, and the flasks were filled to volume with distilled, deionized water. Chloride concentrations were then determined via potentiometric titration using an HBI Digital Chloridometer (Haake Buchler Instruments, Inc.).

Sulfur

For the analysis of feed and fecal sulfur, approximately 3 g of sample was ashed at 500 degrees C for 5 hr. Five ml of a digestion mixture was then added to each flask. The digestion mixture contained 1.7 g ammonium metavanadate

in 1050 ml concentrated nitric acid, 1200 ml of perchloric acid, and 7.5 g of potassium dichromate in 250 ml distilled, deionized water. A funnel was then placed in the neck of each flask, and flasks were heated on a hot plate at 80 degrees C for approximately 15 min until initial reaction was over. The digestion was then continued until the perchloric acid was fuming strongly and an orange-red precipitate appeared. Flasks were removed from heat and allowed to cool. Twenty five ml of an acid mixture, containing 50 ml acetic acid, glacial, 20 ml HCl and 20 ml ortho-phosphoric acid in one L distilled, deionized water, was then added to the flasks, and they were then filled to volume with distilled, deionized water. A stock sulfur standard was made by dissolving 5.4341g of dried potassium sulfate into 1 l distilled deionized H_2O. Working standards were then made by pipetting 1, 2, 3, 4, 6, 8 and 10 ml of stock standard into 100 ml flasks. Two ml of a solution containing 15 mg potassium dichromate/ml, 5 ml of perchloric acid and 50 ml of acid mixture was then added to each flask, and flasks were filled to volume with distilled, deionized H_2O. Two ml of the digested samples were pipetted with 1 ml of a solution containing 100 g barium chloride and 50 ml Tween 80 diluted to 1 L and samples were read on a Gilford Spectrophotometer at 623 nm.

Chromium

Approximately .4 g of fecal and .5 g of feed sample was placed in oven-dried 100 ml beakers, and the air dried sample weight was recorded. Samples were then placed in drying ovens for 24 hours at 60 degrees C. After cooling in dessicators, the beaker and sample were reweighed to determine oven dried sample weight. Samples were then ashed at 500 degrees C for 4 hours. Six ml of an acid mixture (1000 ml DDH, 500 ml H_2SO_4 and 500 ml H_3PO_4) was then added to the ashed sample. Samples were then placed on a hot plate and

brought to a boil at a setting of 6. Three ml of $KBrO_3$ was added, and the sample was boiled for .5 to 1 minute after SO_3 fumes appeared. The beakers were then allowed to cool to room temperature for 10 minutes. Twenty ml of dilute Bromate was then added and the mixture was brought to a boil at a setting of 4. When the sample changed from clear to milky, the beaker was removed from the hot plate and allowed to cool. The sample was then transferred to 100 ml volumetric flasks and filled to volume with DDH. The flasks were then capped and inverted 3 times. Five ml was transferred to centrifuge tubes and 7.5 ml of 5% NaOH was added. After 15 minutes, the tubes were vortexed and allowed to settle for 15 minutes. The sample tubes were then centrifuged at 2000 rpm for 15 minutes. Samples and standards were then analyzed for chromium concentration on a spectrophotometer (Gilford Response Series UV-VIS Spectrophotometer, Ciba Corning Diagnostics Corporation.), and read at 400 nm.

Statistical Analysis

Data for blood gasses and urine pH were analyzed using a repeated measures model, with horse, period and treatment as the main effects and time as the repeated variable. Least squares means over time were then calculated and tested for significance using the pdiff procedure. Significance was declared at $p < .05$ (SAS, 1985). Data for daily excretion of urinary H^+, mineral concentrations, as well as daily fecal mineral concentrations, apparent mineral balances and dry matter digestibility were analyzed using the general linear models procedure with horse, period and treatment as the main effects. Least squared means were then calculated and significance was declared at $p < .05$ using the pdiff procedure (SAS, 1985). Standard errors for urine and fecal excretions and mineral balances were averaged over all treatments because of a

missing cell in the Latin Square design due to one horse being removed from the study after complete refusal of the L-S diet.

CHAPTER IV

RESULTS AND DISCUSSION

Urine pH

The effect of treatment over time on urine pH is shown in Table III and graphically in Figure 1. The effect of DCAB on daily urinary H^+ excretion is shown in Table IV. Least square mean urine pH values were not significantly different for those horses consuming diets L:S and L:Cl, and as expected, were both significantly lower than values for horses consuming the diets with high DCAB. Furthermore, there was no significant difference in least squares mean urine pH values among those horses consuming the high diets at any time post feeding. Least squares means ranged from 5.97 to 6.19 on diet L:S, 5.65 to 5.82 on diet L:Cl, 8.57 to 8.73 for diet H:KC, 8.44 to 8.67 on diet H:KB, 7.96 - 8.79 on diet H:NaC and 8.15 to 8.57 on diet H:NaB. As pH is a log scale of H^+ concentration, a more clearly defined picture of actual H^+ excretion may be gained by charting daily H^+ excretion (Table IV). Least squares means for daily H^+ excretion agreed with the data for urine pH, as those horses consuming diets L:S (22,856.01 neq/d) and diet L:Cl (43,838.26) were not significantly different from one other ($p = .056$). Furthermore, daily H^+ excretion for those horses consuming diets H:KC, H:KB, H:NaC and H:NaB (least squares means of 25.40, 38.50, 44.40 and 55.20, respectively)

were also not significantly different from one another, and again were all significantly lower as compared to diets L:S and L:Cl

These data for urine pH values for horses consuming anionic diets agrees with that of Oetzal, et al. (1991) and Tucker et al. (1991) who observed similar effects when feeding highly anionic diets by the addition of Cl or S. Additionally, the lowered urine pH for those horses consuming diet L:Cl agrees with that of previous experiments in both sedentary and exercising horses (Baker et al., 1992, Wall et al., 1992; Popplewell et al., 1993). This decrease in urinary pH may be attributed to the increase in urinary chloride (L:Cl) and sulfur (L:S) excretion by the kidneys in response to a lowered SID in the blood plasma caused by the increased intake of these anions. Increasing the excretion of Cl and S is the response by which the animal attempts to restore the SID in the plasma to a normal level, and therefore decrease the concentration of circulating H^+ ions. When excess Cl or S is excreted in the urine, the plasma SID is raised, causing H^+ ions to reassociate with OH^- to form H_2O, thereby reducing the concentration of circulating H^+ in the plasma. This same reaction occurs in the filtrate of the kidneys, as excess Cl and S enters, more H_2O dissociates to H^+ and OH^-, with the positive charge of H^+ balancing the negative charge of the Cl^- and S^- ions.

Table III. EFFECT OF DCAB ON URINE pH POST FEEDING*

	Treatment						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
8 AM[a]	6.06[b]	5.76[b]	8.66[c]	8.58[c]	8.55[c]	8.38[c]	0.245
12 PM	5.97[b]	5.66[b]	8.69[c]	8.67[c]	8.63[c]	8.44[c]	0.193
4 PM	6.19[b]	5.65[b]	8.73[c]	8.51[c]	8.31[c]	8.15[c]	0.219
8 PM[a]	6.08[b]	5.80[b]	8.69[c]	8.53[c]	8.46[c]	8.31[c]	0.238
12 AM	6.02[b]	5.67[b]	8.68[c]	8.67[c]	8.79[c]	8.57[c]	0.266
4 AM	6.00[b]	5.82[b]	8.57[c]	8.44[c]	7.96[c]	8.32[c]	0.373

[a] Indicates Feeding Time

[b,c] LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

Table IV. EFFECT OF DCAB ON DAILY URINE H^+ EXCRETION, (neq)*

	Treatment						
	L:S	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
	22856.01^{b}	43838.26^{b}	25.41^{a}	38.52^{a}	44.42^{a}	55.26^{a}	6949.66

^{a,b} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

Blood Acid-Base Status

Blood pH

The effect of DCAB on venous blood pH is shown in Table V and graphically in Figure 2. There was no significant difference detected in blood pH values in those horses consuming diets L:S and L:Cl at 8 of the 12 intervals measured post feeding. Those horses consuming diet L:Cl had lower ($p < .05$) blood pH values as compared to those horses consuming all high diets at 10 of 12 intervals measured, and lower than all but diet H:NaC at the other 2 intervals measured. Those horses consuming diet L:S had lower ($p< .05$) blood pH values as compared to those consuming all high diets at 6 of the 12 intervals measured, and lower than two of the four high diets 5 of the other 6 intervals measured.

There was no difference detected in blood pH values among those horses consuming the four high diets at all intervals measured, except for 0 and 3 hr post feeding. These data agree with that of Oetzal et al. (1991) and Tucker et al. (1991) in dairy cows fed anionic diets supplemented with Cl and S. The data for blood pH in those horses consuming diets L:Cl and the high diets also agrees with previous work in horses (Baker et al. 1992; Stutz et al., 1992; Popplewell et al; 1993).

According to Stewart (1981), H^+ and HCO^-_3 concentrations in the blood are dependent upon the strong ion difference, or SID. According to the theory of electrical neutrality of body fluids, as excess strong cations (Na^+ and K^+) are absorbed from the gastrointestinal tract into the blood, they must be balanced by a loss of a positively charged ion. It is possible that when excess Na^+ and K^+ enter the plasma, this causes H^+ ions to reassociate with a OH^- ion to form H_2O,

thereby causing a reduction in the concentration of H^+ in the plasma, and hence, an increase in blood pH. It has also been postulated that as excess Na^+ is absorbed across the luminal epithelium of the intestine, it is in exchange for a H^+ ion (Best and Taylor, 1991). However, this theory is unlikely because of the significant gap in total concentrations of Na^+ and H^+ in the plasma, as the concentration of H^+ is extremely low relative to that of Na^+. Accordingly, as excess Cl^- or S^-, is absorbed into the blood, they must be balanced by the loss of a negatively charged ion, or the gain of a positively charged ion with osmolarity a limiting factor to the latter. It is posssible that as excess Cl^- or S^- is enters the plasma, H_2O is caused to dissociate to H^+ and OH^-, thereby increasing the concentration of H^+ in the plasma. It is also widely held that excess Cl^- is absorbed across the luminal epithelium of the gastrointestinal tract, it is in exchange for a HCO^-_3 ion (Best and Taylor, 1991), resulting in a decrease in circulating HCO^-_3 in the plasma and a subsequent metabolic acodosis. It is likely that both theories may be at least partially correct as the cause of the increase in concentration of H^+ and decrease in blood pH in those horses consuming the L:S and L:Cl diets.

The osmolarity of blood plasma is approximatley 300 milliosmols/l, or 300 milliequivalents of particles per liter of blood. Of that total, approximately 140 meq are Na, 110 meq are Cl, 28 - 32 meq are HCO_3^-, 3 - 5 meq are K, 2.5 meq are Ca and 5-6 meq are glucose, with the remaining a combination of proteins, free amino acids, urea, sulfates, phosphates and lactate (Best and Taylor, 1991). The body tightly regulates this osmolarity, and keeps the total close to 300 milliosmols/l. In the plasma, there is a balance of ions known as the anion gap, which is defined as: $([Na^+] + [K^+]) - ([Cl^-] + [HCO^-_3])$, and the body will maintain this ratio of ions within a very specific range (Breazile, 1990). As the amount of chloride in the plasma increases, the body will reduce the amount of

TABLE V. EFFECT OF DCAB ON VENOUS BLOOD pH POST FEEDING*

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	7.384^{abc}	7.358^{a}	7.415^{b}	7.413^{bc}	7.384^{ac}	7.414^{b}	.0103
1	7.352^{ac}	7.325^{a}	7.367^{bc}	7.391^{b}	7.389^{b}	7.382^{bc}	.0097
2	7.350^{a}	7.339^{a}	7.386^{b}	7.401^{b}	7.395^{b}	7.401^{b}	.0104
3	7.369^{a}	7.361^{a}	7.402^{bc}	7.381^{ac}	7.397^{bc}	7.413^{b}	.0086
4	7.374^{a}	7.356^{a}	7.415^{b}	7.411^{b}	7.403^{b}	7.407^{b}	.0082
5	7.363^{a}	7.354^{a}	7.410^{b}	7.413^{b}	7.397^{b}	7.408^{b}	.0086
6	7.370^{b}	7.345^{a}	7.403^{c}	7.402^{c}	7.401^{c}	7.398^{c}	.0066
7	7.372^{a}	7.352^{a}	7.416^{b}	7.408^{b}	7.405^{b}	7.422^{b}	.0095
8	7.386^{b}	7.357^{a}	7.414^{c}	7.409^{c}	7.408^{bc}	7.409^{bc}	.0076
9	7.388^{b}	7.358^{a}	7.416^{c}	7.413^{c}	7.408^{bc}	7.410^{bc}	.0073
10	7.389^{ab}	7.369^{a}	7.418^{bc}	7.439^{c}	7.429^{c}	7.432^{c}	.0098
11	7.389^{b}	7.360^{a}	7.413^{c}	7.416^{c}	7.417^{c}	7.418^{c}	.0058

^{a,b,c,d} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

bicarbonate to maintain the anion gap. By definition, a metabolic acidosis, as opposed to a respiratory acidosis, is one caused by a decrease in blood HCO_3^-.

Although the exact mechanism by which plasma H^+ increases with excess Cl^- or S^-, and decreases with excess Na^+ or K^+ is unknown, these data suggest that increased absorption of S has a similar, if not quite as pronounced, effect on decreasing the pH of the blood, while the citrate and bicarbonate forms of both Na and K appear to have the same effect on increasing the pH of the blood.

Blood pCO_2

The effect of DCAB on venous blood pCO_2 is shown in Table VI and graphically in Figure 3. There were no differences detected in blood pCO_2 values in those horses consuming diets L:Cl and L:S at 11 of the 12 intervals measured. Those horses consuming the L:Cl diet had lower ($p< .05$) pCO_2 values as compared to those horses consuming all high diets at 7 of the 12 intervals measured, while those consuming diet L:S had lower ($p< .05$) pCO_2 values as compared to those consuming all high diets at 3 of the 12 intervals measured. There was no difference detected in blood pCO_2 values among those horses consuming any of the high diets at 9 of the the 12 intervals measured. These data are in agreement with previous work in horses (Baker et al., 1992; Stutz et al., 1992; Popplewell et al., 1993).

The decrease in blood pCO_2 values in those horses consuming diet L:Cl is primarily due to the increase in H^+ and concurrent decrease in HCO^-_3 concentration in the plasma. Under these conditions, the body responds by increasing alveolar ventilation which results in a decrease in pCO_2, as the two factors are inversely related (Tucker et al., 1988). This is an example of the respiratory response of the animal to a state of metabolic acidosis.

TABLE VI. EFFECT OF DCAB ON VENOUS BLOOD pCO_2 (mmHg) POST FEEDING*

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	47.91^{ab}	45.61^{a}	48.50^{b}	50.16^{bc}	51.91^{c}	49.56^{bc}	1.02
1	50.60^{ab}	50.30^{a}	55.13^{c}	52.48^{abc}	52.00^{ab}	53.50^{bc}	1.19
2	48.83^{ab}	47.71^{a}	51.90^{b}	48.98^{ab}	49.63^{ab}	49.76^{ab}	1.28
3	48.63^{ab}	47.15^{a}	49.73^{ab}	49.95^{ab}	50.08^{b}	47.73^{ab}	1.19
4	48.04^{bc}	45.51^{a}	47.76^{b}	48.83^{bc}	49.31^{bc}	49.80^{c}	0.82
5	47.55^{ab}	45.21^{a}	48.26^{b}	48.61^{b}	49.40^{b}	49.00^{b}	1.15
6	46.19^{a}	45.60^{a}	49.20^{b}	49.48^{b}	49.35^{b}	50.38^{b}	0.81
7	47.68^{ab}	44.26^{a}	48.53^{b}	49.13^{b}	49.46^{b}	48.23^{b}	1.40
8	46.16^{a}	45.01^{a}	48.63^{b}	49.08^{b}	48.95^{b}	50.10^{b}	0.76
9	46.32^{a}	46.75^{a}	49.28^{ab}	50.15^{b}	50.61^{b}	50.71^{b}	1.17
10	46.31^{a}	46.21^{a}	48.96^{b}	48.15^{ab}	47.51^{ab}	48.46^{ab}	0.95
11	45.99^{a}	46.01^{a}	49.63^{b}	50.20^{b}	48.75^{b}	49.78^{b}	0.95

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

Blood HCO_3^-

The effect of treatment on venous blood HCO_3^- is shown in Table VII and graphically in Figure 4. Horses consuming diet L:Cl had lower ($p< .05$) blood HCO_3^- values as compared to those consuming diet L:S at 6 of the 12 intervals measured. Horses consuming diet L:S had lower ($p< .05$) blood HCO_3^- values as compared to those consuming all high diets at 11 of the 12 intervals measured, while those consuming diet L:Cl had lower values ($p < .05$) as compared to those consuming all high diets at all intervals measured. There was no difference detected in blood HCO_3^- values among those horses consuming the high diets at any interval measured.

When excess chloride or sulfur is absorbed from the gastrointestinal tract, the body must balance the increase of negatively charged strong ions by increasing the dissociation of H_2O to H^+ and OH^-, while decreasing the amount of HCO^-_3 to maintain the anion gap. This HCO^-_3 could come from the dissociation of carbonic acid: $H_2CO_3 \rightarrow H^+$ and HCO^-_3, which is subsequently exchanged at the intestinal lumen level for Cl^-. Again, it is possible that both reactions are responsible for the reduction in HCO_3^- observed when strong anions (Cl^- or S^-) are fed in excess.

It would appear that the increase in blood HCO_3^- concentrations after the feeding of $NaHCO_3^-$ or $KHCO_3^-$ could be directly attributed to the HCO_3^- ion. However, in the stomach, the HCO_3^- is titrated by HCl, resulting in the production of NaCl or KCl and carbonic acid: $NaHCO_3^-$ (or $KHCO_3^-$) + HCl $\rightarrow$ NaCl (or KCl) + H_2CO_3. The carbonic acid then dissociates to H_2O and CO_2. The NaCl or KCl is then absorbed and dissociates into Na^+ (or K^+) and Cl^-. The Na or K then is able to combine with HCO_3^- (exchanged at the parietal cell for Cl^-) resulting in an increase in $NaHCO_3^-$, or $KHCO_3^-$. However, it appears from

TABLE VII. EFFECT OF DCAB ON VENOUS BLOOD HCO_3^- (mmol/l)*

POST FEEDING**

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	28.92^{b}	26.08^{a}	31.38^{c}	32.31^{c}	32.31^{c}	31.28^{c}	.728
1	28.28^{b}	26.48^{a}	31.88^{c}	32.15^{c}	31.68^{c}	32.03^{c}	.499
2	27.19^{a}	26.01^{a}	31.28^{b}	30.75^{b}	30.68^{b}	31.16^{b}	.673
3	28.27^{ab}	27.13^{a}	31.23^{cd}	29.96^{bd}	31.11^{cd}	30.68^{bd}	.830
4	28.34^{b}	25.81^{a}	30.96^{c}	31.31^{c}	31.06^{c}	31.66^{c}	.598
5	27.30^{a}	25.58^{a}	30.95^{b}	31.33^{b}	30.65^{b}	31.18^{b}	.668
6	26.99^{b}	25.21^{a}	31.01^{c}	31.11^{c}	30.91^{c}	31.41^{c}	.497
7	28.01^{b}	24.86^{a}	31.53^{c}	31.26^{c}	31.33^{c}	31.73^{c}	.711
8	27.92^{b}	25.60^{a}	31.48^{c}	31.40^{c}	31.21^{c}	31.98^{c}	.554
9	28.15^{a}	26.63^{a}	31.98^{b}	32.26^{b}	32.23^{b}	32.53^{b}	.574
10	28.13^{a}	27.06^{a}	31.98^{b}	32.63^{b}	31.78^{b}	32.70^{b}	.715
11	28.01^{a}	26.40^{a}	31.95^{b}	32.65^{b}	31.70^{b}	32.46^{b}	.651

^{a,b,c,d} LSMeans in rows with different superscripts differ ($p < .05$)

* Calculated as: $pH + \log_{10} pCO_2 - 7.604$

** Values given are least squares means

these data that the citrate forms of Na^+ and K^+ also result in an equivalent increase in HCO_3^- concentrations in the blood, adding evidence to the theory that the increase in HCO_3^- concentrations can be attributed to the increase in the strong cations Na^+ and K^+.

Blood tCO_2

The effect of DCAB on venous blood tCO_2 is shown in Table VIII. Total carbon dioxide, tCO_2, is the total concentration (both free and bound) of CO_2 in the blood, and is expressed in mmol/l. As with HCO_3^- concentrations, those horses consuming diet L:Cl had lower ($p < .05$) blood tCO_2 concentrations as compared to those consuming diet L:S at 6 of the 12 intervals measured. Horses consuming both the L:Cl and L:S diets had lower ($p < .05$) tCO_2 values as compared to those consuming all the high diets at all intervals measured (except for L:S vs H:KB and H:NaB at 3 hr post feeding). There was no difference detected in blood tCO_2 values among horses consuming any of the high diets at any intervals measured. In blood plasma, tCO_2 is an estimator of HCO_3^- concentrations (Stewart, 1981), as most CO_2 in the blood is in the form of HCO_3^-, hence tCO_2 is another parameter that is dependent upon the SID.

Base Excess, Blood (BEb) and Base Excess, Extracellular Fluid (BEecf)

The effect of DCAB on base excess in venous blood, (BEb), is shown in Table IX and graphically in Figure 5. Base excess is an indicator of the overall buffering capacity of the blood (HCO_3^- concentration), and takes into account the buffering capacity of Hb and its carrying of CO_2. Horses consuming diet L:Cl

TABLE VIII. EFFECT OF DCAB ON VENOUS BLOOD tCO_2 (mmol/l)*

POST FEEDING**

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	30.38^{b}	27.48^{a}	32.88^{c}	33.81^{c}	32.90^{c}	33.48^{c}	.746
1	29.85^{b}	28.05^{a}	33.58^{c}	33.76^{c}	33.26^{c}	33.66^{c}	.514
2	28.70^{a}	27.48^{a}	32.86^{b}	32.25^{b}	32.20^{b}	32.71^{b}	.694
3	29.79^{ab}	28.58^{a}	32.78^{c}	31.46^{bc}	32.65^{c}	32.15^{bc}	.853
4	29.84^{b}	27.21^{a}	32.41^{c}	32.83^{c}	32.60^{c}	33.21^{c}	.617
5	28.77^{a}	26.95^{a}	32.43^{b}	32.83^{b}	32.16^{b}	32.68^{b}	.699
6	28.38^{b}	26.63^{a}	32.51^{c}	32.60^{c}	32.43^{c}	32.95^{c}	.500
7	29.49^{b}	26.21^{a}	33.00^{c}	32.76^{c}	32.88^{c}	33.20^{c}	.737
8	29.30^{b}	26.98^{a}	32.96^{c}	32.90^{c}	32.73^{c}	33.51^{c}	.567
9	29.61^{a}	28.06^{a}	33.50^{b}	33.83^{b}	33.81^{b}	34.08^{b}	.606
10	29.57^{a}	28.31^{a}	33.46^{b}	34.10^{b}	33.25^{b}	34.18^{b}	.744
11	29.43^{a}	27.83^{a}	33.48^{b}	34.20^{b}	33.18^{b}	33.98^{b}	.677

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Calculated as: $[HCO_3^-] + .0307(pCO_2)$

** Values given are least squares means

had lower ($p < .05$) BEb values as compared to those consuming diet L:S at 8 of 12 intervals measured, and to those consuming all high diets at all intervals measured. Those horses consuming diet L:S had lower ($p < .05$) BEb values as compared to those consuming all high diets at all intervals measured (except for diet H:KB at 3 hr post feeding). Furthermore, there were no differences detected in BEb values among those horses consuming all the high diets at any interval measured. This decrease in BEb in those horses consuming diets L:Cl and L:S, and increase in BEb in those consuming the high DCAB is another indication of the decrease and increase, respectively, of the buffering capacity of the blood when horses are consuming the low and high DCAB, respectively, regardless of the source of anion (Cl or S), or source (citrate or bicarbonate) of Na and K.

Base excess, extracellular fluid (BEecf) is also an indicator of the buffering capacity of the blood, however, it does not take into account Hb. The effect of DCAB on venous blood BEecf is shown in Table X and graphically in Figure 6. As in BEb, those horses consuming diet L:Cl had lower ($p < .05$) BEecf values as compared to those consuming diet L:S at 7 of the 12 intervals measured, and those consuming all the high diets at all intervals measured. Those consuming diet L:S had lower ($p < .05$) BEecf values as compared to those consuming all the high diets at all intervals measured (except for diet H:KB at 3 hr post feeding). There was no differences detected on BEecf values among those horses consuming any of the high diets at any interval measured. Once again, this decrease in BEecf in those horses consuming diets L:Cl and L:S, and increase in BEecf in those horses consuming the diets with high DCAB is an indication of the changes in the buffering capacity of the blood brought about by the intake of either strong anions (Cl or S) or cations (Na or K) regardless of the citrate or HCO_3^- forms of those cations.

TABLE IX. EFFECT OF DCAB ON VENOUS BLOOD BASE EXCESS (mmol/l)* POST FEEDING**

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	3.540 ^{b}	0.750 ^{a}	6.233 ^{c}	6.916 ^{c}	5.450 ^{c}	6.700 ^{c}	.745
1	2.360 ^{b}	0.300 ^{a}	5.550 ^{c}	6.300 ^{c}	5.850 ^{c}	6.000 ^{c}	.517
2	1.426 ^{a}	0.210 ^{a}	5.483 ^{b}	5.383 ^{b}	5.233 ^{b}	5.766 ^{b}	.680
3	2.748 ^{ab}	1.650 ^{a}	5.810 ^{c}	4.316 ^{bc}	5.616 ^{c}	5.616 ^{c}	.795
4	2.908 ^{b}	0.433 ^{a}	5.900 ^{c}	6.066 ^{c}	5.733 ^{c}	6.283 ^{c}	.613
5	1.778 ^{a}	0.216 ^{a}	5.800 ^{b}	6.166 ^{b}	5.250 ^{b}	5.933 ^{b}	.630
6	1.700 ^{b}	-0.266 ^{a}	5.633 ^{c}	5.716 ^{c}	5.533 ^{c}	5.883 ^{c}	.484
7	2.571 ^{b}	-0.383 ^{a}	6.400 ^{c}	5.983 ^{c}	6.000 ^{c}	6.683 ^{c}	.639
8	2.805 ^{b}	0.300 ^{a}	6.316 ^{c}	6.100 ^{c}	5.950 ^{c}	6.583 ^{c}	.566
9	3.060 ^{b}	1.183 ^{a}	6.716 ^{c}	6.900 ^{c}	6.750 ^{c}	7.050 ^{c}	.504
10	3.081 ^{a}	1.733 ^{a}	6.750 ^{b}	7.633 ^{b}	6.866 ^{b}	7.650 ^{b}	.721
11	2.970 ^{b}	1.033 ^{a}	6.633 ^{c}	7.283 ^{c}	6.516 ^{c}	7.133 ^{c}	.593

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Calculated as: $(1-0.014[Hb])([HCO_3^-]-24) + (1.43[Hb]+7.7)(pH-7.4)$

** Values given are least squares means

TABLE X. EFFECT OF DCAB ON VENOUS BLOOD BASE EXCESS, extracellular fluid (mmol/l)* POST FEEDING**

	TREATMENT						
Time	L:S	L:Cl	H:KC	H:KB	H:NaC	H:NaB	S.E.
0	3.635^{b}	0.416^{a}	6.650^{c}	7.533^{c}	6.016^{c}	7.233^{c}	.839
1	2.545^{b}	0.283^{a}	6.350^{c}	7.033^{c}	6.500^{c}	6.730^{c}	.577
2	1.406^{a}	0.033^{a}	6.083^{b}	5.766^{b}	5.600^{b}	6.200^{b}	.765
3	2.826^{ac}	1.516^{a}	6.300^{b}	4.633^{bc}	6.083^{b}	5.900^{b}	.923
4	2.921^{b}	0.116^{a}	6.200^{c}	6.500^{c}	6.133^{c}	6.783^{c}	.691
5	1.715^{a}	-0.166^{a}	6.116^{b}	6.533^{b}	5.616^{b}	6.350^{b}	.734
6	1.521^{b}	-0.650^{a}	6.083^{c}	6.150^{c}	5.933^{c}	6.416^{c}	.554
7	2.575^{b}	-0.900^{a}	6.833^{c}	6.416^{c}	6.450^{c}	7.100^{c}	.749
8	2.708^{b}	-0.083^{a}	6.750^{c}	6.550^{c}	6.350^{c}	7.150^{c}	.637
9	2.953^{b}	0.950^{a}	7.250^{c}	7.500^{c}	7.366^{c}	7.700^{c}	.594
10	2.993^{a}	1.550^{a}	7.250^{b}	8.183^{b}	7.266^{b}	8.216^{b}	.820
11	2.855^{a}	0.783^{a}	7.183^{b}	7.950^{b}	6.983^{b}	7.750^{b}	.699

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Calculated as: $[HCO_3] - 25 + 16.2(pH - 7.400)$

** Values given are least squares means

Serum Mineral Status

Serum Sodium

The effect of treatment over time on least squares mean serum Na concentration is shown in Table XI. At time 0, 3 and 8 hours post feeding, there was no significant difference detected in serum Na concentrations between treatments. At 2 and 4 hours post feeding, those horses consuming diet L:Cl had lower ($p < .05$) serum Na concentration as compared to those consuming diet H:KB, while at times 6 and 10 hr post feeding, those horses consuming diet L:Cl had higher ($p < .05$) serum Na concentrations as compared to those consuming diet H:KC. While some significant differences were observed between treatments, serum Na concentrations varied widely, and ranged from 114 to 137 mmol/l in all treatments. These data generally agree with work in dairy cows (Tucker et al., 1991; Jackson et al., 1992) that showed no effect of DCAB on serum Na concentration. The concentration of Na in the plasma is tightly regulated so that the osmolarity of the blood remains largely unchanged, even with large changes in dietary intake of Na. Additionally, Na concentration is regulated by antidiuretic hormone. As levels become high in the plasma, osmoreceptors in the circulatory system will cause an increase in sodium excretion via the kidneys to help regulate the plasma concentration.

Serum Potassium

The effect of treatment over time on serum K concentration is shown in Table XII. There were no differences detected in serum K concentrations between those horses consuming diets L:Cl and L:S, or between those

TABLE XI. EFFECT OF DCAB ON SERUM SODIUM (mmol/l)

POST FEEDING*

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	118.83	120.00	127.50	126.50	124.66	121.00	5.76
2	130.26^{ab}	117.83^{a}	130.16^{ab}	133.66^{b}	119.66^{ab}	123.50^{ab}	6.32
3	130.41	119.83	115.00	127.66	123.00	113.83	6.63
4	129.35^{bc}	116.50^{ab}	127.66^{c}	137.33^{c}	118.00^{ab}	114.50^{a}	5.40
6	119.36^{abc}	129.66^{bc}	116.16^{a}	133.16^{c}	122.00^{abc}	118.83^{ab}	5.57
8	120.71	123.66	121.50	145.33	114.16	119.16	5.26
10	133.73^{c}	126.66^{bc}	115.66^{a}	135.33^{c}	126.83^{bc}	122.16^{ab}	4.07

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

consuming diets H:NaC and H:NaB, at any interval measured. Those horses consuming diet H:KB had higher ($p < .05$) serum K concentrations as compared to those horses consuming diet: H:NaB at 2, 3, 4, 6, 8 and 10 hr post feeding; L:Cl at 2, 3, 4, 8 and 10 hr post feeding; L:S at 4, 6 and 8 hr post feeding. Those horses consuming diet H:KC had higher serum K concentration as compared to those consuming diet: L:Cl at 0, 2 and 4 hr post feeding; H:NaB at 2 and 4 hr post feeding. Potassium concentration in the extracellular fluid is very tightly regulated by the body, because of the deleterious effects of high extracellular potassium on the resting membrane potential of both skeletal muscle cells and, even more critically, cardiac muscle cells. In this instance, two mechanisms play a part to increase the amount of potassium excreted in the distal nephron of the renal tubular cells: one, the metabolic alkalotic effect on the plasma, and two, the increase in K itself. Both situations will cause an increase in K excretion via the kidney when K intake is increased above the daily requirement. The primary hormone responsible for this increased K excretion is aldosterone, which is stimulated by increased extracellular levels of K, and causes an increase in the amount of K secreted by the distal nephron while at the same time causing increased reabsorption of Na ions.

Serum Chloride

The effect of treatment over time on serum chloride concentrations is shown in Table XIII. There were no treatment effects observed ($p < .05$) at 0 and 2 hr post feeding. Those horses consuming diet H:NaB had lower ($p < .05$) serum Cl concentrations as compared to those horses consuming diet L:S at 3 and 4 hr post feeding, and as compared to those consuming the L:Cl

TABLE XII. EFFECT OF DCAB ON SERUM POTASSIUM (mmol/l) POST FEEDING*

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	3.195^{a}	3.233^{a}	3.616^{bc}	3.483^{ac}	3.533^{ac}	3.333^{ac}	.151
2	3.153^{ab}	3.000^{a}	3.583^{b}	3.650^{b}	3.183^{ab}	2.950^{a}	.238
3	3.555^{ab}	3.116^{a}	3.383^{ab}	3.750^{b}	3.466^{ab}	3.233^{a}	.187
4	3.446^{a}	3.216^{a}	3.800^{b}	4.083^{b}	3.400^{a}	3.233^{a}	.130
6	3.340^{a}	3.566^{ab}	3.400^{a}	3.933^{b}	3.516^{ab}	3.283^{a}	.179
8	3.411^{a}	3.350^{a}	3.483^{a}	4.116^{b}	3.216^{a}	3.266^{a}	.174
10	3.530^{ab}	3.300^{a}	3.250^{a}	3.666^{b}	3.516^{ab}	3.366^{a}	.120

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

diet at 6 and 10 hr post feeding. Also, those horses consuming diet H:NaC had lower ($p < .05$) Cl concentrations as compared to those horses consuming diet L:Cl at 8 hr post feeding. This trend for lower Cl concentrations in the serum in those horses consuming the Na supplemented diets agrees with work in dairy cows (Jackson et al., 1992) and with sodium bicarbonate dosing work in horses (Kline et al., 1993; Hanson et al., 1993). However, other researchers in dairy cows observed no effect on serum Cl concentrations with increasing DCAB (Romo, et al., 1991).

Serum Calcium

The effect of treatment over time on serum Ca concentrations is shown in Table XIV. There were no treatment effects observed on serum Ca concentrations at 0, 2 and 3 hr post feeding. Those horses consuming diet H:KB had greater ($p < .05$) serum Ca concentrations as compared to those horses consuming diet H:KC at 6, 8 and 10 hr post feeding. These data tend to agree with work in dairy cows (Gaynor et al., 1989; Romo et al., 1991; Jackson and Hemken, 1994) who observed no effect of DCAB on plasma Ca concentrations, but contrasts with that of Block (1984), Oetzal et al., (1988) and Tucker et al. (1991). In a Ca kinetic study in sheep, Takagi and Block (1991b) fed DCAB of +339, +35 and -127 meq/kg, and found no difference in the concentration of total Ca in the plasma. However, they observed that feeding the reduced DCAB diets increased the Ca flux through the exchangeable pool with no changes in the size of the Ca pool. The calcium homeostatic mechanism operates very stringently to maintain extracellular Ca within physiological ranges (approximately 8 - 11 mg/dl). In the present study, only total Ca in the serum was measured, and all values were within the physiological range, indicating that the body maintains

TABLE XIII. EFFECT OF DCAB ON SERUM CHLORIDE (mmol/l) POST FEEDING*

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	89.88	92.16	94.00	91.16	90.50	86.83	4.63
2	98.26	89.83	95.16	94.33	88.16	88.33	4.99
3	98.16^{b}	90.16^{ab}	83.83^{a}	93.66^{ab}	90.00^{ab}	83.16^{a}	4.44
4	96.85^{bc}	89.00^{ab}	93.50^{bc}	100.33^{c}	86.33^{ab}	83.33^{a}	4.19
6	91.00^{ab}	100.33^{b}	85.16^{a}	97.66^{b}	90.00^{ab}	86.33^{a}	4.35
8	92.21^{ab}	95.66^{b}	90.00^{ab}	106.16^{c}	83.66^{a}	87.33^{ab}	3.84
10	102.05^{d}	97.33^{cd}	85.16^{a}	97.33^{cd}	93.33^{bc}	89.33^{ab}	3.21

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

the size of the Ca pool under conditions of nutritionally induced acidosis or alkalosis.

Serum Magnesium

The effect of treatment over time on serum magnesium concentrations is shown in Table XV. No differences in serum Mg due to treatment were observed at 0, 2, 3 or 10 hr post feeding. Those horses consuming diet H:NaB had lower ($p < .05$) serum Mg concentrations as compared to those consuming diet H:KB at 4, 6 and 8 hr post feeding. Serum Mg concentrations ranged from 1.20 - 1.58 mmol/l for those horses consuming diet L:S, 1.23 - 1.48 for those consuming diet L:Cl, 1.04 - 1.47 for those consuming diet H:KC, 1.24 - 1.55 for those consuming diet H:KB, 1.11 - 1.37 for those consuming diet H:NaC and 1.14 - 1.38 for those consuming diet H:NaB. These data agree with previous work in dairy cows (Romo et al., 1991; Tucker et al., 1991) that showed no significant treatment effect on serum Mg concentrations. However, others (Oetzal et al., 1988; Gaynor et al., 1989; Jackson et al., 1992) observed a decrease in serum Mg concentrations with increasing DCAB in dairy cows. While in the present study values for serum Mg tended to be numerically lower in those horses consuming diets H:NaC and H:NaB as compared to those consuming diets L:S and L:Cl, there was no significant trend detected. Factors involved in Mg homeostasis have not been clearly defined, although extracellular Mg concentration is regulated by renal excretion (Guyton, 1986).

TABLE XIV. EFFECT OF DCAB ON SERUM CALCIUM (mmol/l) POST FEEDING*

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	9.14	9.15	9.91	9.52	9.96	9.50	.532
2	9.71	8.76	9.88	10.00	8.92	8.92	.608
3	10.35	8.90	8.99	9.87	9.85	8.96	.630
4	10.09^{bcd}	9.33^{ac}	10.19^{cd}	10.87^{d}	9.05^{ab}	8.59^{a}	.423
6	8.93^{a}	10.05^{ab}	8.91^{a}	10.66^{b}	9.88^{ab}	9.45^{ab}	.589
8	9.79^{a}	9.82^{a}	9.61^{a}	11.33^{b}	8.55^{a}	9.01^{a}	.570
10	10.21^{cd}	9.36^{bc}	8.83^{ab}	10.41^{d}	9.93^{cd}	9.57^{abcd}	.354

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

TABLE XV. EFFECT OF DCAB ON SERUM MAGNESIUM (mmol/l) POST FEEDING*

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	1.28	1.23	1.30	1.24	1.30	1.18	.083
2	1.58	1.29	1.47	1.55	1.37	1.38	.128
3	1.48	1.33	1.24	1.44	1.35	1.26	.109
4	1.44^{ab}	1.23^{ab}	1.34^{ab}	1.51^{b}	1.27^{ab}	1.21^{a}	.097
6	1.20^{ab}	1.48^{c}	1.18^{a}	1.43^{bc}	1.24^{ab}	1.15^{a}	.079
8	1.23^{a}	1.29^{ab}	1.17^{a}	1.55^{b}	1.11^{a}	1.22^{a}	.090
10	1.29	1.36	1.04	1.25	1.18	1.14	.089

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

Serum Phosphorus

The effect of treatment over time on serum phosphorus concentrations is shown in Table XVI. There were no treatment effects on serum P concentrations at 0, 2, 3 or 6 hr post feeding. Those horses consuming diet H:KB had higher ($p < .05$) serum P concentrations as compared to those consuming diet H:NaB at 4, 8 and 10 hr post feeding. While some significant differences were detected between diets, overall these data tend to agree with previous work in dairy cows (Romo et al., 1991; Tucker et al., 1991; Jackson et al., 1992; Jackson and Hemken, 1994;) that showed no significant treatment effect on serum P concentrations. If lowering the DCAB resulted in an increase in PTH release (as shown by some dairy researchers), P would be mobilized from bone, but the threshold for reabsorption of phosphate in the renal tubules also would be reduced (Guyton, 1986) so that more P would be lost in the urine. Therefore, plasma P may not be affected.

Serum Cation-Anion Balance

The effect of treatment over time on serum cation-anion balance (expressed as meq (Na + K) - Cl/l) is shown in Table XVII and graphically in Figure 7. Those horses consuming diet L:Cl had lower ($p < .05$) serum cation-anion balance (SCAB) as compared to those consuming diet: H:KB at 0, 2, 4, 6, 8 and 10 hr post feeding; H:KC at 0, 2 and 4 hr post feeding; H:NaB at 0 and 2 hr post feeding; H:NaC at 0 and 10 hr post feeding. Furthermore, horses consuming diet H:KB had higher ($p < .05$) SCAB as compared to those consuming L:S at 0, 2, 4, 6, 8 and 10 hr post feeding. Although there appeared

TABLE XVI. EFFECT OF DCAB ON SERUM PHOSPHORUS (mmol/l) POST FEEDING*

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	2.95	2.93	3.41	3.23	3.26	2.99	.246
2	3.16	2.86	3.30	3.27	2.80	2.78	.248
3	3.14	2.91	2.88	3.00	2.82	2.55	.224
4	3.11^{abc}	2.93^{abc}	3.15^{bc}	3.33^{c}	2.74^{ab}	2.52^{a}	.194
6	2.81	3.13	2.79	3.18	2.77	2.66	.229
8	2.89^{a}	2.93^{a}	3.03^{ab}	3.50^{b}	2.62^{a}	2.69^{a}	.182
10	3.04^{ab}	3.03^{ab}	2.89^{ab}	3.32^{b}	2.97^{ab}	2.74^{a}	.192

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

TABLE XVII. EFFECT OF DCAB ON SERUM CATION-ANION BALANCE (meq/l) POST FEEDING*

	TREATMENT						
Time	**L:S**	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
0	32.14^{ab}	31.06^{a}	37.11^{bc}	38.81^{c}	37.70^{c}	37.50^{bc}	1.75
2	35.15^{ab}	31.00^{a}	38.58^{bc}	42.98^{c}	34.68^{ab}	38.11^{bc}	2.20
3	35.80	32.78	34.55	37.75	36.46	33.90	2.46
4	35.94^{b}	30.71^{a}	37.96^{bc}	41.08^{c}	35.06^{ab}	34.40^{ab}	1.53
6	31.70^{a}	32.90^{a}	34.40^{a}	39.43^{b}	35.51^{ab}	35.78^{ab}	1.64
8	31.91^{a}	31.35^{a}	34.98^{a}	43.28^{b}	33.71^{a}	35.10^{a}	1.86
10	35.21^{ab}	32.63^{a}	33.75^{ab}	41.66^{c}	37.01^{b}	36.20^{ab}	1.41

^{a,b,c} LSMeans in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

to be no significant trend in the absolute values of the individual ions, this data indicates that those horses consuming the L:Cl and L:S diets had lower SCAB values as compared to at least two of the high diets at 4 of the intervals measured. This data agrees with that of Tucker et al. (1988, 1991b) who observed higher plasma CAB in dairy cows as compared to those cows consuming anionic diets supplemented with either Cl or S.

Mineral Excretion

Dry Matter Digestibility

The effects of DCAB on daily fecal output and dry matter digestibility are shown in Table XVIII. Total daily fecal output, g/d, was calculated as: (g Cr fed/d * 100)/% fecal Cr). Dry matter digestibility was calculated as: (g DM intake/d-g DM fecal excretion/d) / g DM intake/d. There were no differences detected in dry matter digestibility between treatments. Least squares mean dry matter digestibilities ranged from 47.69 - 52.73%. This data contrasts with that of Nelson and coworkers (1981) who reported a decrease in dry matter digestibility in chicks fed a higher cation-anion ratio. This data also contrasts with that of Baker et al. (1993) who observed exercising horses consuming the lowest DCAB had lower DM digestibility as compared to those horses consuming the highest DCAB. These data also contrast with that of Yen et al. (1981) who showed a decreased feed intake, feed efficiency and weight gain in barrows fed a diet with 4% cacium chloride added, and also with Haydon and West (1990) who reported a linear relationship between DCAB and apparent ileal digestibility of energy, dry matter, N, and amino acids (with the exception of alanine and

TABLE XVIII. THE EFFECT OF DIETARY CATION-ANION BALANCE ON DM INTAKE, FECAL OUTPUT AND DRY MATTER DIGESTIBILITY (g/d) IN SEDENTARY HORSES*

	Treatment						
	L:S	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
DM Int, g/d	7860.8	7860.8	7860.8	7860.8	7860.8	7860.8	
Fecal Output, g/d	3884.9	4102.0	4093.2	3721.2	4094.0	3914.3	161.6
DM Digestibility	**50.69**	**47.69**	**48.08**	**52.73**	**47.91**	**50.08**	1.98

[a,b] LSMean fecal outputs in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

methionine) in diets with DCAB's of -50 to 400 meq/kg of diet dry matter. However, these researchers noted that nutrient and amino acid digestibilities were similar when measured over the entire tract.

Sodium Balance

After supplementation with sodium citrate and sodium bicarbonate, least squares mean daily intake of Na was 116.70 and 136.30 mg/kg BW for those horses consuming diets H:NaC and H:NaB, respectively. The daily requirement for horses of this size is approximately 7 g/d (NRC, 1989). The effect of DCAB on sodium balance is shown in Table XIX and graphically in Figure 8. Those horses consuming diet H:NaC excreted more Na in the urine ($p < .05$) as compared to those consuming all other diets. Additionally, those horses consuming diet H:NaB excreted more ($p < .05$) Na in the urine as compared to those consuming all other diets (with exception of H:NaC). Those horses consuming diets H:NaC and H:NaB excreted more Na in the feces ($p < .05$) as compared to all other diets. Those horses consuming diet H:NaB had a greater ($p < .05$) apparent daily Na balance as compared to those horses consuming diets H:NaC and H:KC. These findings are in partial agreement with that of Schryver et al. (1987) who demonstrated that urinary excretion was the primary pathway for sodium loss in sedentary horses consuming 1, 3 and 5% sodium chloride. The author stated that sodium intake was directly related to urinary sodium excretion but had no effect on fecal excretion, intestinal absorption or retention of sodium. However, In contrast to the study by Schryver et al. (1987), it appears that the horse responds to an increased Na intake by also increasing fecal excretion of Na to maintain sodium homeostasis in the body.

TABLE XIX. THE EFFECT OF DIETARY CATION-ANION BALANCE ON SODIUM BALANCE IN SEDENTARY HORSES (mg/kg BW)*

	Treatment						
	L:S	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
Intake, g/d	40.10 ^{a}	39.10 ^{a}	38.30 ^{a}	37.90 ^{a}	116.70 ^{b}	136.30 ^{c}	.700
Urine, g/d	30.60^{a}	31.10 ^{a}	41.50 ^{a}	31.70 ^{a}	113.50^{c}	84.90^{b}	.600
Fecal, g/d	23.30 ^{a}	16.70 ^{a}	22.90 ^{a}	27.00 ^{a}	43.70 ^{b}	46.70^{b}	2.90
Balance, g/d	-13.80^{abc}	-8.70^{bc}	-26.00^{ab}	-20.70^{abc}	-40.50^{a}	4.60^{c}	1.60

^{a,b,c} Means in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

These data are also in agreement with Baker et al. (1993) who reported an increase in urinary Na excretion in both sedentary and exercising horses consuming a high DCAB with supplemental sodium.

Potassium Balance

After supplementation with potassium citrate and potassium bicarbonate, those horses consuming diets H:KC and H:KB had daily K intakes of 256.50 and 244.20 mg/kg BW, as compared to an average intake of 121 mg/kg BW for horses consuming all other diets. The effect of DCAB on potassium balance is shown in Table XX and graphically in Figure 9. Those horses consuming diets H:KC and H:KB excreted more ($p < .05$) K in the urine as compared to those consuming all other diets. Those horses consuming diets H:NaC and H:NaB excreted less ($p < .05$) K in the feces as compared to those horses consuming all other diets. Furthermore, those horses consuming diets H:KC and H:KB had lower ($p < .05$) least squares mean apparent daily K balance as compared to those consuming all other diets. Although apparent daily K balance was negative for those horses consuming diets H:KC and H:KB, it is physiologically impossible for the animal to be in a true negative daily balance. Instead, these data indicate the powerful response of the body to an increased extracellular concentration of K, and the subsequent increase in urinary excretion of K in order to maintain normal physiologic levels of K in the extracellular fluid. The negative values seen may be due to an underestimation of K in the diet, or an overestimation of K excreted. These results agree with that of Baker et al. (1993) who reported an increased daily urinary potassium excretion in both sedentary and exercising horses consuming rations with a high DCAB containing 1.25 - 1.39% K. The NRC

TABLE XX. THE EFFECT OF DIETARY CATION-ANION BALANCE ON POTASSIUM BALANCE IN SEDENTARY HORSES (mg/kg BW)*

	Treatment						
	L:S	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
Intake, g/d	116.50	124.00	256.50	244.20	121.00	122.90	1.10
Urine, g/d	77.10^{a}	79.70^{a}	261.70^{b}	275.20^{b}	96.20^{a}	95.40^{a}	13.40
Fecal, g/d	41.60^{b}	43.30^{b}	41.70^{b}	39.80^{b}	24.80^{a}	22.70^{a}	3.00
Balance, g/d	-2.10^{b}	0.900^{b}	-46.80^{a}	-70.80^{a}	0.05^{b}	4.70^{b}	12.70

^{a,b,c} Means in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

(1989) lists the potassium requirement for horses at maintenence at 1.52 g/Mcal of DE, therefore a 500 kg (1100 lb) horse would require 25 g/d of dietary potassium. The horses in this trial received dietary potassium well above the minimum requirements, which is common in most rations fed today, due to the high K content in medium to high quality hays.

Chloride Balance

After formulation of the L:Cl diet with NH_4Cl, least squares mean daily intake of Cl for those horses consuming diet L:Cl was 115.20 mg/kg BW, as compared to approximately 78 mg/kg BW for those horses consuming all other diets. The effect of DCAB on chloride balance is shown in Table XXI and graphically in Figure 10. Those horses consuming diet L:Cl excreted more ($p< .05$) Cl in the urine as compared to horses consuming all other diets. Those horses consuming diet L:Cl also excreted less ($p< .05$) Cl in the feces as compared to those horses consuming diets H:KC, H:KB, H:NaC and H:NaB. These data agree with previous work in both sedentary and exercising horses (Topliff et al., 1989; Wall et al., 1992; Baker et al., 1993; Popplewell et al., 1993;) that showed an increased urinary Cl excretion in horses consuming highly anionic diets supplemented with Cl. Apparently, the increase in urinary chloride excretion in those horses consuming diet L:Cl was enough to offset the increased intake of Cl, as least squares mean apparent daily Cl balance was not different across treatments. The NRC (1989) states that chloride requirements are presumed to be met when the sodium requirements are met with sodium chloride. Younget al. (1989) fed approximately 1.5 times more chloride than sodium to exercised miniature horses and still experienced a chloride deficiency. In the present study, those

TABLE XXI. THE EFFECT OF DIETARY CATION-ANION BALANCE ON CHLORIDE BALANCE IN SEDENTARY HORSES (mg/kg BW)*

	Treatment						
	L:S	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
Intake, g/d	79.10 ^{b}	115.20 ^{c}	78.60 ^{b}	77.70 ^{ab}	76.70 ^{a}	78.70 ^{b}	.030
Urine, g/d	73.00^{a}	114.20^{b}	74.30^{a}	75.80^{a}	73.80^{a}	66.30^{a}	5.40
Fecal, g/d	2.90^{ab}	2.60^{a}	3.50^{bc}	3.40^{bc}	3.40^{bc}	3.80^{c}	0.02
Balance, g/d	3.10	-1.60	0.80	-1.50	-0.50	8.40	5.40

^{a,b,c} Means in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

horses consuming diets L:Cl, H:KB and H:NaC were in an apparent negative daily Cl balance, however, this author believes this to be of minor practical significance, as the standard error for the balance LSMeans allows for a range for these balances to be positive.

Magnesium Balance

Magnesium has been previously implicated as having a possible role in the DCAB equation in dairy cattle (Tucker, 1988). Therefore, in the present study dietary it was attempted to hold Mg intakes constant across treatments. The effect of DCAB on magnesium balance is shown in Table XXII. Those horses consuming diet L:Cl had higher ($p < .05$) daily urinary Mg excretions as compared to those consuming diets L:S, H:KC and H:NaC. Those horses consuming diet H:KC had greater ($p < .05$) daily fecal excretions of Mg as compared to those consuming diet H:NaB. Further, those horses consuming diet H:KB had a lower ($p < .05$) apparent daily Mg balance as compared to those horses consuming diets L:S, L:Cl, H:NaC and H:NaB. The differences in urinary and fecal excretions of Mg between treatments is not believed to be of any practical significance. However, these data are in agreement with Baker and others (1993) who reported similar results in daily magnesium balance in sedentary horses consuming diets varying in DCAB. Thus, it is possible that the NRC requirements for sedentary horses may be inadequate. The NRC (1989) suggests a magnesium intake of .46 g/Mcal DE to meet the horse's requirement. Therefore, the horses in this trial would require approximately 10 g/d. All diets used in the present trial should have been sufficient in meeting the magnesium requirement, however, these data suggest that this value may be inadequate

TABLE XXII. THE EFFECT OF DIETARY CATION-ANION BALANCE ON MAGNESIUM BALANCE IN SEDENTARY HORSES (mg/kg BW)*

	Treatment						
	L:S	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
Intake, g/d	25.10	27.00	25.00	23.00	25.10	25.00	0.10
Urine, g/d	8.50^{ab}	10.10^{c}	8.00^{a}	10.10^{c}	8.30^{a}	9.70^{bc}	0.47
Fecal, g/d	17.40^{ab}	18.00^{ab}	18.90^{b}	16.20^{ab}	17.90^{ab}	16.20^{a}	0.75
Balance, g/d	-0.70^{b}	-1.10^{b}	-1.80^{ab}	-3.40^{a}	-1.20^{b}	-0.90^{b}	0.74

^{a,b,c} Means in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

independent of DCAB, because of the apparent negative daily balances for horses on all treatments.treatments in the present study. Further research is needed to accurately quantify the magnesium requirements of sedentary horses

Phosphorus Balance

It was attempted by the author to hold the intake of phosphorus constant across all treatments. The effect of DCAB on phosphorus balance is shown in Table XXIII. There were no differences detected between daily urinary P excretions. Urinary excretions were extremely low, ranging from 0.30 to 0.40 mg/kg BW. These urinary P values agree with those of Baker et al. (1993) who reported similar values in both sedentary and exercising horses consuming diets with varying DCAB. There were no differences in daily fecal excretion of P across treatments. Those horses consuming diet H:NaC had a lower ($p < .05$) apparent daily P balance as compared to those horses consuming diet H:NaB. The NRC (1989) suggests a phosphorus requirement of .87 g/Mcal DE. Therefore, the horses in this study required approximately 37.0 mg/kg BW. Each of the diets used in this study appear to have supplied adequate phosphorus, and all daily balances were positive with the exception of those horses consuming diet H:NaC (least squares mean apparent daily balance of -0.16 mg/kg BW).

Calcium Balance

The effect of DCAB on calcium balance is shown in Table XXIV and graphically in Figure 11. Diets were formulated to have equivalent amounts of

TABLE XXIII. THE EFFECT OF DIETARY CATION-ANION BALANCE ON PHOSPHORUS BALANCE IN SEDENTARY HORSES (mg/kg BW)*

	Treatment						
	L:S	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
Intake, g/d	43.00	44.20	42.80	41.80	41.80	46.00	0.11
Urine, g/d	0.300	0.400	0.400	0.400	0.400	0.300	0.04
Fecal, g/d	37.9	40.60	39.90	37.50	41.60	39.50	1.60
Balance, g/d	4.80 ^{ab}	3.20 ^{ab}	2.40 ^{ab}	3.70 ^{b}	-0.16 ^{a}	6.00 ^{b}	1.88

^{a,b,c} Means in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

calcium in each treatment. Actual Ca intakes ranged from 80.90 to 86.30 mg/kg BW across all treatments. Those horses consuming diet L:Cl had greater ($p < .05$) daily urinary Ca excretion as compared to those consuming all other diets. There were no significant differences detected in daily fecal Ca excretion across treatments. Those horses consuming diet L:Cl also had a lower ($p < .05$) apparent daily Ca balance (-29.40 mg/kg BW) as compared to all other treatments, while those horses consuming diet L:S had a lower ($p < .05$) apparent daily Ca balance as compared to those horses consuming diet H:NaB.

These data on urinary excretion of calcium agree with other data in horses (Topliff et al., 1989; Baker et al., 1993), rats (Newell and Beauchene, 1975; Petito and Evans, 1984; Goulding and Campbell, 1984; Barzel and Jowsey, 1989), rabbits (Thacker, 1959), dairy cattle (Freeden et al., 1988; Tucker et al., 1988, 1991a, 1991b; Gaynor et al., 1989; Oetzal et al., 1991, Wang and Beede, 1992; Jackson and Hemken, 1994), and sheep (Takagi and Block, 1991a,b) that show animals consuming diets with a lower DCAB have increased urinary Ca excretion. In 1991, Goff and others demonstrated that parathyroid hormone has a greater effect on renal production of 1,25 dihydroxyvitamin D_3 in dairy cows fed highly anionic diets, resulting in increased intestinal calcium absorption. Furthermore, osteoclastic bone resorption was more responsive to parathyroid hormone as plasma hydroxyproline concentration was higher in those cows fed the low DCAB diet. It has also been suggested that renal tubular reabsorption of calcium may be inhibited by the acidotic state and low pH induced by the lower DCAB diets (Beck and Webster, 1976). Furthermore, an increase in bone mobilization of Ca a few days prepartum has been observed by dairy researchers (Block, 1984; Leclerc and Block, 1989; Goff, 1991) when DCAB was reduced.

TABLE XXIV. THE EFFECT OF DIETARY CATION-ANION BALANCE ON CALCIUM BALANCE IN SEDENTARY HORSES(mg/kg BW)*

	Treatment						
	L:S	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
Intake, g/d	82.70 ^{b}	80.90 ^{a}	83.10 ^{b}	81.10 ^{a}	81.20 ^{a}	86.30 ^{c}	0.35
Urine, g/d	52.80^{a}	75.40^{b}	44.70^{a}	40.00^{a}	43.60^{a}	40.10^{a}	4.20
Fecal, g/d	35.70	34.90	37.40	37.70	37.10	38.30	1.60
Balance, g/d	-5.80^{b}	-29.40^{a}	1.00^{bc}	3.30^{bc}	0.40^{bc}	7.90^{c}	3.90

^{a,b,c} Means in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

The NRC (1989) suggests that the calcium requirement is 1.22 g/Mcal DE/d. The horses in the present trial would therefore have required approximately 26 g/d of dietary calcium. The calcium intake in this trial was purposely exceeded so that these horses consuming the L:S and L:Cl diets would not be predisposed to a daily negative calcium balance. However, these data demonstrate that as the DCAB is lowered, daily calcium balance decreases, and that a low DCAB formulated with S has a similar, if not as powerful anionic effect on calcium balance in sedentary horses. If this condition were prolonged, these animals could be predisposed to an osteoporotic weakening of the skeletal system that has been demonstrated in poultry (Leach and Neshium, 1965, 1972; Hurwitz et al., 1973; Sauveur and Mongin, 1978; Hamilton and Thompson, 1980; Mongin, 1981; Halley et al., 1987), and rabbits (Thacker, 1959).

Sulfur Balance

Due to supplementation of diet L:S with S (as SO_4), daily intake of S for those horses consuming diet L:S was 121.30 mg/kg BW. The effect of treatment over time on daily S balance is shown in Table XXV. Those horses consuming diet H:NaB had a lower daily urinary excretion of S compared to those consuming all other diets. Those horses consuming diet L:S had greater ($p < .05$) daily fecal excretion of S as compared to all other diets. This increased daily fecal excretion of S by those horses consuming diet L:S was apparently not enough to offset the increased inake of S, however, as least squares mean apparent daily balance was higher (56.90 mg/kg BW, $p < .05$) for horses consuming diet L:S as compared to horses consuming all other diets. It is thought by this author that this apparent large positive daily S balance is due to a "carryover effect". Although mineral excretions were not measured after

horses were removed from these experimental diets, it is thought that those horses that were consuming diet L-S would continue to be in an apparent positive daily balance after removal from these diets, but that this apparent positive daily balance would decrease to a point equal to that observed in those horses consuming diets with normal S intakes.

Although S requirements for the horse have not been established, adequate, high quality dietary protein usually provides at least 0.15% organic S (NRC, 1989). The horses in the present study received a lower quality native prairiegrass hay that may have been lower in S than some other high quality hays. However, all horses in the present study were in a positive apparent daily S balance. This data contrasts with that of Baker et al. (1993) who reported that exercising horses consuming approximatley 9 g/d of S were in an apparent negative daily S balance. However, this discrepancy may be due to a difference in S metabolism between sedentary and exercising horses.

Table XXV. THE EFFECT OF DIETARY CATION-ANION BALANCE ON SULFUR BALANCE IN SEDENTARY HORSES (mg/kg BW)*

	Treatment						
	L:S	**L:Cl**	**H:KC**	**H:KB**	**H:NaC**	**H:NaB**	**S.E.**
Intake, g/d	121.30 ^{e}	17.50^{c}	16.30 ^{b}	17.40 ^{c}	15.30 ^{a}	18.40 ^{d}	0.24
Urine, g/d	8.60 ^{b}	4.80 ^{b}	4.90 ^{b}	7.40 ^{b}	5.90 ^{b}	3.90 ^{a}	1.40
Fecal, g/d	55.60^{b}	8.90^{a}	10.60^{a}	7.70^{a}	8.90^{a}	7.90^{a}	4.10
Balance, g/d	56.90^{b}	3.70^{a}	0.70^{a}	2.30^{a}	0.50^{a}	6.50^{a}	4.00

^{a,b,c} Means in rows with different superscripts differ ($p < .05$)

* Values given are least squares means

CHAPTER V

SUMMARY AND CONCLUSIONS

In summary, these results indicate that the feeding of excess strong cations in relation to anions, regardless of whether the form is with bicarbonate or citrate, results in an increase in the acid-base status of the animal, as can be seen by the similar increases in urine pH, daily urine H^+ excretion, blood pH, bicarbonate, base excess, blood and base excess, extracellular fluid in those horses consuming the H:KC, H:KB, H:NaC and H:NaB diets. Furthermore, those horses consuming the L:S diet had similar, but not as pronounced deleterious effects on the acid-base status of the horse as that of diet L:Cl, as evidenced by the lowering of urine pH, increase in daily H^+ excretion and blood pH.

Treatments had no significant effect on absolute values of serum minerals, however, those horses consuming diet L:Cl had lower serum cation-anion balances as compared to those horses consuming diet H:KB at 6 of the 7 intervals measured, and also as compared to those consuming diets H:KC (3 of 7 intervals measured), H:NaC and H:NaB (2 of 7 intervals measured, respectively). There were no differences detected in daily fecal output or dry matter digestibility due to treatment. Those horses consuming diets H:NaC and H:NaB excreted more Na in the urine as compared to all other diets, indicating that urinary Na excretion is dependent on intake. Those horses consuming diet L:Cl excreted more Cl in the urine as compared to those consuming all other

diets, and this increased excretion was apparently enough to offset the increased intake as apparent daily Cl balance was not different between treatments. Those horses consuming diets H:KC and H:KB also excreted more K in the urine as compared to those consuming all other diets, indicating that urinary K excretion is also dependent upon intake. Those horses consuming diet H:KB had lower apparent daily Mg balance as compared to those consuming diets H:NaC and H:NaB. However, all apparent daily Mg balances were negative, and these balance values agree with earlier work in sedentary horses, indicating that the daily Mg requirements as recommended by the NRC may need further review. Those horses consuming diet H:NaC had a lower apparent daily P balance as compared to those consuming diet H:NaB and H:KB, however, this difference is probably not of any practical significance. Those horses consuming diet L:S had lower daily Ca balance as compared to those consuming diet H:NaB, while those horses consuming diet L:Cl had greater daily urinary Ca excretion, and lower daily Ca balance as compared to horses consuming all other diets. This data, along with previous work in horses, indicates that when formulating rations for Ca requirements, the DCAB needs to be taken into careful consideration, as it has been consistently shown that feeding diets with a low cation-anion balance results in an apparent decreased retention of Ca, even when fed above requirements.

Those horses consuming diet H:NaB excreted more S in the urine compared to those consuming all other diets. Those horses consuming diet L:S had greater daily fecal excretions of S as compared to horses consuming all other diets, however, this increased fecal excretion apparently was not enough to offset the increased intake, as apparent daily S balance was greater in those horses consuming diet L:S as compared to those consuming all other diets.

As indicated by this data, the feeding of the strong cations Na or K in the form of citrate or bicarbonate are equally effective in raising the acid-base status and buffering capacity of the sedentary horse. These data also support the theory that H^+ and HCO_3^- concentrations are dependent upon the strong ion difference of the diet. The feeding of diets with added sodium citrate or sodium bicarbonate at a DCAB above 350 meq/kg DM could prove a valuable alternative to the use of sodium bicarbonate dosing with concurrent furosemide use. However, this data indicates that the feeding of high potassium diets leads to increased potassium excretion, which could compound the potassium excretory effect of furosemide. Therefore, it is this authors opinion that the feeding of high potassium diets to improve the buffering capacity of the blood with concurrent use of furosemide is not recommended. Sulfur appears to have similar, but not as pronounced, effects as that of chloride on the urine pH, daily H^+ excretion and blood pH in sedentary horses. However, there may need to be a modifying factor used with S in the DCAB equation, as its digestibility and absorption may depend upon the form of sulfur being fed.

This data indicates that the concentrations of H^+ and HCO_3^- are dependent upon the strong ion difference (SID). Further research is needed to study the effects of sodium citrate, potassium citrate and potassium bicarbonate in exercising horses to test if these forms have the same beneficial effect on acid-base status.

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APPENDIX

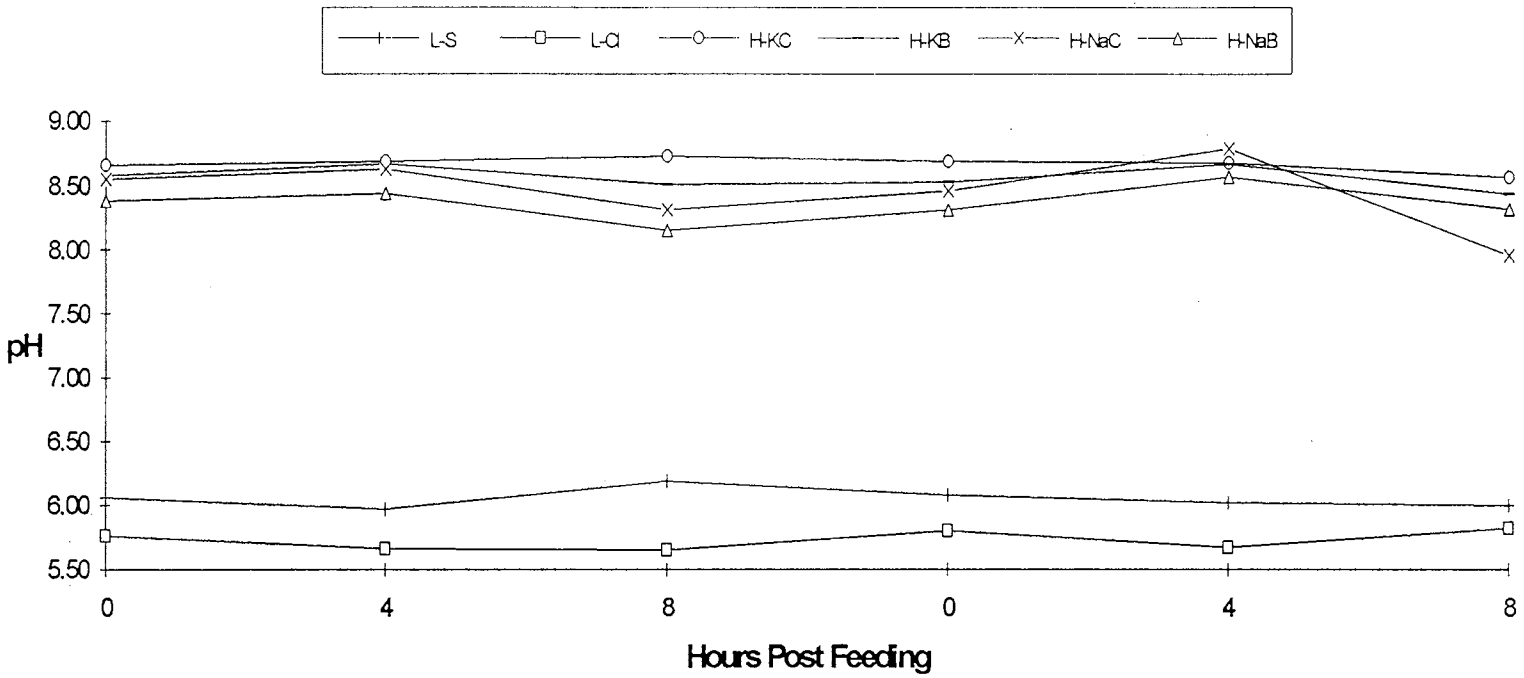

Figure 1. EFFECT OF DIETARY CATION-ANION BALANCE ON LSMean URINE pH POST FEEDING

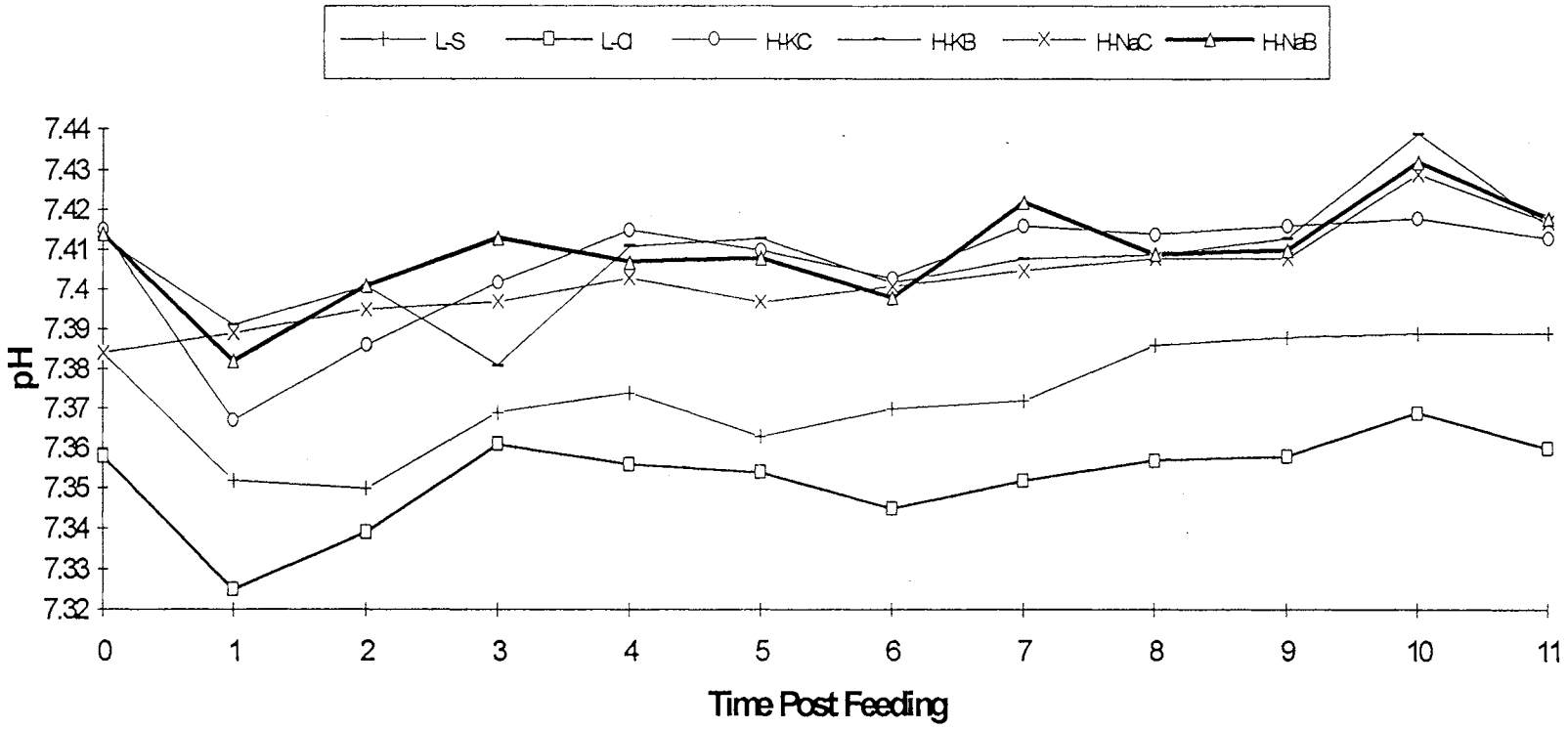

Figure 2. EFFECT OF DIETARY CATION-ANION BALANCE ON BLOOD pH POST FEEDING

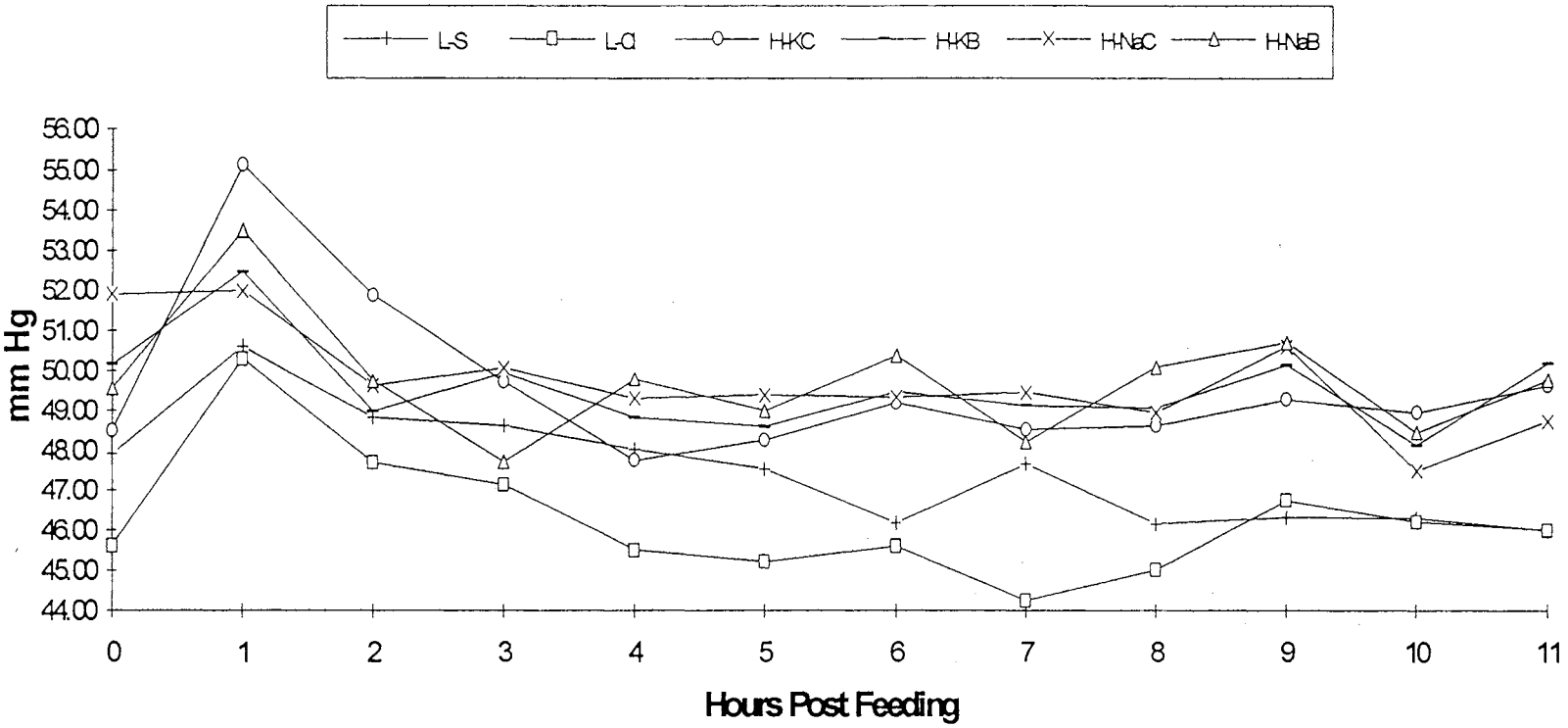

Figure 3. EFFECT OF DIETARY CATION-ANION BALANCE ON BLOOD pCO_2 POST FEEDING

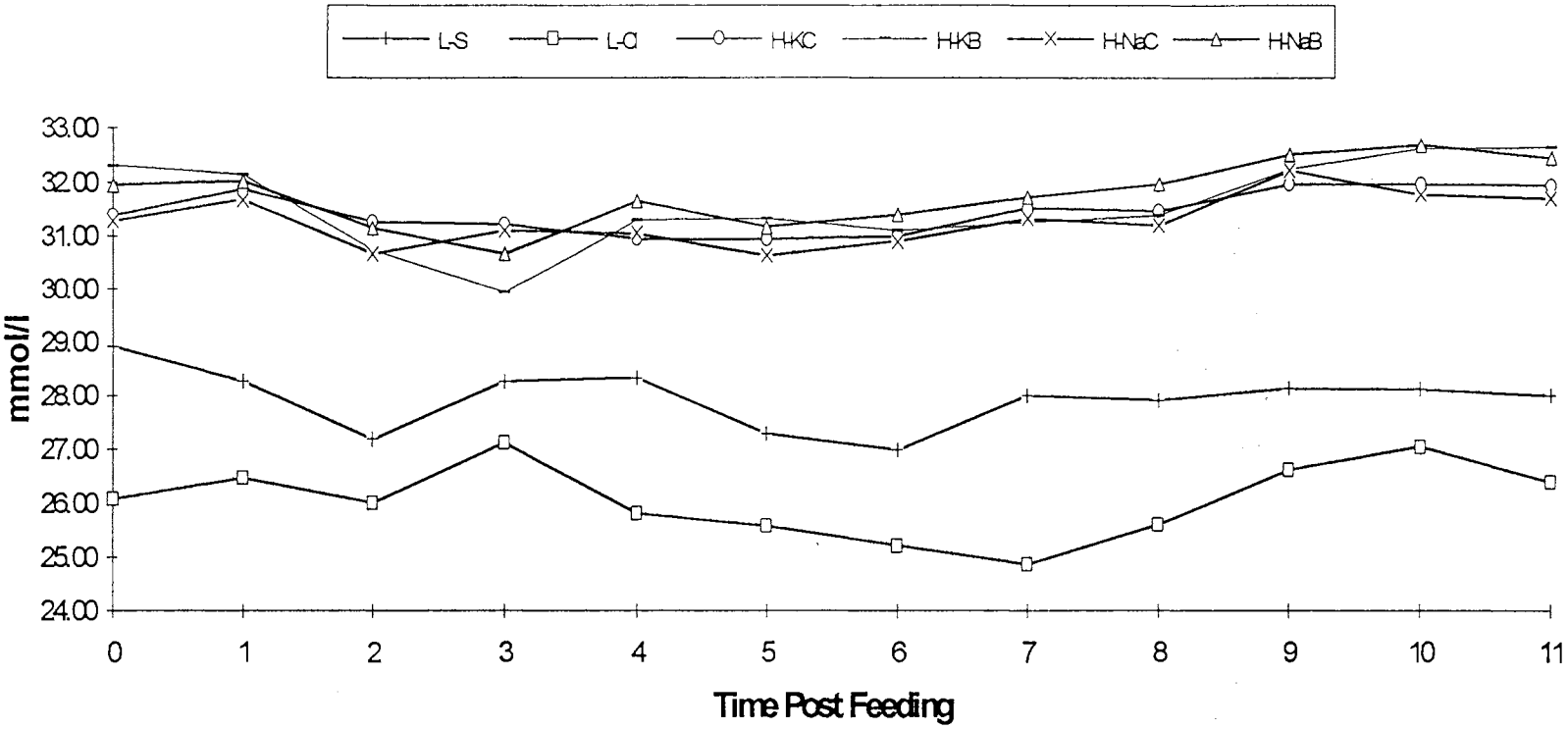

Figure 4. EFFECT OF DIETARY CATION-ANION BALANCE ON BLOOD HCO_3 POST FEEDING

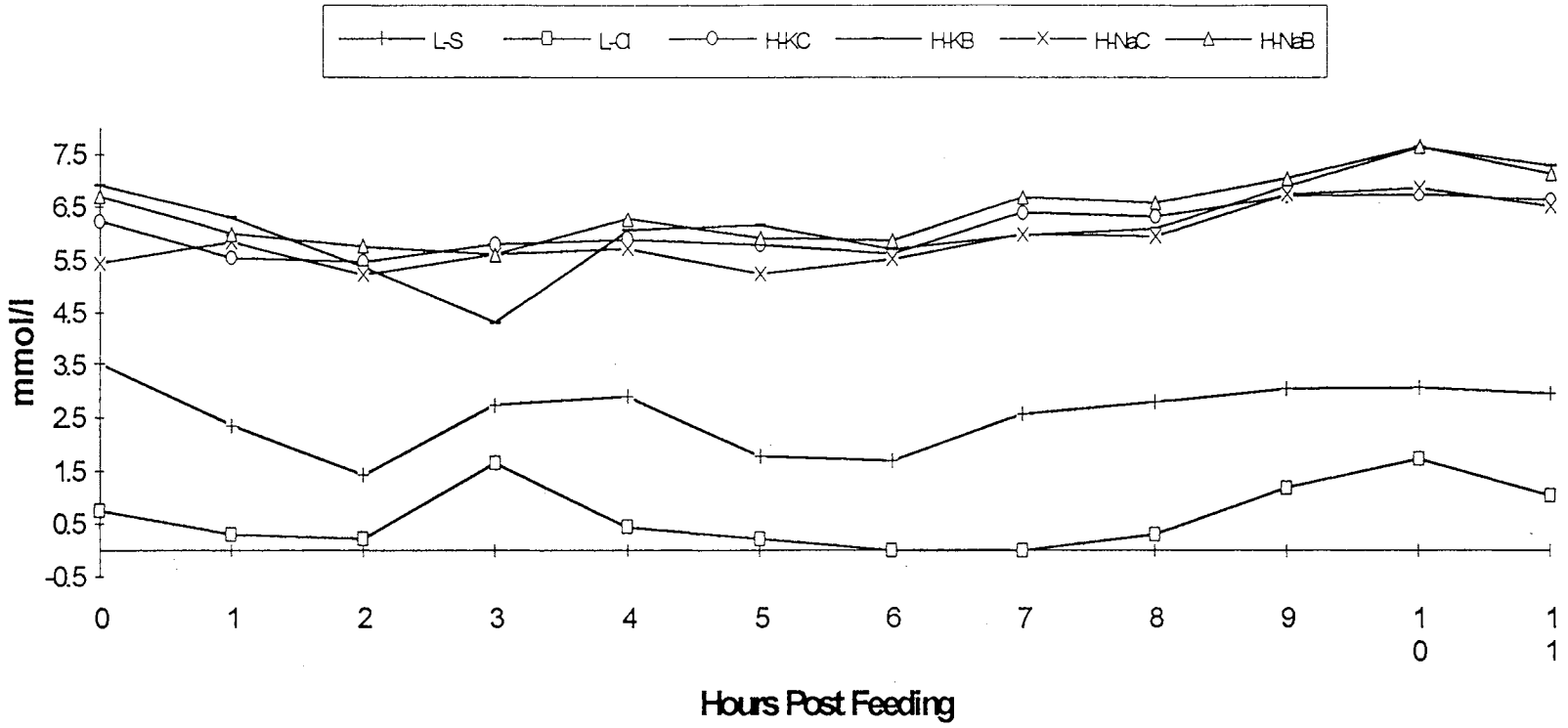

Figure 5. EFFECT OF DIETARY CATION-ANION BALANCE ON BLOOD BASE EXCESS POST FEEDING

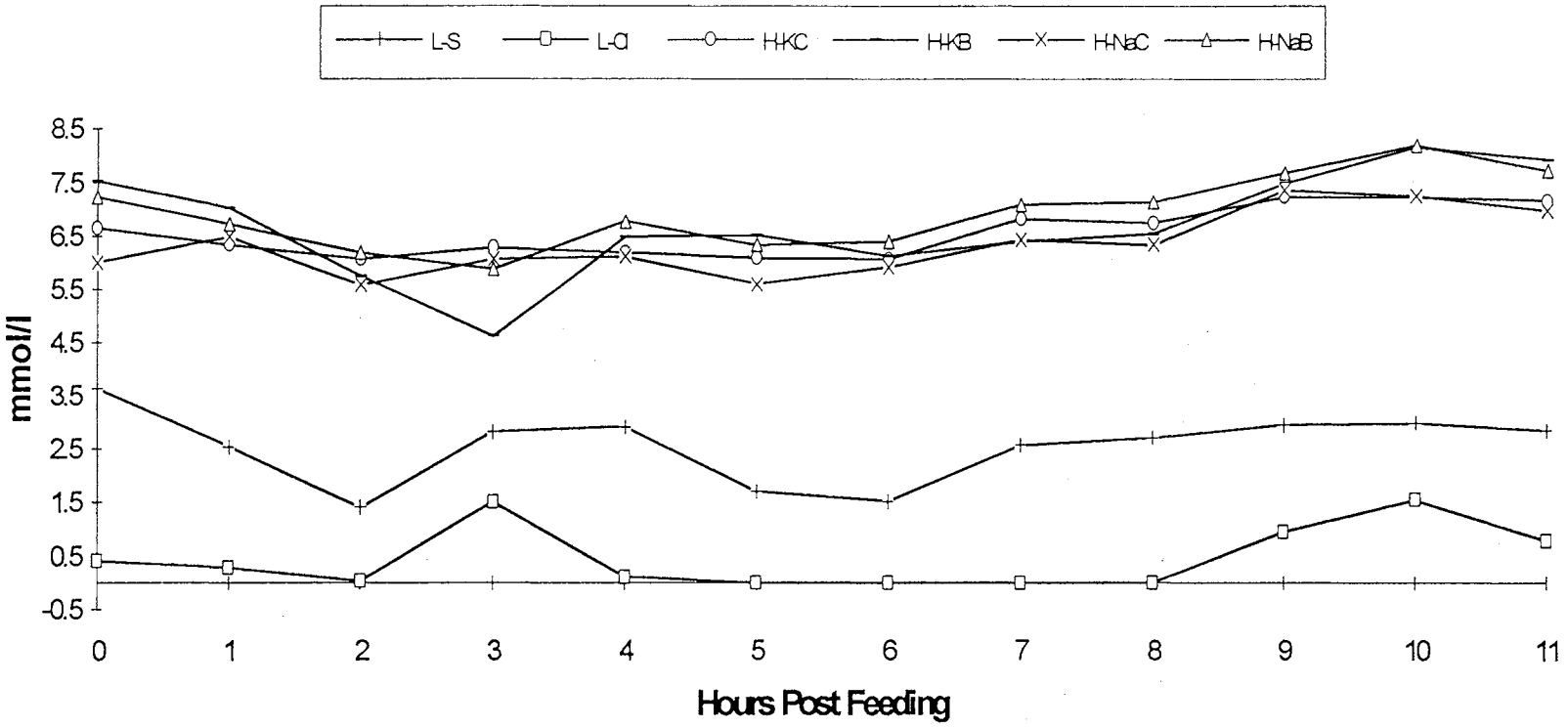

Figure 6. EFFECT OF DIETARY CATION-ANION BALANCE ON BLOOD BASE EXCESS, extracellular fluid POST FEEDING

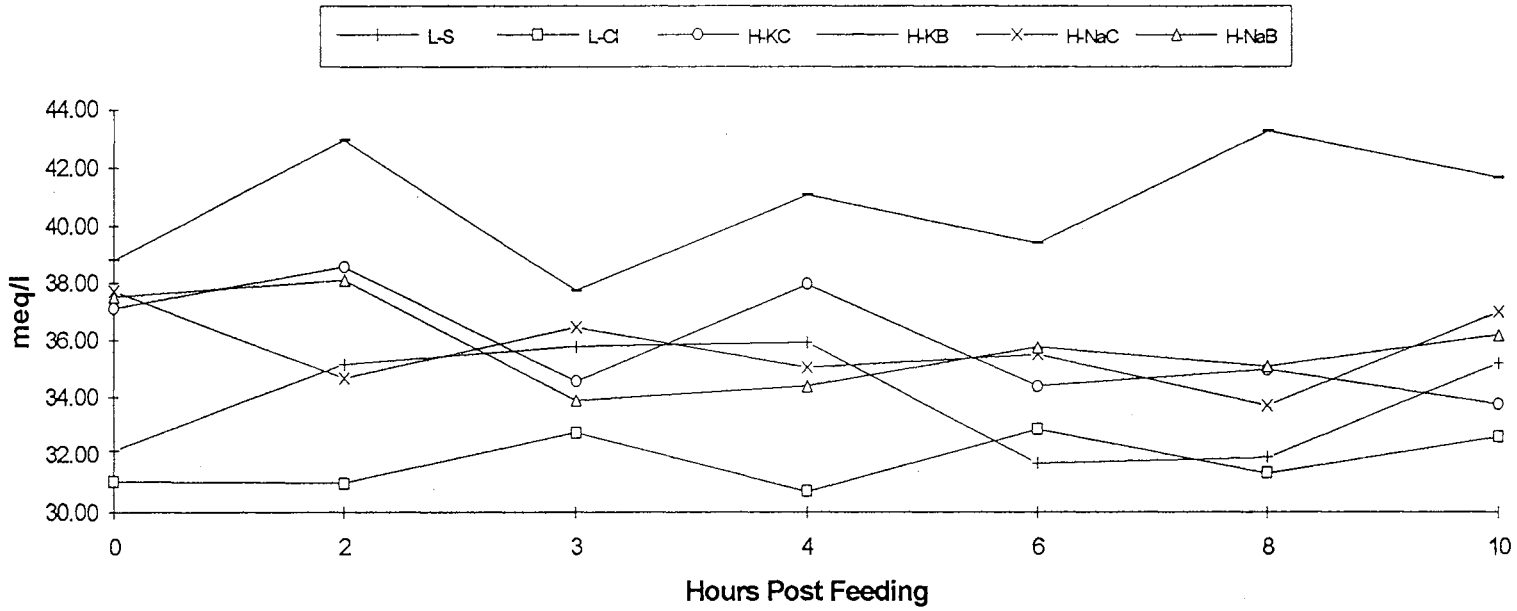

Figure 7. EFFECT OF DIETARY CATION-ANION BALANCE ON SERUM CATION-ANION BALANCE POST FEEDING

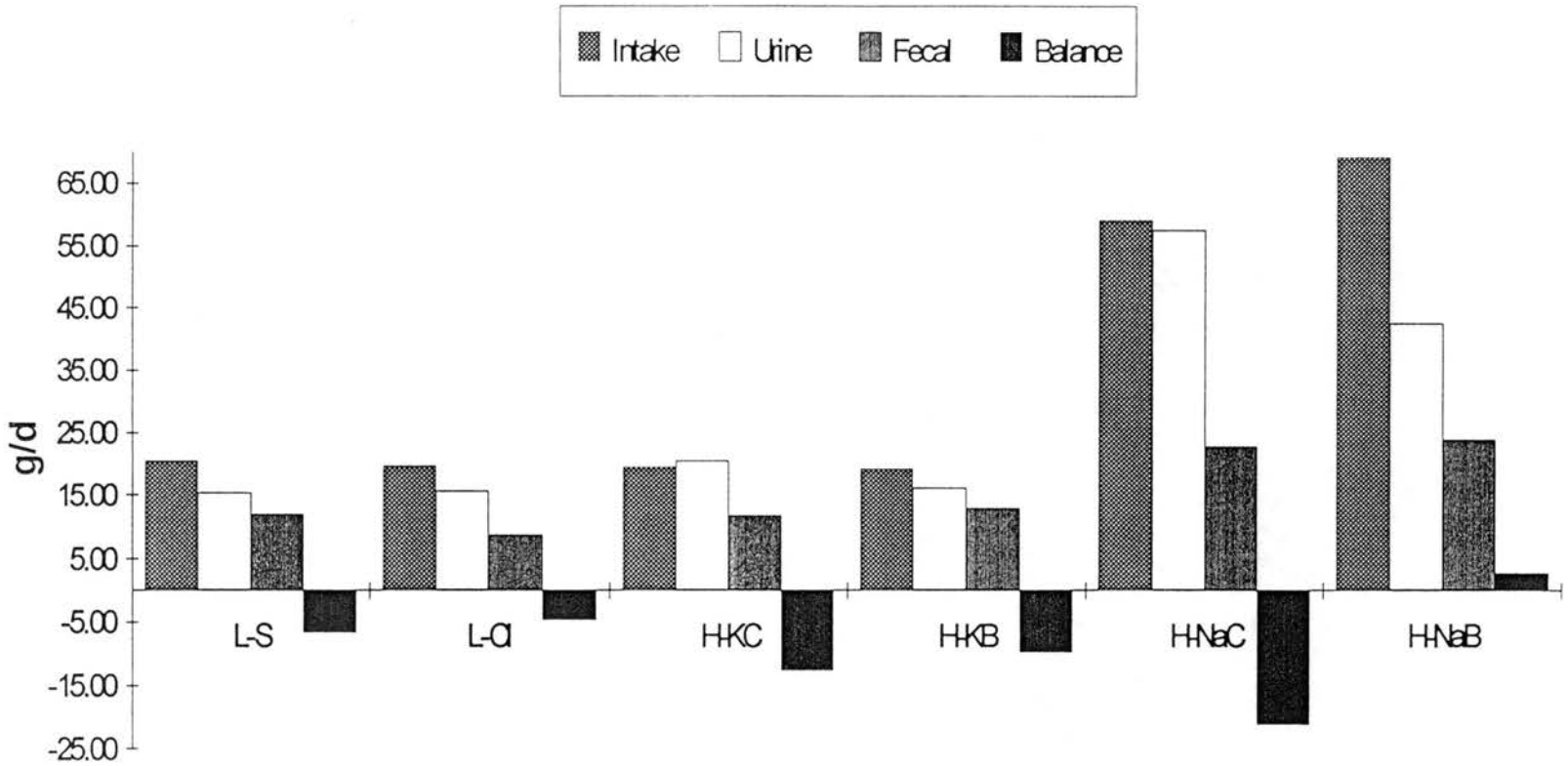

Figure 8. THE EFFECT OF DIETARY CATION-ANION BALANCE ON SODIUM BALANCE IN SEDENTARY HORSES

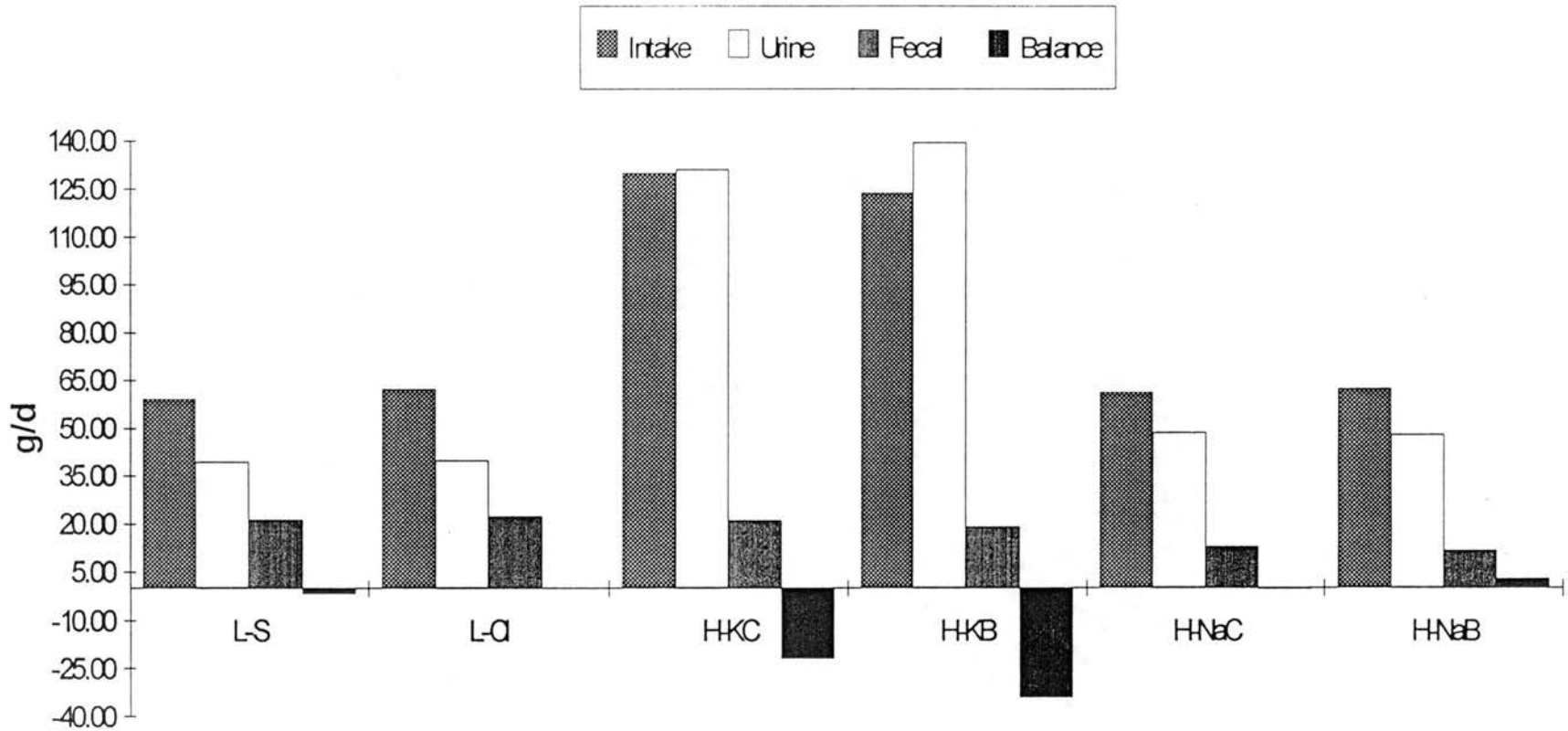

Figure 9. THE EFFECT OF DIETARY CATION ANION BALANCE ON POTASSIUM BALANCE IN SEDENTARY HORSES.

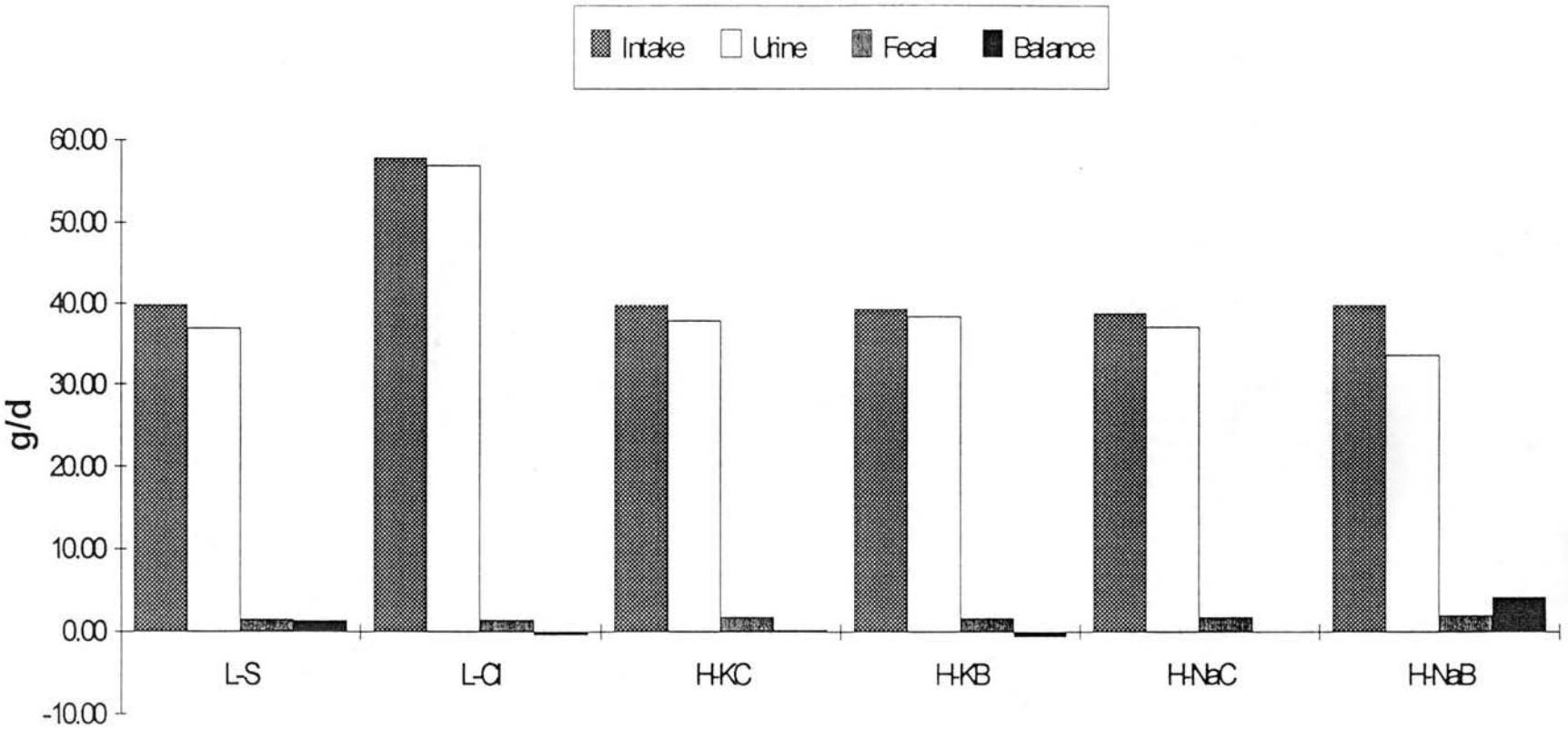

Figure 10. THE EFFECT OF DIETARY CATION-ANION BALANCE ON CHLORIDE BALANCE IN SEDENTARY HORSES.

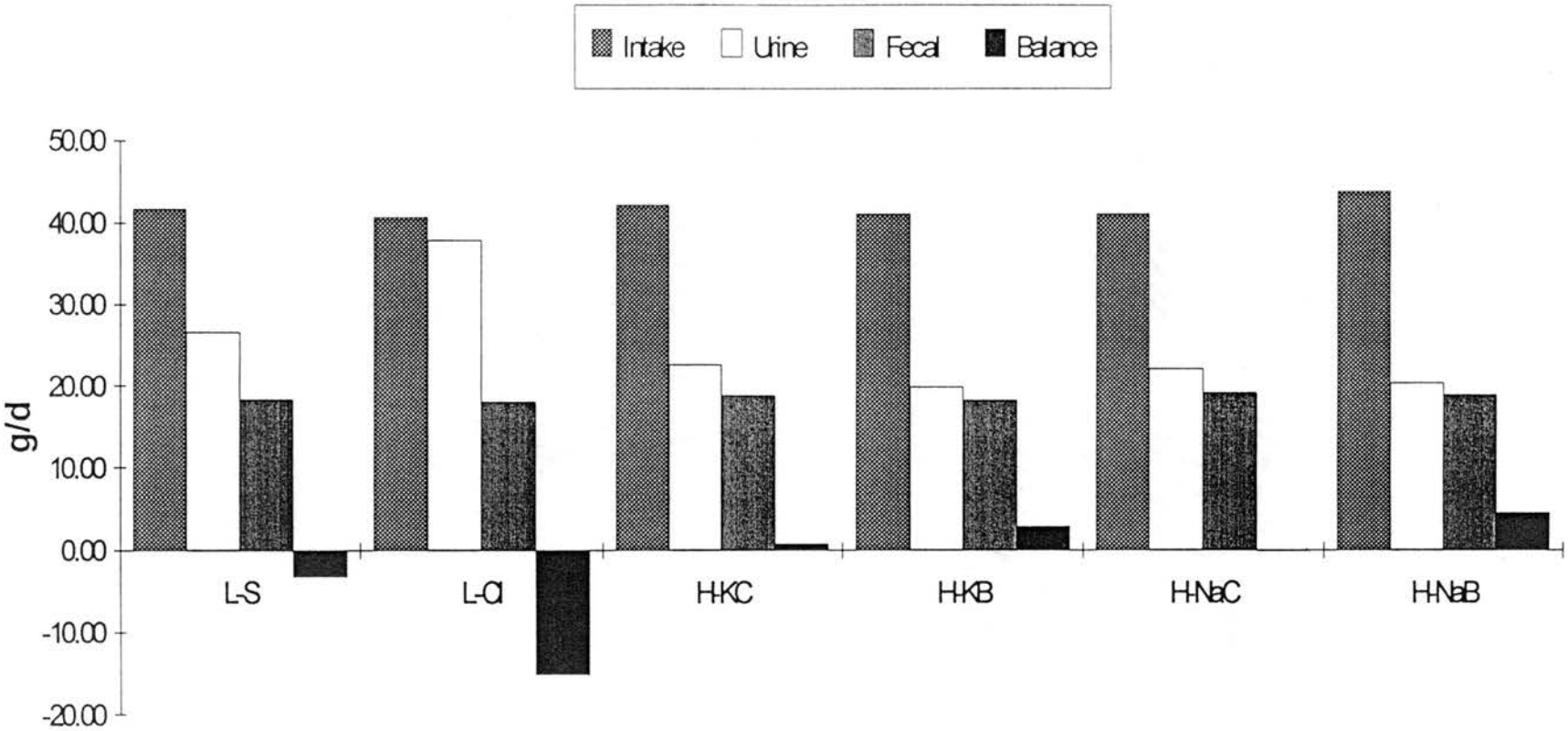

Figure 11. THE EFFECT OF DIETARY CATION-ANION BALANCE ON CALCIUM BALANCE IN SEDEMTARY HORSES.

VITA

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Doctor of Philosophy

Thesis: THE COMPARISON OF TWO FORMS OF SODIUM AND POTASSIUM AND CHLORIDE VERSUS SULFUR IN THE DIETARY CATION-ANION BALANCE EQUATION AND SUBSEQUENT EFFECTS ON ACID-BASE STATUS AND MINERAL BALANCE IN SEDENTARY HORSES.

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