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EFFECT OF DIETARY CATION-ANION DIFFERENCE ON ACID-BASE STATUS ENERGY DIGESTIBILITY AND MINERAL BALANCE IN SEDENTARY HORSES FED VARYING LEVELS AND SOURCES OF STARCH

By

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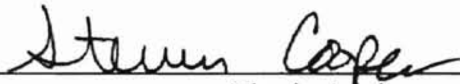

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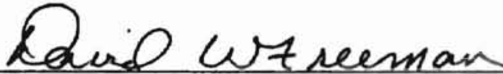

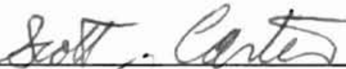

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CHAPTER I

INTRODUCTION

To be successful in the horse industry today, producers must raise champions in the show pen and on the racetrack, while watching the bottom line. An area that affects this a great deal is the money spent on the feed to produce these winners. Grain prices fluctuate like the Stock Market, and what may be profitable one day, may cause bankruptcy the next. Therefore, a great deal of research focuses on the process of digestion in the animal. The more investigators understand digestion, the more information they can pass on to the producer, and ultimately reduce the costs associated with feeding. If an animal gets out of its normal homeostatic range, production can suffer. There have been many studies in horses and other species that have established the link between the Dietary Cation-Anion Difference (DCAD) and the subsequent alterations in acid-base status, mineral metabolism and growth of the animal. Currently there is no recommended DCAD level for horses set forth by the NRC. However several companies are starting to utilize research studies and incorporate DCAD into their ration balancing formulas.

The purpose of the current study was to determine the effect of DCAD on the acid base status, energy digestibility and mineral balance of sedentary horses consuming varying sources of starch and starch intakes. Several studies have linked high starch diets to metabolic acidosis, but have neglected to address the mineral content of these diets, and thus the DCAD effect on acid/base parameters. Therefore the objective of this study was to evaluate the effects of DCAD in horses fed varying starch intake and sources to investigate which factor most effects metabolic homeostasis.

CHAPTER II

LITERATURE REVIEW

Acid-Base Physiology

To sustain life, animals must maintain the acid-base balance of the body within a very narrow range. The critical reason is that excessive protein denaturation will occur due to elevated levels of hydrogen ions (Rose, 1994). Thus, maintaining extra-cellular fluid (ECF) pH is vital for normal body function. The definition of pH is the inverse logarithm of the hydrogen ion concentration of a system (pH = -log [H+]). In physiologic systems, acids are compounds that donate protons (H+), whereas bases are those substances that accept protons. For physiological purposes, the most important weak acid and conjugate base compounds are carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-), respectively. To maintain the desired pH, animals must maintain these two compounds in a proper ratio in accordance with the Henderson-Hasselbalch equation, which states that the $pH = 6.1 + \log [HCO_3^-]/[H_2CO_3]$. Unfortunately, the quantification of carbonic acid is very difficult. Therefore, we estimate its concentration by taking the partial pressure of CO_2 (due to CO_2 reacting with water to form carbonic acid) and multiplying it by its solubility coefficient of 0.03. This results in the equation being rewritten as $pH = 6.1 + \log [HCO_3^-]/(.03 * pCO_2)$. The stable ratio of 20 parts bicarbonate to one part dissolved CO_2 is the principle determining factor to body pH, and any alteration of this ratio must be corrected. A more accurate determination would also include hemoglobin, plasma protein and phosphate buffer systems. Another way to estimate the pH of the body and its subsequent homeostasis is to look at the system's electrolyte balance. Under normal conditions the sum of plasma sodium and potassium will exceed chloride and bicarbonate, and is known as the anion gap (Duke, 1993).

A depression in ECF pH beyond normal (7.4) is called acidosis, while an increase in pH above normal is termed alkalosis. To further divide these categories, excess addition of acid or loss of base from the ECF resulting in a decreased pH is referred to as metabolic acidosis. On the other hand, an increase in pH due to decreased acid or base excess, is termed metabolic alkalosis. If the acidosis is due to a decrease in alveolar ventilation resulting in reduced expiration of CO_2, respiratory acidosis develops. When alveolar ventilation and CO_2 expiration increases above normal, respiratory alkalosis may occur (Duke, 1993).

The body uses three mechanisms to counteract these conditions and include chemical buffering, changing respiration rate, and excretion via the kidney. Chemical buffering entails the use of the bicarbonate buffer system that will produce more weak acid or conjugate base in response to increased strong base or strong acid, respectively. In the second system, respiration rate can be altered to either expel more or less CO_2 than is produced, thus again altering the ratio of acid to base. In the final system, hydrogen ions and bicarbonate ions are filtered through the kidney. Excretion of one of these ions and reabsorption of the other, depending on the state of the animal, will result in the ultimate return to a homeostatic condition (Duke, 1993).

In the intestinal lumen, sodium and potassium ions are absorbed into the blood in exchange for a hydrogen ion (H^+) which enters the lumen. In a similar situation, Cl^- will be absorbed from the lumen in exchange for an HCO_3^- ion being released into the lumen. Therefore it is evident that the absorption of ions from the gastrointestinal tract can have a dramatic effect on the acid/base status of the body. (Duke, 1993)

Dietary Cation-Anion Difference

Knowing that gastrointestinal absorption of cations and anions could alter the body's acid-base status; a great deal of research has evaluated the ratio of cations to anions in the diet. The ratio of these ions in the diet is known as the dietary cation-anion difference (DCAD). Using this knowledge, many researchers have looked at these levels and studied the effects on the body. Several numerical equations have been developed from this research to quantify DCAD. Several studies have utilized the cations sodium (Na^+) and potassium (K^+) and the anion chloride (Cl^-) to calculate DCAD using the following equation: meq $[(Na^+ + K^+) - Cl^-]$/ kg dietary DM (Baker et al., 1992; Patience et al., 1987; Fredeen et al., 1988b). Due to its acidogenic nature, researchers also started including sulfur into the anion portion of this equation (Tucker et al., 1991). Currently, DCAD is calculated as: meq $(Na^+ + K^+) - (Cl^- + S^-)$/ kg DM. (Block, 1984; Popplewell et al., 1993; Baker, et al., 1997). The principle of the DCAD equation assumes the complete dietary availability of Na^+, K^+, and Cl^-, and the acidifying properties of sulfur (Tucker et al., 1988; Block 1991). Using this equation, researchers can formulate diets with variable DCAD values and study the resulting alterations in homeostasis and mineral metabolism.

Effects on Acid/Base Parameters

Blood pH

Many researchers have found that altering the DCAD level of the diet can have dramatic effects on body acid/base physiology.

Looking at blood pH, many studies have shown a very dominant correlation between the arterial and venous pH values and the DCAD of the diet. Escobosa et al., (1984) fed a high Na^+ diet (DCAD +320 meq/kg DM) and a high Cl^- diet (DCAD -191 meq/kg DM) to lactating dairy cows and found blood pH values lower than normal for the high Cl^- diet, and elevated pH readings on the high Na^+ diet. Further research in dairy cows (Tucker et al., 1988) showed that pH varied from 7.369 for cows on a -100 meq/ kg DM diet, up to 7.427 for the cows on a +200 meq/ kg DM diet. Research in swine (Patience et al., 1987) has shown that growing pigs, fed diets high (+341) and low (-85) in DCAD, had a mean blood pH of 7.21 and 7.09, respectively. Studies in sheep (Abu Damir et al., 1990) have shown that when ammonium chloride (↓DCAD) was added to the diet, blood pH was significantly lower ($P<.05$) than sheep that consumed diets with added sodium bicarbonate (↑ DCAD). In horses, several studies have shown results that are dramatically similar. Baker et al. (1992) fed four diets with a calculated DCAD of +21, +125, +231, and +351 meq/ kg DM, and found that arterial and venous blood pH values increased as DCAD increased. Stutz and coworkers (1992) found that the blood pH was significantly lower for horses consuming a -50 meq/kg DM diet as opposed to either a +150 or +250 meq/kg DM diet . Other studies have also shown that horses consuming a low DCAD diet tended to exhibit an induced metabolic acidosis (Wall et al., 1995; Popplewell et al., 1993).

Blood pCO_2

Because of the connection between blood pH and blood pCO_2, it would seem logical that DCAD would have a very similar response on pCO_2.

In dairy cattle, Escobosa and coworkers (1984) found that the pCO_2 values varied from 30.57 mm Hg on the low DCAD diet to 32.98 mm Hg on the high diets. Further work with dairy calves has shown that pCO_2 increases linearly ($P<.0004$) as the DCAD of the diet increases (Jackson et al., 1992). Patience et al., (1987) also showed a response in swine, with an increase in pCO_2 from 73.9 mm Hg to 74.2 mm Hg as the DCAD increased from -85 to +341 meq/kg DM. Stutz et al.(1992) found that the pCO_2 in horses increased from 46.75 mm Hg to 55.2 mm Hg as the DCAD increased from -50 to +250 meq/kg DM. Further studies showed that horses consuming a –21 meq/kg DM diet had significantly lower pCO_2 levels than horses consuming a +231 meq/kg DM diet (Baker et al., 1992). This alteration is pCO_2, is due an increase in alveolar respiration (or decrease depending on the state) during metabolic acidosis, to result in removal of CO_2 and returning the ration of conjugate base to weak acid back to normal (Duke, 1993)

Blood Bicarbonate

The levels of bicarbonate (HCO_3^-) in the blood also have been found to vary with the DCAD of the diet. Patience et al. (1987) found that blood HCO_3^- concentration of swine showed a linear ($P<.001$) and quadratic ($P<.022$) relationship to DCAD. Dairy cows also showed a response in HCO_3^- increasing from 18.06 to 24.64 meq/l when fed a -191 and 320 meq/kg DM diet, respectively (Escabosa et al., 1984). Tucker et al., (1988) showed a similar response in dairy cattle to low and high DCAD diets. Horses have also shown a significantly lower ($P<.05$) mean arterial HCO_3^- concentration when consuming a +21 meq/ kg DM diet as compared to +125,+231, or +350 meq/kg DM diets (Baker et al., 1992). In a later study, Baker and coworkers (1997) again found that bicarbonate levels were lower ($P <.05$) when horses consumed a low DCAD diet as opposed to a high diet.

Additional studies have demonstrated a possible trend toward lower bicarbonate concentrations in exercising horses consuming a diet low in sodium and potassium, as opposed to a high sodium and potassium diet (Stutz et al., 1992).

Urine pH

The pH value of urine is also a good indication of the acid/base status of the body. The final regulatory mechanism is either H^+ or HCO_3^- excretion through the urine in response to acidosis or alkalosis, respectively. In horses, Wall and coworkers (1995) showed that DCAD had a significant effect on urine pH. The pH means ranged from 5.38 to 8.34 as the DCAD increased from +13 to +367 meq/kg DM . Baker et al. (1992) also showed that horses exhibited a significant response in urine pH to the varying DCAD of the diet. Additional studies show that the urine pH was significantly lower for horses consuming a 10 meq/kg DM diet as opposed to a 95 meq/kg DM diet. In the same study, urine pH was increased ($P<.05$) as the DCAD of the diet increased from 165 meq/kg DM to 295 meq/kg DM. Furthermore, work in dairy cattle reveals a decrease ($P<.01$) in urinary pH as the DCAD of the diet decreases (Escabosa et al., 1984). Tucker et al. (1988) found a linear and quadratic ($P<.05$) response in urine pH as the DCAD of the diet increased.

Effect on Mineral Metabolism

The effect of dietary cation anion balance on mineral absorption and excretion has also been very well studied. Exercising horses had a linear increase in urinary calcium excretion as the DCAD of the diet decreased from 327 meq/ kg DM to 5 meq / kg DM (Wall et al., 1992).

Baker et al. (1997) also found that horses consuming an anionic diet, achieved through ammonium chloride addition, had a lower ($P<.05$) apparent daily calcium balance as compared to all other diets. An increase in urinary sodium excretion was observed in sedentary and exercising horses as the sodium content and thus DCAD of their diet was increased (Baker et al.,1993). In this study, urinary excretion of Ca^{++} and Cl^- increased in horses consuming a low DCAD diet. Further studies in weanlings also show an increased urinary excretion of Ca^{++} and Cl^- when horses consume a –25.69 meq/kg DM diet (Cooper et al., 1995). This same study found that horses on a high DCAD diet (+379 meq/kg DM) had higher ($P<.01$) urinary excretion of Na^+ and K^+. Wall et al. (1992) found that horses consuming low DCAD diets (high levels of chloride), excreted more ($P < .05$) chloride in their urine than horses consuming diets high in DCAD (low levels of chloride). On the other hand, this same study found that DCAD had no effect on the urinary excretion of magnesium, phosphorus, potassium, or sulfur. Fecal calcium excretion shows dramatic decreases for horses consuming low DCAD diets which resulted in an overall increase in Ca^{++} balance on the low diets (Wall et al., 1997).

In growing lambs, Abu Damir et al. (1990) found that lambs fed a sodium bicarbonate supplemented diet retained 1.46, 1.26, and 1.21 times the calcium, phosphorus, and magnesium, respectively, than those animals fed an ammonium chloride supplemented diet. Further research in sheep has shown that sheep infused with hydrochloric acid (HCl) produced increase urinary excretion of Ca^{++}, Na^+, and Cl^- when compared to a control group (Scott et al., 1981).

In lactating goats, it was found that urinary calcium excretion was increased on anionic diets when compared to a cationic diet (Fredeen et al., 1988a). During lactation, these goats on the anionic diet also had an apparent increase in calcium absorption, as evidenced by a decreased fecal excretion. The incidences of milk fever have been found to decrease on low DCAD diets (Block, 1984). It was found that if dairy cows consumed a –12 meq/kg DM diet there was no milk fever during the study.

If the diet increased to a +33 meq/kg DM, the incidence of milk fever increased to 47.4 % throughout the study. It is postulated that the acidifying nature of low DCAD diets will increase bone mobilization of Ca^{++} to offset the Ca^{++} lost in the milk.

The mechanism for altered excretions of these minerals is not fully understood. For the minerals sodium, potassium and chloride, intake is the chief determinant of amount excreted. The major source of these minerals is intestinal absorption from food or water, thus balance is a factor of intake. In the case of calcium, phosphorus and magnesium, the body has a significant concentration of these minerals already present in the bone. Therefore, mineral balance is a function of gastrointestinal absorption as well as the amount deposited and/or removed from bone (bone turnover). Calcium, magnesium, and phosphorus excretion via the kidney is altered primarily by parathyroid hormone (PTH) levels. In the intestinal lumen, the compound 1,25 $(OH)_2D_3$ (active form of vitamin D_3) will promote the absorption of calcium and phosphorus (Best and Taylor, 1985). However, there are many contradicting reports as to how acidosis or alkalosis will effect these mechanisms. One study found that rats in an induced metabolic acidosis had increased serum calcium levels when compared to control and alkalotic rats. It was determined that this rise in Ca^{++} was due to increased levels or efficacy of PTH in response to the acidotic state, thereby increasing the mobilization of Ca^{++} from bone. Furthermore this same study found that acidotic rats had an overall increase in urinary excretion of calcium, possibly due to acidosis inhibiting renal reabsorption of calcium. However, this decrease in reabsorption diminished in acidotic rats with the administration of exogenous PTH.

Thus while the acidosis directly inhibits renal reabsorption, the increased levels and efficacy of PTH due to the acidosis, could partially decrease the level of inhibition (Beck and Webster, 1976).

Energy Metabolism

Energy is required to drive body processes. The ultimate energy source of all living organisms is adenosine triphosphate (ATP). ATP is derived from many sources depending on the organism. Plants transfer the energy of sunlight into ATP during photosynthetic reactions. Animals must consume substances, break them down into smaller molecules, and form ATP to gain energy for life. Many processes involved in the final production of ATP require energy themselves, thus the amount energy consumed is not the same as the amount of energy available for body functions. Gross energy is the amount of energy available in a substance, determined by a total combustion of the substance and measuring the amount of heat given off. As feedstuffs are consumed, some of this stored energy is not digested ,and thus lost in feces. From there significant amounts of the digestible energy are lost in urine and production of gases. Of the remaining energy, a great deal is lost in the form of heat, thus leaving smaller amounts of energy available to the animal for body processes (Lewis, 1995)

Heat produced during complete combustion	Amount of energy that is absorbed from the intestine	Amount of energy available to the cells for metabolism	Amount of energy available that the body can use for production and body maintenance
Gross Energy (GE) ⇨	**Digestible Energy (DE)** ⇨	**Metabolizable Energy (ME)** ⇨	**Net Energy**
Energy lost in feces	Energy lost in urine and gases	Energy lost in heat	

Carbohydrate Digestion

The main energy sources for today's livestock are vegetative sources of carbohydrates. There are many forms of carbohydrate that can be utilized including structural carbohydrates, sucrose, lactose, and starches. Structural carbohydrates make up the rigid supporting structures of the plant cell, and are made up of cellulose and hemicellulose. Cellulose is an unbranched polymer of glucose linked by β- 1,4 bonds which can only be broken down with the aid of microbial digestion. Sucrose and lactose are disaccharides that are made up of glucose and fructose (sucrose) and glucose and galactose (lactose). These sugars are degraded in the gastrointestinal tract and absorbed as their respective monosaccharides. Starch is a polymer of glucose units connected by α-1,4 glucosyl bonds to form linear chains and α-1,6 glucosyl bonds form branches in the polymer (Duke, 1993).

Starch Utilization

For horses, starch is a major energy source that is generally fed in high levels via concentrate diets. The major source of starch in these diets is from the endosperm of seed grains such as corn, oats and barley, which can typically have 75 %, 58 % and 64 % starch, respectively (Herera-Saldana et al., 1990). The extent and site of digestion of different starch sources is very similar in the equine gastrointestinal tract. In a study by Potter et al. (1992), they found that when horses were fed corn, oats, barley and sorghum there were no differences in the extent of starch digestion. For these grains, the starch digestibility averaged 98.6 % with 86.3 % of digestion occurring in the small intestine. This study also showed that as the dietary starch component increased, more starch was degraded in the cecum.

Potter also found that as starch content exceeded 0.4% of the body weight per feeding, an upper limit to small intestinal digestion of starch was reached, resulting in an increased amount of undigested starch reaching the cecum. Starch degradation in the cecum, via anaerobic microbial digestion, can result in a lactic acidosis, very similar to carbohydrate overload in ruminants (Garner et al., 1977). This lactic acidosis in horses could result in severe lameness and even possible death.

For the starch to be available to the body cells, it must be broken down to glucose, which is the major source of cellular energy. The breakdown of a majority of these starches in the intestinal lumen is due to the pancreatic enzyme *α-amylase*. This enzyme cleaves the interior α-1,4 linkages of glucose yielding the oligosaccharides, maltose (two glucose units), maltotriose (three glucose units), and remaining glucose units containing α-1,6 bonded glucoses, known as α-dextrin. Little free glucose is formed by *α-amylase* in the intestinal lumen. Glucose is not formed until enzymes within the brush border membranes utilize *oligosaccharidase* enzymes to allow further breakdown. A group of enzymes collectively known as *maltases* will degrade maltose and maltotriose into glucose, and *α-dextrinase* will breakdown α-dextrins into glucose. Now that glucose is present, it must be absorbed into the bloodstream to be distributed throughout the body. This is accomplished through the active cotransport (with Na^+) of glucose across the brush border membrane into the portal blood system. The energy for this transport is derived from the sodium-potassium ATPase pump, thus sodium has to be present in a ratio of two moles of sodium per one mole of glucose, in order for maximum glucose transport to occur. Upon glucose being delivered to the cell it is then available for energy metabolism and ATP production via glycolysis (Gray, 1992)

Fiber Utilization

For the forage content of the diet to be digested in the horse, the large intestine, namely the cecum becomes greatly involved. The insoluble fiber portion of the diet (cellulose and hemicellulose) is very similar to starch, except it is a β linked glucose chain instead of α linked. There are no enzymes produced by the animal that are capable of breaking this bond. However, microbes namely bacteria, protozoa, and some fungi, do produce such an enzyme and are present in high concentrations in the cecum of the horse. Thus, a symbiotic relationship exists between the host animal and the microbes. The primary products of microbial digestion of forages are the volatile fatty acids (VFA's) (Duke, 1993) As the forage to concentrate ratio changes to include more concentrate there is an increase in the cecal concentration of propionate and a decrease in acetate, compare to high forage diets (Hintz et al., 1972) These VFA's (acetic, proprionic, and butyric) are consequently absorbed by the large intestine and are utilized as a source of energy (Duke, 1993)

Starch Research

Significant research has been done in the animal to study the effects of altering dietary forage:concentrate ratios. Hintz et al. (1972) found no decrease in forage digestibility due to addition of grain. They also found that the plasma glucose levels showed no differences with forage to grain ratios of 1:0, 3:2 or 1:4. Stull et al. (1988) found that as the equine diet changed from 100 % alfalfa to 100% corn there was no difference in plasma glucose, cortisol or insulin levels. In terms of acid-base status several studies have also been done with some profound results. Dairy heifers consuming a 90 % corn diet had lower blood pH and bicarbonate than those heifers consuming a 100 % alfalfa diet (Roby et al., 1987).

Furthermore, this same study showed that the depressed pH and bicarbonate levels could be reversed by the addition of 2 % sodium bicarbonate. Ralston et al. (1997) further demonstrated in horses that a reduced post feeding acidemia (P=.01) and higher levels of blood bicarbonate (P=.004) could be obtained with the addition of 1 % sodium bicarbonate to a grain and alfalfa hay diet fed in a 50:50 ratio.. Grains typically have a low cation content (Na^+, K^+, Ca^{++}) and a high anion (Cl^-) content, thus resulting in a low DCAD for that particular grain (Corn ≈ 58 meq/kg DM, Oats ≈ 73 meq/kg DM(NRC, 1989)). Forages, on the other hand, generally have elevated levels of cations, therefore they have an increased DCAD (Alfalfa ≈329 meq/kg DM, Bermuda grass hay ≈ 427 meq/kg DM(NRC, 1989)). Thus, it is very plausible that the increased acid load of animals consuming a high grain diet is due to a concurrent decrease in DCAD in association with the increased grain portion of the diet (Abu Damir et al., 1990).

Objectives

Since grains are a significant portion of the diets of horses, it is vital that researchers and producers understand how they can affect the body's homeostasis. Maximum production occurs when the body is functioning within its normal parameters. Thus, anything the owner can do to achieve maximum performance would be of benefit. Therefore, the purpose of the current trial was to investigate the effect of DCAD on acid-base status, mineral balance and energy digestibilities of sedentary horses fed varying intakes and sources of starch. The objectives of this study include 1) measurement of acid-base parameters such as blood pH, pCO_2, HCO_3^-, and urine pH; 2) determine the actual energy digestibilies of the treatment diets; and 3) determination of daily mineral balances of sodium, potassium, chloride, magnesium, phosphorus, and calcium.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

Six mature sedentary horses (3 mares & 3 geldings) were used in a 6 x 6 Latin square design experiment to study the effects of DCAD on the acid-base status, energy digestibility, and mineral balance of mature horses fed varying starch intakes and sources. The Latin square was designed so as to reduce any statistical effect of carryover between the experimental diets. Each diet preceded and /or followed each other diet only once (Table 1). Thus if carryover did occur when moving from one diet to another, that combination would only occur once, thus having little effect on the overall outcome. (John, 1971) During each of the six experimental periods, horses were fed one of the six experimental diets during an 11-d adjustment period followed by a 72 h collection period. The six diets were formulated utilizing one of three energy sources combined with one of two DCADs.

Horses were exercised 15 minutes/day on a mechanical walker at a brisk trot. On days one, five and eleven of the adjustment periods all horses were weighed prior to the morning feeding. Routine deworming were completed throughout the study.

Experimental Rations

All diets were formulated to equalize daily intakes of energy, protein calcium and phosphorus.

Table 1.
Latin Square Design

	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6
Period 1	HC	LC	HO	LO	HH	LH
Period 2	LC	HO	LO	HH	LH	HC
Period 3	LH	HC	LC	HO	LO	HH
Period 4	HO	LO	HH	LH	HC	LC
Period 5	HH	LH	HC	LC	HO	LO
Period 6	LO	HH	LH	HC	LC	HO

HC→ High DCAD-Corn Diet

LC→ Low DCAD-Corn Diet

HO→ High DCAD-Oats Diet

LO→ Low DCAD-Oats Diet

HH→ High DCAD-Hay Diet

LH→ Low DCAD-Hay Diet

The treatments were as such: 1) rolled corn with a DCAD of >300 meq/kg DM (HC); 2) rolled corn with a DCAD of <100 meq/kg DM (LC); 3) whole oats with a DCAD of >300 meq/kg DM (HO); 4) whole oats with a DCAD of <100 meq/kg DM (LO); 5) dehydrated alfalfa with a DCAD of >300 meq/kg DM (HH), and 6) dehydrated alfalfa with a DCAD of <100 meq/kg DM (LH). Native prairie grass was fed at 30 % of daily intake for the four grain diets and 50 % of daily intake on the alfalfa diets (Table 2) The DCAD of the HH diet is the naturally occurring DCAD level. Sodium bicarbonate was added to the HC and HO diets to achieve the DCAD levels above 300 meq/kg DM. On the LC, LO and LH diets, ammonium chloride as added to result in the low DCAD values. Horses were fed at 7:00 a.m. and 7:00 p.m. and supplied with fresh water at all times. Daily feed intake was constant within a treatment regardless of animal weight (Table 3). Horses were given ½ of the previous diet and ½ of the current diet for the first three days of the adjustment period in order to decrease the chances of gastrointestinal problems.

Mineral Balance

During the 72 h collections, total urine production was collected via urine harness. Urine was collected at the time of urination, immediately measured for volume and analyzed for pH (Cole-Parmer® Hand-held pH meter). A sample representing 1% of the volume was then frozen and composited for later analysis. Fecal samples were obtained via rectal palpation to represent every 2 h post feeding. Samples were composited on equal weight basis, and dried for 72 h at 40°C.

Table 2.
Diet composition, as fed basis

	Treatments					
Ingredient %	**HC**	**LC**	**HO**	**LO**	**HH**	**LH**
Starch sources						
Rolled corn	45.5	45.5	----	-----	-----	-----
Whole oats	----	-----	48	48	-----	-----
Pellet						
Dehy. alfalfa	----	-----	-----	-----	49.1	48.1
Soybean meal	18.7	19.5	16.8	17.5	-----	-----
Cottonseed hulls	1.2	1.2	1.1	1.1	-----	-----
Grd.Limestone	2.9	2.9	2.6	2.7	-----	-----
DiCal phosphate	.12	.12	----	-----	.15	.15
Trace Mineral premix	.06	.06	.06	.06	.06	.06
Feed flavoring	.02	.02	.02	.02	.02	.02
Sodium bicarb.	1.2	-----	1.3	-----	-----	-----
Ammon. Chloride.	-----	.24	-----	.25	-----	1.0
Sodium Chloride	-----	.24	-----	.23	.50	.50
Prairie hay	30	30	30	30	50	50

Table 3.
Daily intakes, as fed basis

kg/d	Treatment					
	HC	**LC**	**HO**	**LO**	**HH**	**LH**
Rolled Corn	3.37	3.37	-----	-----	-----	-----
Whole Oats	-----	-----	4.00	4.00	-----	-----
Dehydrated alfalfa pellet	-----	-----	-----	-----	5.45	5.45
Supplement pellet	1.82	1.82	1.82	1.82	-----	-----
Prairie Hay	2.27	2.27	2.45	2.45	5.45	5.45

Blood Collection

On the last day of the collection period, blood samples were drawn via jugular venapuncture at times 0, 2, 4, and 6 h post-feeding. Samples for blood gas analysis were collected in lithium heparin syringes, immediately placed on ice, and analyzed within 15-30 min with a Ciba-Corning 288 Blood Gas analyzer. Samples for blood lactate analysis were drawn into lithium heparin vacutainers, placed on ice, and measured within 15-30 min, using a YSI 1500 Lactate Analyzer. Blood samples for glucose analysis were drawn into potassium oxalate vacutainers (inhibits glycolysis), centrifuged, plasma harvested and frozen for later evaluation.

Laboratory Procedures

Digestible Energy Determination

Experimental gross energy values for all feed and fecal samples were determined using bomb calorimetry. All samples were prepared into approximately one gram pellets using a Parr pellet press. Samples were then placed in a Parr 1180 Oxygen bomb canisters with a 10 cm. fuse wire and placed under 20 to 25 atmospheres of oxygen. The bomb canister was then placed into a Parr 1261 Automatic Isoperibol Bomb Calorimeter in 2,000 ml of water. Upon sample ignition, the calorimeter calculates a preliminary gross energy value determined by the raise in the water temperature during ignition. The canister is then rinsed and any acid that is produced during combustion is determined via titration. Furthermore, the unburned fuse wire is measured.

The amount of acid produced and the amount of fuse burned is then reentered into the calorimeter, and the preliminary energy value is adjusted for these factors.

Starch analysis

To determine the starch content of the corn, oats, pellets, and prairie hay, the Megazyme (Bray , Co. Wicklow, Ireland) Total Starch Assay kit was used. This procedure has been proven reliable and reproducible to give quantitative starch measurements (AACC Method 76-12). Thermostable *α-amylase* and *amyloglucosidase* was used to degrade the starch and result in a colorimetric reaction. The pellet and prairie hay samples were ground through a .5 mm screen on a No. 3 Willey Mill. The corn and oats samples were ground with a mortar and pestle (Willey Mill resulted in significant portions of the sample not being ground) to achieve a sample that would pass a .5 mm screen. One hundred mg of sample was placed in a tube with aqueous ethanol to aid in dispersion. Due to the presence of resistant starch the corn and oat samples had to be treated with Dimethyl Sulfoxide (DMSO) and placed in a boiling water bath for five minutes to get accurate starch readings. The thermostable *α-amylase* diluted with a MOPS buffer solution was added to the sample and incubated in a boiling water bath for six minutes. A sodium acetate buffer and *amyloglucosidase* enzyme was added to each sample and incubated at 50°C for 30 minutes. An aliquot of the samples was then diluted with water and centrifuged. Aliquots of the diluted solution were then mixed with a GOPOD color reagent and incubated at 50°C for 20 minutes. The absorbance of each sample, blank and standard were then read at 510 nm on a Gilford Spectrophotometer.

Blood Gas Analysis

Blood values for pH, pCO_2, pO_2, and HCO_3^- were determined using a Ciba Corning 288 Blood Gas analyzer. All reported values were adjusted for the rectal body temperature of the animal at time of collection by the analyzer. Samples were placed on ice and analyzed within 15-30 min, (Clinical Pathology division of the Oklahoma State University Veterinary Teaching Hospital). The analyzer goes through two self-calibrations per hour, and is fully maintained by the clinical pathology staff.

Urine Mineral Analysis

Urine mineral values for sodium (Na^+), potassium (K^+), chloride (Cl^-), phosphorus (P^+), calcium (Ca^{++}), and magnesium (Mg^{++}) were analyzed using the composited frozen urine samples. Samples were thawed and analyzed by the staff of the Clinical Pathology division of the Oklahoma State University Veterinary Teaching Hospital. The urine values for Na^+, K^+, and Cl^- were determined using a Beckman's System E4A™ Electrolyte Analyzer. The values for Ca^{++}, Mg^{++}, and P^+, were determined colorimetrically using the EKTACHEM 700 Analyzer C Series.

Fecal Mineral Analysis

The fecal mineral values were determined at the Northeast Dairy One Forage Lab, in Ithaca, NY. The mineral contents for sodium, potassium, phosphorus, calcium, and magnesium were determined using inductively coupled plasma spectroscopy (ICP). Duplicate samples were ashed in a muffle furnace at 500°C for four hours. Three ml of 6N HCl are added to the ashed residue and evaporated to dryness on a 100°C hot plate.

Minerals were extracted with an acid solution of 1.5NHNO_3 + .5 N HCl, and determined using and ICAP 61 (Thermo Jarrell Ash Corporation, Franklin, Mass.). Chloride concentration is determined via potentiometric titration with $AgNO_3$ and utilizing a Brinkman Metrohm 716 Titrino Titration unit with a silver electrode.

Chromic Oxide Analysis

Chromium analysis of feed and fecal samples was completed using inductively coupled plasma spectroscopy (ICP). The samples were digested in nitric acid in a microwave oven at pressures of 20, 40, 80, 135, and 175 PSI for five minutes at each pressure. Samples were then diluted and read on an ICAP 61.

Blood Lactate Analysis

Values for blood lactate concentrations were obtained using the YSI (Yellow Springs, OH) 1500 Lactate Analyzer. The analyzer was property of the Oklahoma State University Veterinary Teaching Hospital, and on loan throughout the trial. Each morning, prior to collection the machine was calibrated using a 5 mmol/L lactate standard and linearity checked using a 30 mmol/L lactate standard. Following that, a calibration was completed prior to the next collection time. Each blood sample was analyzed twice and the mean values reported for that sample.

Plasma Glucose Analysis.

Plasma glucose was determined using the Sigma Diagnostics (St. Louis, MO) Blood Glucose Procedure 510-DA. Frozen plasma samples were allowed to thaw in a refrigerator the evening prior to analysis.

Duplicate aliquots of plasma were deproteinized by adding 1.0 ml barium hydroxide solution and 1.0 ml zinc sulfate solution and mixing well. Samples were centrifuged and a clear .5 ml aliquot was taken from sample and mixed with 5.0 ml of a combined enzyme-color reagent solution and allowed to sit at room temperature for 45 minutes. Samples were then transferred to cuvettes and absorbance was read on a Gilford Spectrophotometer at 475 nm and compared against a standard glucose solution

Statistical Analysis

Statistical analysis was determined using the General Linear Models (GLM) procedure of SAS (1990). Data for urine pH, mineral balances, digestible energies, and blood data were analyzed using orthogonal contrasts which compared, high vs low DCAD, corn vs oats (starch source and intake comparison), corn vs hay (starch intake comparison), oats vs hay (starch intake comparison), and corn and oats vs hay (starch intake comparison). Blood data was analyzed using a repeated measures model to determine any response over time, with horse, period, and diet as the main effects and time as the repeated measure. Least squared means were then calculated and tested for significance using the pdiff procedure of SAS (1990). Statistical significance was declared at an alpha level of $P<.05$.

CHAPTER IV

RESULTS AND DISCUSSION

All horses maintained good physical health throughout the trial. One mare did have a cracked hoof and was unable to be exercised on the walker for 1wk, until lameness subsided. There was no significant dietary effect on animal weights. The average gain while on any specified diet was .93 kg. The initial mean weight was 550.3 kg, with this increasing to 563.6 kg by trials end (Table 4).

Ration Analysis

Due to unexpected nutrient contents, analyzed daily intakes of nutrients ended up being more variable than calculated values. (Table 5 & 6). There was a significant difference in starch intakes between diets. The HC and LC diets had a mean daily intake of 4.378 g starch/kg BW/day. The HO and LO diets had a mean daily intake of 3.96 g starch/ kg BW / day. The HH diet had an average daily intake of .3187 g starch /kg BW/day, whereas the LH diet had an average daily intake of .2591g starch/ kg BW/day. These levels are all below the threshold intake (0.4% of BW of starch/ feeding) above which increasing amounts of starch are digested in the cecum (Potter et al., 1992). The highest level in the current study was on the HC and LC diets with a %of BW intake of 0.2 % of BW of starch /feeding. Thus it can be assumed that there is minimal starch digestion taking place in the cecum, thereby minimizing the potential for a lactic acidosis due to rapid fermentation of starch.

Table 4
Effect of diet on body weight

kg	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6
Period 1	HC	LC	HO	LO	HH	LH
Day 1	615	495	590	531	509	559
Day 5	605	499	597	540	501	552
Day 11	616	486	582	540	495	550
Period 2	LC	HO	LO	HH	LH	HC
Day 1	613	493	592	550	504	535
Day 5	612	495	591	550	504	532
Day11	624	497	596	550	507	536
Period 3	LH	HC	LC	HO	LO	HH
Day 1	619	502	589	547	503	543
Day 5	619	505	597	549	508	540
Day11	620	509	601	550	513	539
Period 4	HO	LO	HH	LH	HC	LC
Day 1	619	507	604	557	501	540
Day 5	621	513	600	559	500	536
Day11	622	520	597	555	506	536
Period 5	HH	LH	HC	LC	HO	LO
Day 1	627	531	590	550	509	538
Day 5	627	531	590	545	514	540
Day11	618	522	592	552	513	546
Period 6	LO	HH	LH	HC	LC	HO
Day 1	614	534	601	545	522	545
Day 5	618	535	604	550	518	550
Day11	621	538	600	547	520	552

Table 5
Daily nutrient intake, DM basis.

	Treatment					
Nutrient	**HC**	**LC**	**HO**	**LO**	**HH**	**LH**
DE (mcal/d)	23	24	24	24	22	22
Starch (g/d)[a]	2426.7	2427.5	2195.7	2192.7	176.7	143.7
CP (g/d)	1127	1147	1435	1461	1240	1355
Ca (g/d)	132	128	144	143	100	88
P (g/d)	23	25	30	32	22	22
Mg (g/d)	12	12	15	16	24	23
K (g/d)	68	67	78	82	190	180
S (g/d)	10	12	15	15	15	16
Na (g/d)	26	15	27	10	23	32
Cl (g/d)	10	43	14	35	69	144

[a]Starch intake values are analyzed values obtained with the Megazyme Total Starch Assay Kit.(Wicklow, Ireland)

Table 6
Nutrient analysis, DM basis.

	Treatment					
Nutrient (%)	**HC**	**LC**	**HO**	**LO**	**HH**	**LH**
CP (%)	15.1	15.4	17.4	17.7	11.4	12.4
DE (Mcal/kg)[a]	3.11	3.26	2.88	2.89	2.0	2.0
Ca (%)	1.78	1.72	1.75	1.74	.92	.81
P (%)	.31	.35	.37	.38	.20	.20
Mg (%)	.16	.16	.19	.20	.22	.21
K (%)	.92	.90	.94	.99	1.75	1.66
S (%)	.14	.16	.18	.18	.14	.14
Na (%)	.34	.20	.33	.13	.21	.29
Cl (%)	.14	.58	.17	.42	.64	1.32
DCAD (meq/kg DM)	+318	+124	+305	+154	+333	+152

[a]Digestible energy values in this table are from Dairy One Forage Lab Analysis

Acid-Base Status

Blood Lactate

Blood lactate showed no significant response due to treatment or over time (Figure 1). Mean lactate values for HC, HO, HH, LC, LO, and LH are .7545, .7546, .8063, .7513, .7533, and .7650 mmol/L, respectively (Table 7). These feeding and post feeding values are similar to basal lactate values (no excess lactic acid production) of horses in other studies prior to feeding or exercise. (Garner et al., 1977; Popplewell et al., 1993). Thus it is unlikely that any acid-base response could be attributed to lactic acidosis from starch overload in the cecum.

Plasma Glucose

There was no significant effect of treatment on plasma glucose levels (Figure 2 & Table 7). This is in agreement with Stull et al. (1987) who found no difference in blood glucose values when horses consumed diets of 100 % alfalfa, 50% alfalfa and 50% corn, 100% corn, or 90% corn and 10 % corn oil. Hintz and coworkers (1972) further found no significant difference in plasma glucose levels as the forage to grain ratio was altered from 1:0, 3:2 and 1:4. The HO diet at feeding had a significantly lower plasma glucose level than the LO and HH diets. There was a significant increase in plasma glucose levels between feeding and two hours post feeding for the HC and HO diets. Furthermore this same diet showed a significant decrease from two hours post feeding to six hours post feeding

Table 7
Effect of diet on blood lactate, glucose, pH, pCO_2, and HCO_3^-.

	Treatment						
Item[a]	**HC**	**LC**	**HO**	**LO**	**HH**	**LH**	**S.E.M.**
Lactate (mmol/L)	.754	.751	.754	.753	.806	.765	.02618
Glucose (mmol/L)	6.066	6.129	6.027	6.617	6.160	5.980	.25745
PH	7.384[b]	7.368[c]	7.376[b]	7.368[c]	7.379[b]	7.373[c]	.00379
HCO_3^- (mmol/L)	31.39[b]	29.94[c]	31.06[b]	29.90[c]	30.30[b]	29.98[c]	.25551
pCO_2 (mm Hg)	53.00	52.34	53.58	52.38	51.70	51.88	1.0805

[a] Values are least square means.
[bc] Means within a row with different superscripts differ (P<.05) with respect to DCAD.
[de] Means within a row with different superscripts differ (P<.05) with respect to source or intake of starch.

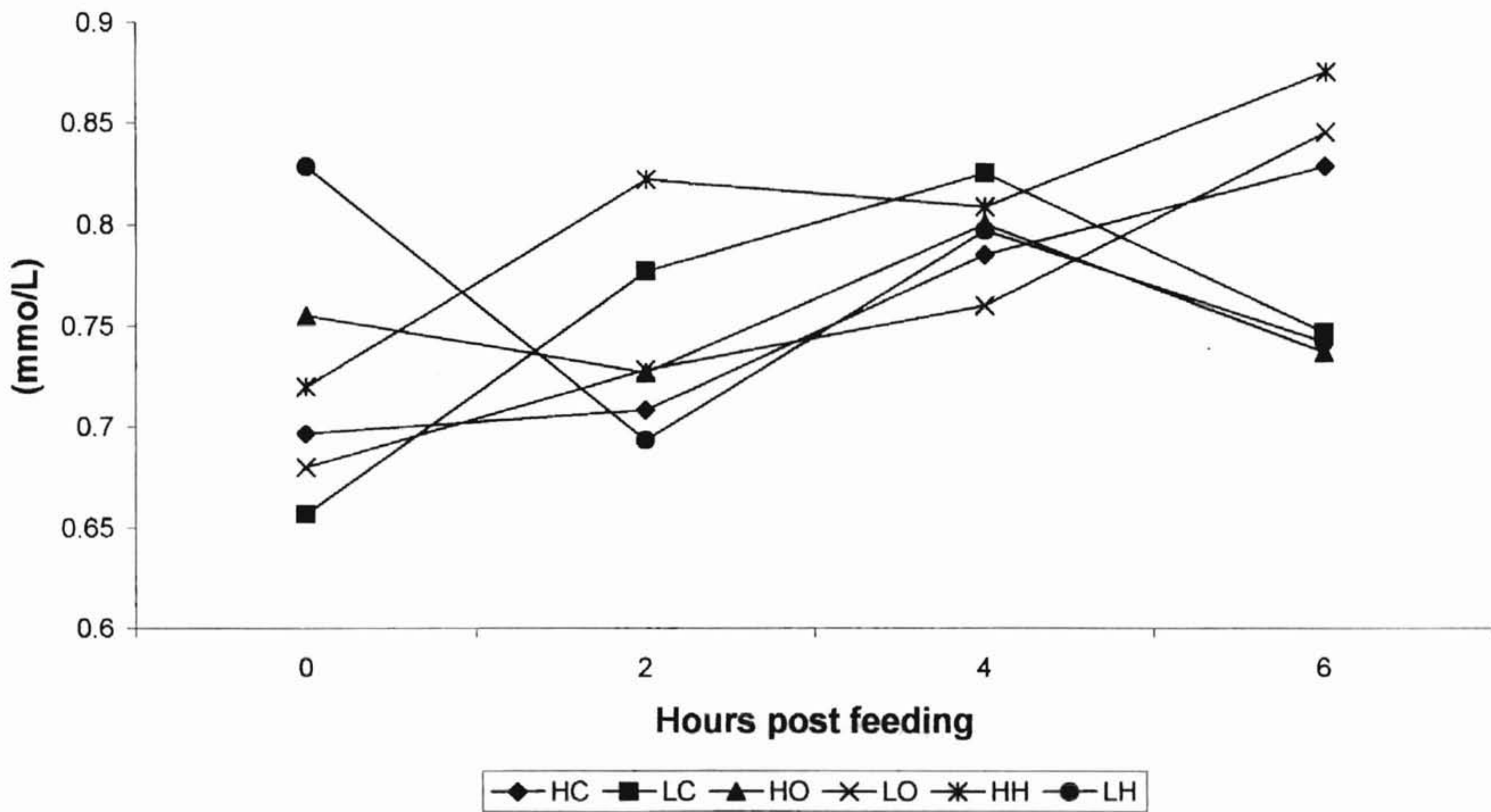

Figure 1
Effect of diet on blood lactate

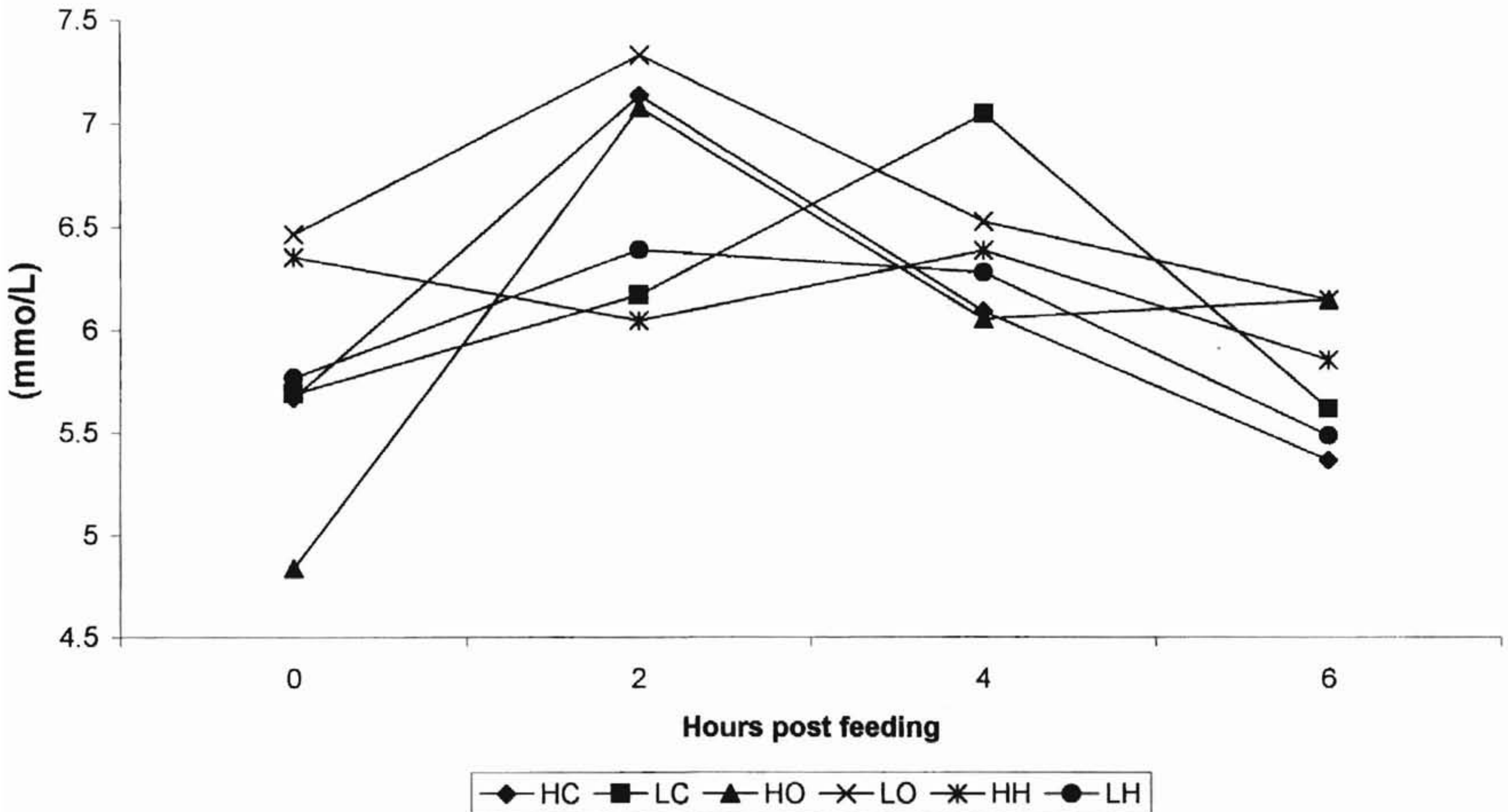

Figure 2
Effect of diet on plasma glucose

Blood pH

Horses consuming low DCAD diets had a lower ($P<.01$) mean blood pH than the horses on the high DCAD diets (Table 7). Since anions are absorbed from the intestinal lumen in exchange for HCO_3- from the blood, there is an increase in blood hydrogen ions thus decreasing pH. Conversely cations are absorbed in exchange for H^+, thus raising blood pH (Baker et al., 1992; Tucker et al., 1990). This response agrees with several studies showing that low DCAD diets result in a depression in blood pH (Baker et al., 1992; Wall et al., 1995; Patience et al., 1987) There was no significant differences in blood pH when comparing starch source or level of starch intake. This finding is in contradiction with Ralston et al. (1993) who found a lower blood pH in horses consuming 5 kg of grain and 2.7 kg of grass hay, versus horses consuming .45kg of gain and 7.7kg of grass hay, irrespective of DCAD. All horses in the present study tended to show a drop in pH 2 h post feeding regardless of diet (Figure 3 & Figure 4). This may signal the time of peak Cl^- absorption, thus the highest concentration of H^+ in the blood. These values were increased at 4 and 6 h post-feeding for all diets, except HO which was still lower at 4 h and increased at 6 h post feeding.

Blood Bicarbonate

The horses on the low DCAD diets had significantly lower mean blood bicarbonate (HCO_3^-) values than did the horses on the high DCAD diets (Table 7). This is also due to the counterexchange of anions for HCO_3^- between the intestine and blood. These findings agree with several studies showing that low DCAD diets tend to decrease the HCO_3^- levels in the blood (Baker et al., 1992; Tucker et al., 1988).

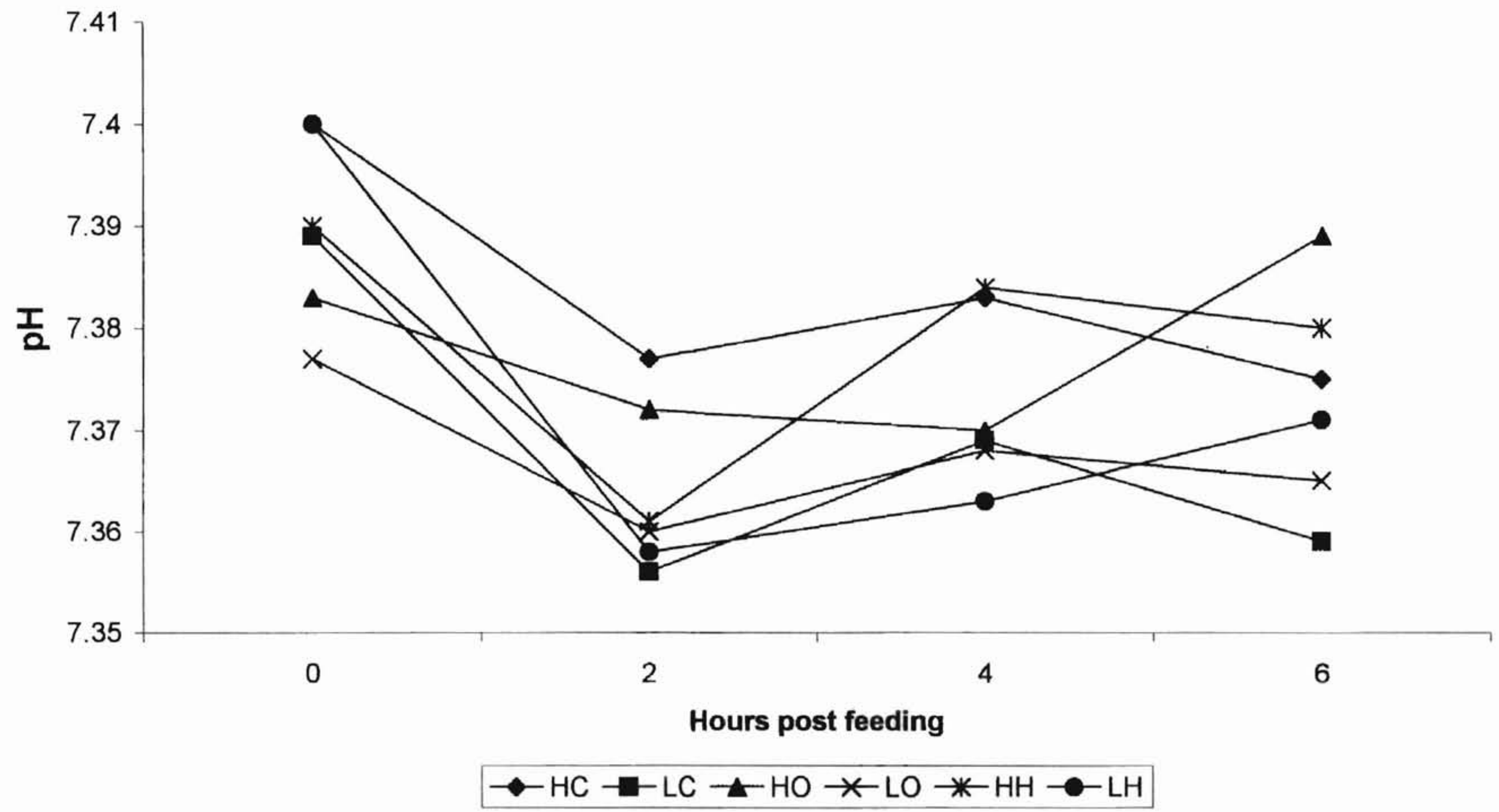

Figure 3
Effect of diet on blood pH

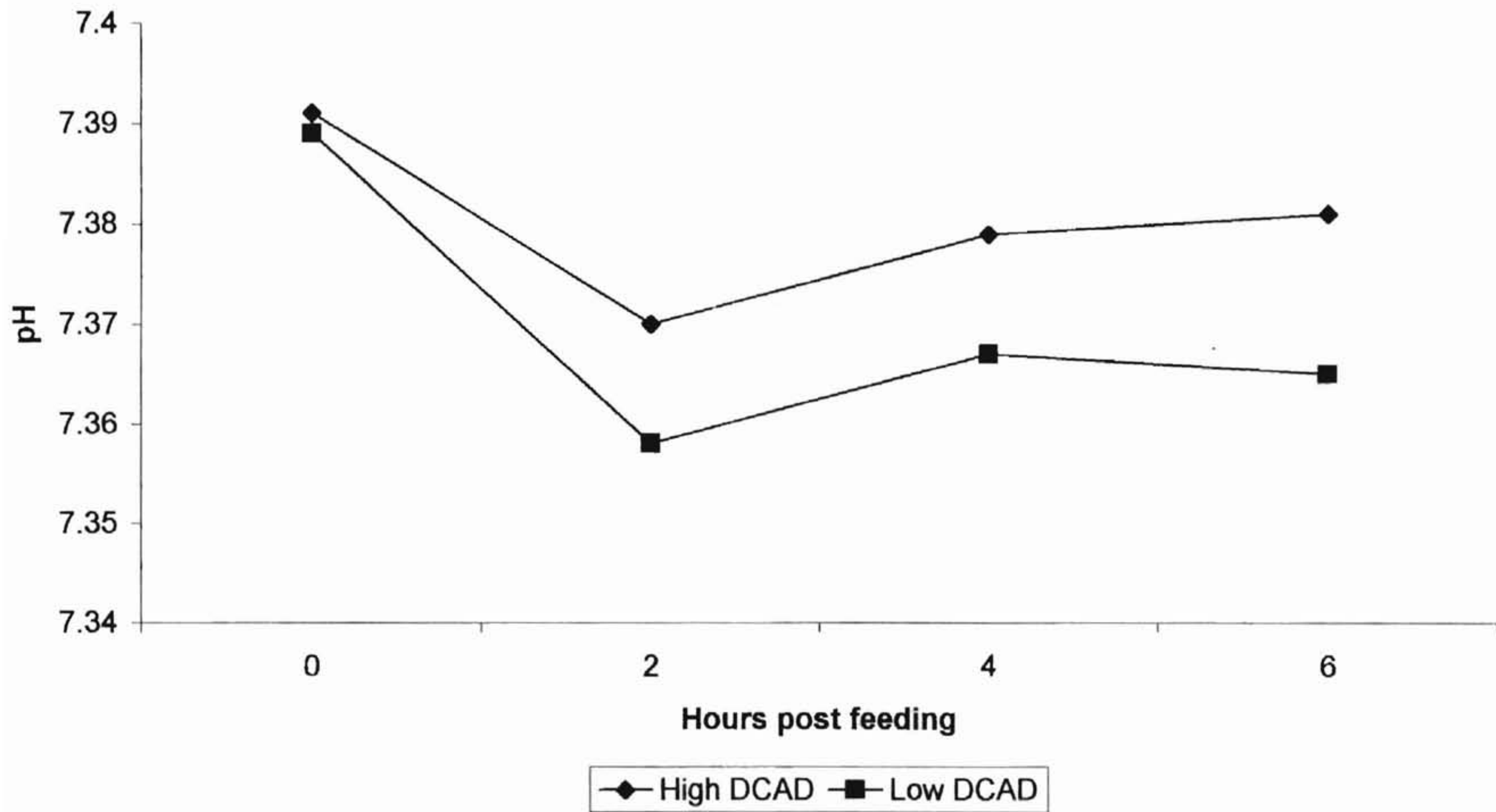

Figure 4
Effect of DCAD on blood pH

Bicarbonate also shows a significant dietary effect over time (Figure 5 & Figure 6). At time of feeding the LO and LH diets started out with a lower ($P<.05$) HCO_3^- level than did the HC diet. Two hours post feeding there was no significant differences among treatments. However at 4 h post feeding all the low DCAD diets had a significantly lower HCO_3^- concentration than did the HC diet. The low DCAD diets also had a mean drop in bicarbonate from feeding to 6 h post feeding of 1.70 mmol/L, whereas the high DCAD diets only dropped an average of .7167 mmol/l.

Blood pCO_2

Diet had no significant effect on mean pCO_2 readings (Table 7). There was also no effect of diet on pCO_2 values over time (Figure 7). Mean pCO_2 values ranged from 51.7 mm Hg to 53.58 mm Hg. These results are in contradiction with many researchers (Baker et al., 1992; Stutz et al., 1992). This finding may be due to the fact that the alteration in blood pH and HCO_3^-, while statistically different, may not have been a sufficient change for the body to correct the pH by altering alveolar respiration.

Urine pH

Urine pH was higher ($P<.0001$) for horses on the high DCAD diets, versus the horses on the low DCAD diets (Figure 8). Since the final mechanism to reestablish acid-base balance is urinary excretion of H^+ in cases of acidosis, and HCO_3^- in cases of alkalosis, it is evident why urine pH behaves in this manner. Horses consuming diets HC, HO, and HH had mean urine pH values of 7.75, 7.94, and 7.67 respectively, where as horses on diets LC, LO, and LH had mean pH values of 7.13, 7.11, and 6.93, respectively.

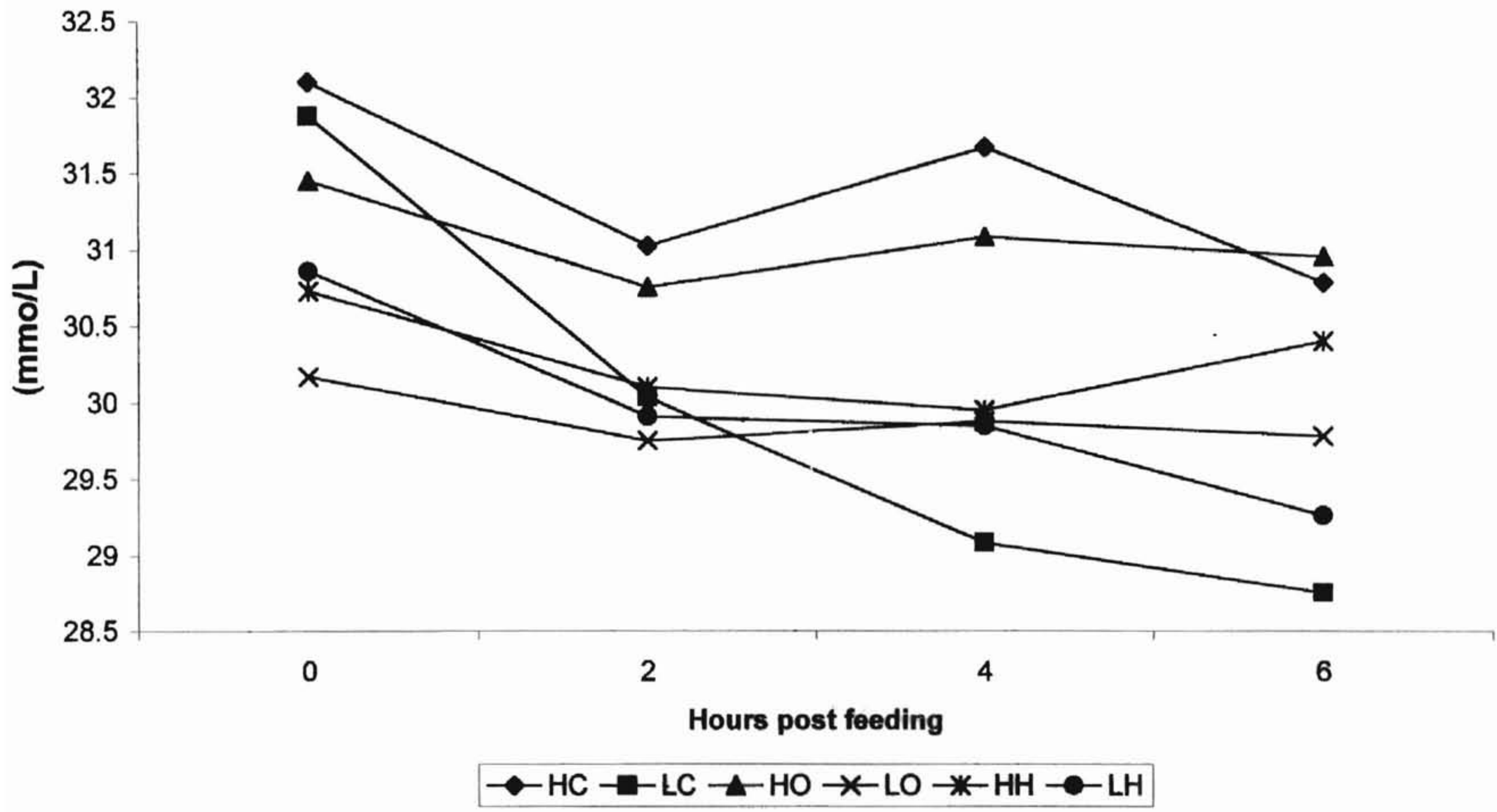

Figure 5
Effect of diet on blood bicarbonate

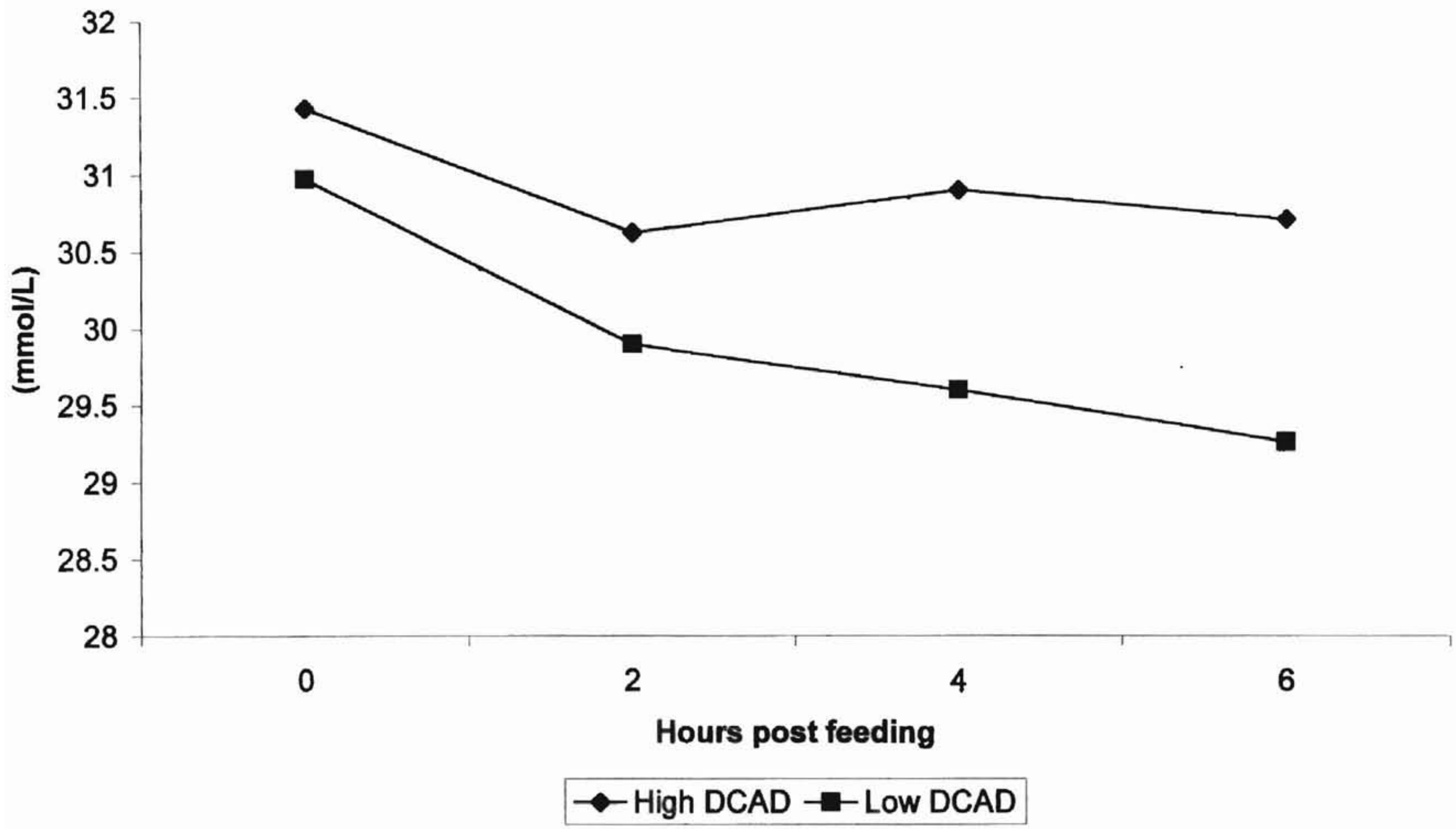

Figure 6
Effect of DCAD on blood bicarbonate

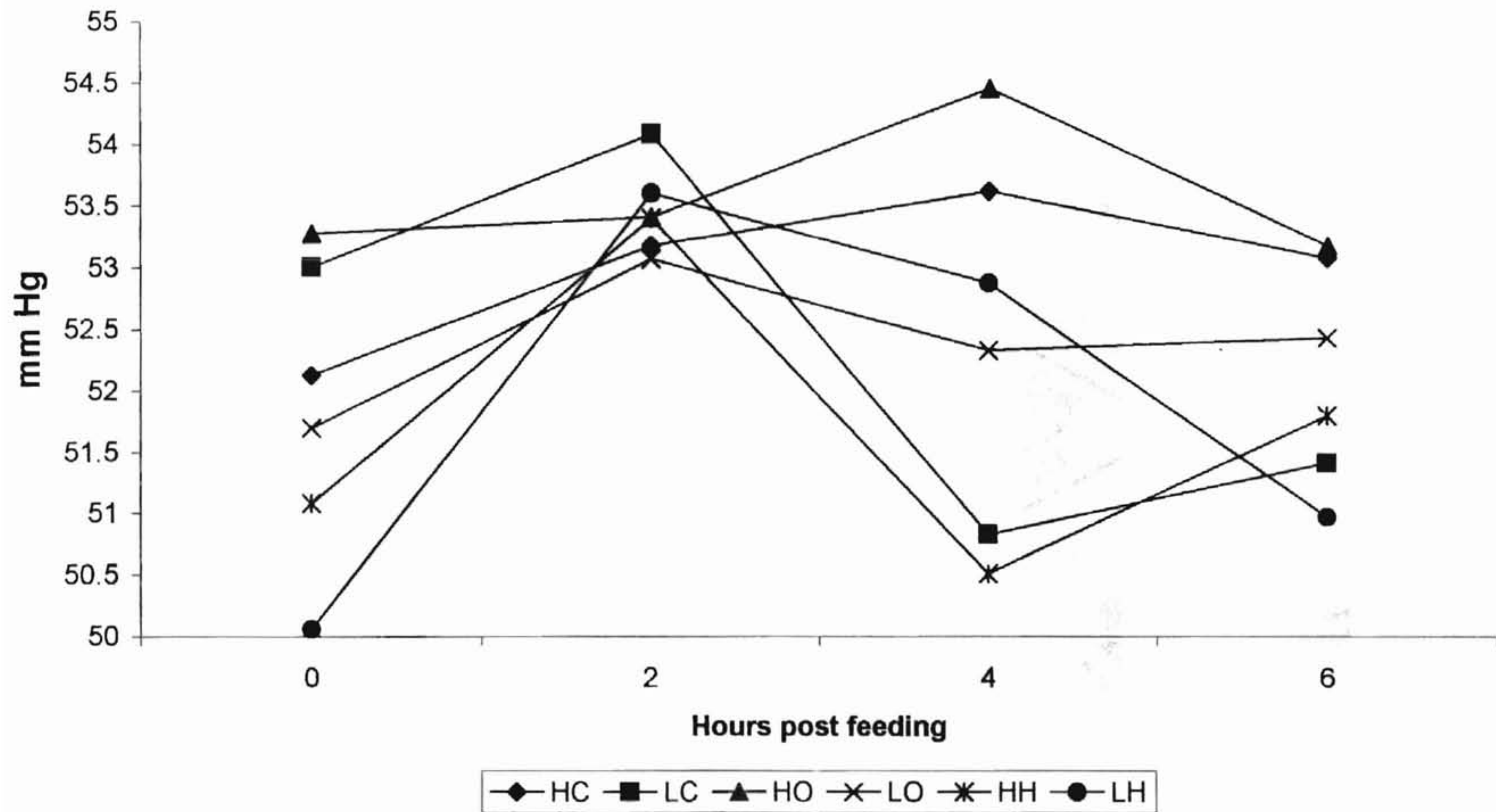

Figure 7
Effect of diet on blood pCO_2

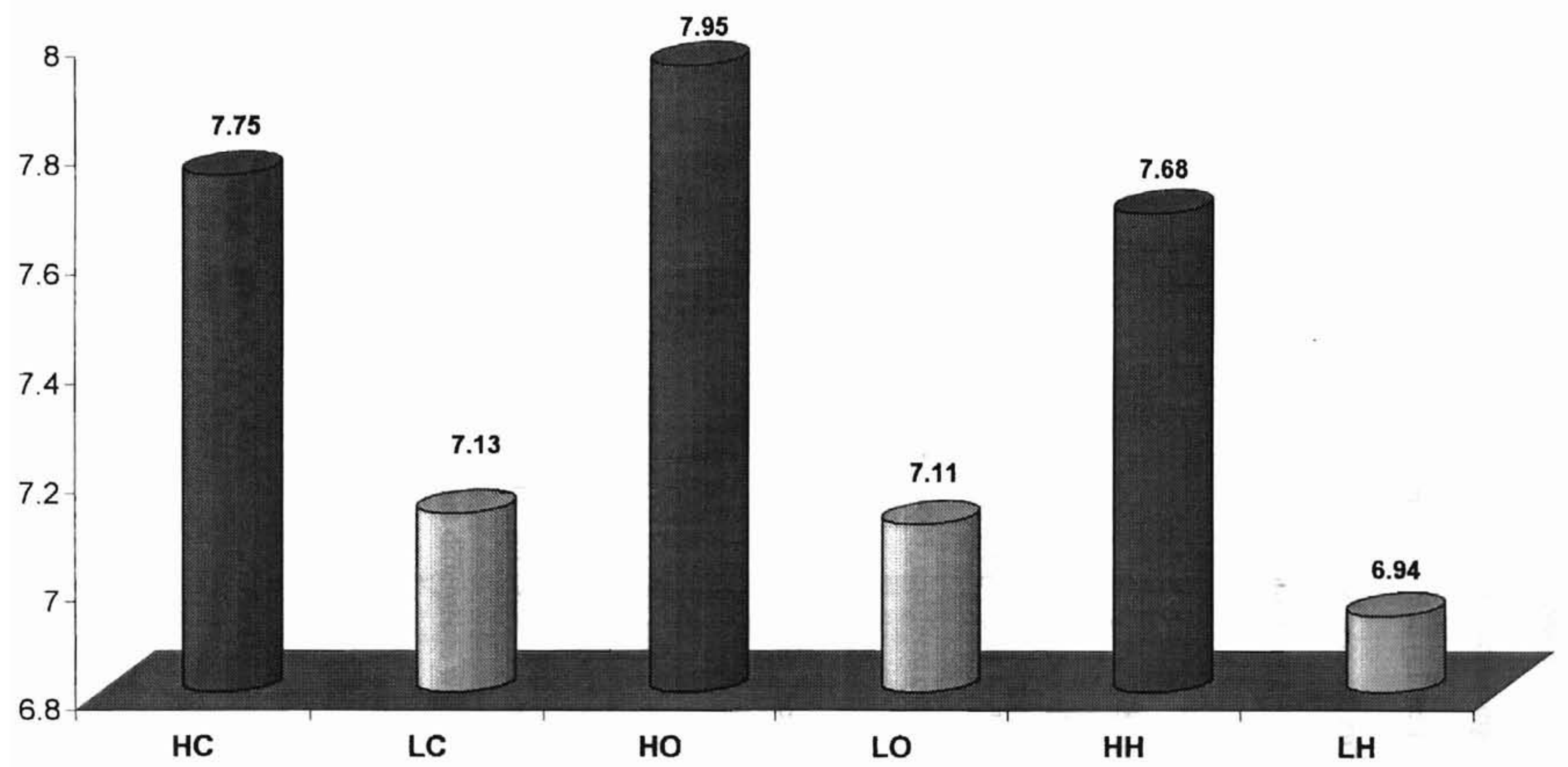

Figure 8
Effect of diet on urine pH

No significant difference in urine pH was detected between the corn and oat based diets (HC &LC vs HO & LO), or between the corn and hay based diets (HC & LC vs HH & LH). However, urine pH was higher (P<.01) in horses consuming the high starch diets versus the low starch diets. However, the urine pH values of the LH diet were considerably lower than all other readings, which could account for this difference.

Dry Matter Digestibility

The effect of treatment on dry matter digestibility and fecal output is shown in Table 8. Fecal dry matter output was calculated by taking the g/d intake of chromium and dividing that by the g of chromium per g of feces. Dry matter digestibility was calculated by taking the dry matter intake minus fecal output and dividing by dry matter intake. Dry matter digestibility was higher (P<.05) and fecal output was lower (P<.05) for the high starch versus the low starch diets. This alteration in dry matter digestibility agrees with Hintz et al. (1972), where dry matter digestibility increased with increasing grain content of the diet. Furthermore there was a significant difference due to starch source (HC & LC vs. HO & LO). There was no DCAD effect on dry matter digestibility or fecal output. This data agrees with Baker et al. (1997) who found that dry matter digestibility didn't vary with respect to DCAD. However, Baker et al. (1993) did find that dry matter digestibility did show significant differences with respect to DCAD. Fecal output also disagrees with Baker et al. (1993) which found that DCAD had a significant effect on fecal output.

Table 8
Effect of diet on dry matter digestibility, fecal output and urine volume.

Item	Treatment						
	HC	**LO**	**HO**	**LO**	**HH**	**LH**	**SEM**
Dry Matter Intake kg/d	6.72	6.72	7.48	7.47	10.01	9.96	
DM Digestibility %	73.31^{f}	70.91^{f}	67.81^{g}	66.66^{g}	57.86^{e}	57.38^{e}	2.26
Feces g/d	1805.87^{f}	1960.44^{f}	2412.09^{g}	2488.92^{g}	4211.09^{e}	4259.08^{e}	194.15
Urine ml/d	10454.01	10251.68	10558.85	8474.558	9462.85	11740.64	1124.004

[a]Values are least square means
[bc]Means within a row with different superscripts differ ($P<.05$) with respect to DCAD
[de]Means within a row with different superscripts differ ($P<.05$) with respect to starch intake
[fg]Means within a row with different superscripts differ ($P<.05$) with respect to starch source (excludes HH and LH diets)

Energy Digestibility

The effect of treatment of energy digestibility is shown in Table 9. Energy digestibility was calculated by taking the gross energy intake/day minus the gross energy of fecal output/day and dividing by the gross energy intake/day. There was no effect of DCAD on energy digestibility. However, there was a significant difference with respect to starch intake with the high starch diets having significantly higher energy digestibilities. The HH and LH diets did have significantly higher DE intake values than the HC, LC, HO or LO diets. Digestible energy intakes on the HH and LH diets were 26.62 Mcal/d and 25.95 Mcal/d respectively. Whereas the DE values on the HC, LC, HO, and LO diets ranged from 23.31 Mcal/day, 22.68 Mcal/d, 24.15 Mcal/d, and 24.35 Mcal/day respectively. This difference may have been a result of the increased dry matter intake and therefore increased gross energy intake for the hay diets, and not the starch intake.

Mineral Balance

Due to the lack of variation in daily feed intakes between horses for each diet, statistical analysis could not be completed for mineral intake values. Thus, the values that are given are absolute values, and no orthogonal comparisons could be made.

Sodium Balance

The effect of treatment on sodium balance is shown in Table 10. In order to achieve the elevated DCADs on the HC and HO diets, $NaHCO_3$ was used.

Table 9
Effect of diet on fecal energy loss and digestible energy.

Item[a]	Treatment						
	HC	**LC**	**HO**	**LO**	**HH**	**LH**	**SEM**
GE[b] Intake Mcal/d	30.43	30.73	34.24	34.26	44.42	44.29	
GE feces Mcal/d	7.11[eg]	8.04[eg]	10.09[eh]	9.91[eh]	7.80[f]	18.44[f]	.985
DE Intake Mcal/d	23.31[g]	22.68[g]	24.15[g]	24.35[g]	26.62[f]	25.95[f]	.984
Energy Digestibility %	76.80[e]	73.93[e]	70.56[e]	70.99[e]	59.85[f]	58.57[f]	2.54

[a]Values are least square means.
[b]Gross energy as determined by oxygen bomb calorimetry.
[cd]Means within a row with different superscripts vary ($P<.05$) with respect to DCAD.
[ef]Means within a row with different superscripts vary ($P<.05$) with respect to starch intake.
[gh]Means within a row with different superscripts vary ($P<.05$) with respect to starch source (excludes HH and LH diets).

Table 10
Effect of diet on mineral balances

Mineral g/d[a]	Treatment						
	HC	**LC**	**HO**	**LO**	**HH**	**LH**	**SEM**
Sodium							
Intake	25.71	14.93	27.64	10.35	23.05	32.05	
Urine	16.66^{b}	7.41^{c}	18.89^{b}	4.92^{c}	12.87^{b}	13.76^{c}	1.181
Fecal	9.61^{d}	6.62^{d}	10.81^{d}	9.39^{d}	12.16^{e}	13.53^{e}	1.176
Balance	-.57	.89	-2.05	-3.96	-1.978	4.75	1.612
Potassium							
Intake	68.22	66.80	78.01	82.14	190.55	180.88	
Urine	43.44^{df}	41.08^{df}	51.43^{dg}	47.35^{dg}	108.17^{e}	102.62^{e}	2.406
Fecal	10.27^{d}	13.73^{d}	10.43^{d}	13.93^{d}	30.34^{e}	32.93^{e}	2.287
Balance	14.51^{d}	11.97^{d}	16.14^{d}	20.86^{d}	52.04^{e}	45.33^{e}	2.915
Chloride							
Intake	10.29	42.99	13.95	34.99	69.32	144.11	
Urine	9.81^{bd}	30.46^{cd}	9.14^{bd}	32.78^{cd}	57.69^{be}	116.62^{ce}	2.835
Fecal	1.54^{d}	1.40^{d}	1.34^{d}	1.37^{d}	2.35^{e}	2.38^{e}	.293
Balance	-1.06^{d}	11.13^{d}	3.47^{d}	.85^{d}	9.28^{e}	25.13^{e}	2.840
Magnesium							
Intake	12.28	12.28	15.39	16.25	24.47	23.37	
Urine	2.86^{bd}	3.61^{cd}	2.99^{bd}	4.04^{cd}	5.46^{be}	8.07^{ce}	.366
Fecal	4.05	4.80	3.63	4.18	4.06	3.90	.423
Balance	5.36^{bdf}	3.87^{cdf}	8.77^{bdg}	8.02^{cdg}	14.95^{be}	11.39^{ce}	.511
Phosphorus							
Intake	23.35	25.84	30.23	31.76	22.17	22.10	
Urine	.162^{d}	.200^{d}	.196^{d}	.167^{d}	.052^{e}	.071^{e}	.056
Fecal	14.23^{d}	16.79^{d}	11.80^{d}	13.74^{d}	6.84^{e}	7.81^{e}	1.398
Balance	8.95^{df}	8.85^{df}	18.23^{dg}	17.86^{dg}	15.28^{e}	14.22^{e}	1.380
Calcium							
Intake	132.71	128.27	144.44	143.81	100.76	88.17	
Urine	9.22^{b}	14.44^{c}	8.39^{b}	14.50^{c}	8.24^{b}	22.52^{c}	1.516
Fecal	58.15^{df}	40.17^{df}	31.21^{dg}	38.99^{dg}	15.43^{e}	15.77^{e}	5.268
Balance	65.34^{f}	73.65^{f}	104.84^{g}	90.31^{g}	77.07	49.86	5.600

[a]Values are least squares means.
[bc]Means within a row with different superscripts differ (P<.05) with respect to DCAD.
[de]Means within a row with different superscripts differ (P<.05) with respect to starch intake.
[fg]Means within a row with different superscripts differ (P<.05) with respect to starch source (excludes HH and LH diets).

The DCAD of the HH diet is the naturally occurring DCAD. This resulted in the high DCAD diets having a higher DCAD along with elevated daily sodium intakes. The intake on the LH diet was elevated as well due to the supplementation of NaCl in order to meet NRC sodium requirements. Horses on the high DCAD diets did have a significant increase in daily sodium urinary excretion over the horses on the low DCAD diets. High DCAD diets resulted in sodium excretion least square means of 16.66, 18.89 and 12.87 g/d for the HC, HO, and HH diets respectively. The LC diet had a daily excretion of 7.41 g/d with the LO and LH diet excretion of 4.92 and 13.76 g/d respectively The elevated sodium urine excretion on the high DCAD diets agrees with Cooper et al. (1995) and Wall et al. (1992) who demonstrated that daily sodium excretion is a function of sodium intake. This is further shown in the current study by the elevated urinary sodium excretion on the LH diet, which had an elevated daily intake due to NaCl supplementation even with a low DCAD. There was no significant effect of starch source or intake on urinary sodium loss.

Daily fecal excretion of sodium did not vary with DCAD, starch source or intake. The values ranged from 6.62 g/d on the LC diet to 13.53 g/d on the LH diet. The lack of a significant difference due to treatment on the fecal sodium excretions disagrees with Baker et al. (1993), who found an increase ($P<.05$) in daily fecal sodium output as the DCAD of the diet increased

The sodium balance calculated as the daily urinary sodium excretion plus daily fecal sodium excretion minus daily sodium intake, did not vary with DCAD, starch source or starch intake. The horses in the current study did demonstrate a very low or even negative sodium balance.

Potassium Balance

The effect of diet on potassium balance is shown in Table 10. All potassium intakes are due to naturally occurring potassium, thus resulting in the elevated daily potassium intake on the HH and LH diets due to the high percentage of dehydrated alfalfa in the ration. The potassium intakes for the HC, LC, HO, and LO diets are all at least ½ the daily intake of the HH and LH diets. Starch source and starch intake had a significant effect on the daily urinary potassium excretion. The HH and LH (low starch intake) diets had a significantly higher excretion than the HC, LC, HO and LO (high starch intakes) diets. The HO and LO diets were also higher ($P<.05$) than the HC and LC diets.

Low starch intake diets (HH & LH) also resulted in an increased ($P<.05$) daily fecal potassium excretion compared to the high starch intake diets. Furthermore, there was no DCAD or starch source effect. The overall potassium balance mirrored the fecal excretion with starch intake showing a significant difference in grams per day. The potassium excretion and overall balance is similar to sodium in the fact that excretion is a function of intake. These data agree with Tucker et al. (1990) and Baker et al. (1997) who further demonstrated that the potassium excretion is dependent upon intake.

Chloride Balance

Chloride was the only anion that was manipulated to alter the DCAD. The LC and LH diets had .24 and .25% ammonium chloride additions respectively, and the LH diet had 1.0% ammonium chloride added. The effect of diet on Cl^- balance is shown in Table 10. The low DCAD diets had a significantly higher daily urinary chloride excretion.

The urinary chloride excretion data agree with Cooper et al. (1995) which found that daily chloride excretion was elevated on low DCAD diets, due to the increased daily intake. Starch intake also resulted in a significantly lower urinary chloride loss on the high starch diets when compared to the low starch diets.

Fecal chloride losses were lower ($P<.05$) on the high starch diets versus the low starch diets. Furthermore, overall chloride balance also was higher ($P<.05$) on the low starch diets as compared to the high. There was no effect of DCAD on fecal chloride losses or daily chloride balances. Baker et al. (1993) found that fecal chloride excretion in sedentary horses did not vary with respect to DCAD. This study also showed that the overall daily chloride balance showed no significant difference due to DCAD. These data all support the findings of the current study.

Magnesium Balance

No alterations in dietary magnesium concentrations were made during this trial; therefore all intakes are from naturally occurring sources. Daily intakes ranged from a low of 12.28 g/d on the HC and LC diets to 24.47 g/d for the HH diet (Table 10). The high DCAD diets did result in a decrease ($P<.0002$) in daily urinary magnesium excretions. The least square means for the high diets (HC, HO, and HH) were 2.86, 2.99, and 5.46 g/d respectively. This is compared to the low DCAD diets (LC, LO, and LH) which had a daily urinary magnesium loss of 3.61, 4.04 and 8.07 g/d, respectively. Starch intake also resulted in a significant increase in daily magnesium loss via the urine. The effect of DCAD on urinary and overall balance is in contradiction with other studies (Baker et al., 1993; Baker et al., 1997; Wall et al, 1997).

This may be explained by the fact that renal magnesium handling parallels calcium, thus with increased urinary calcium losses and decreased balances on low DCAD diets, the same effect occurs in magnesium (Best and Taylor, 1985).

The magnesium lost through the feces did not vary with treatment. This is in contradiction with Baker et al. (1993) who found that as the DCAD of the diet increased the daily fecal magnesium excretion declined in exercising and sedentary horses. Overall daily magnesium balance was significantly higher on the high DCAD diets. The low starch diets also resulted in an increased ($P<.05$) daily magnesium balance compared to the high starch diets.

Phosphorus Balance

A concise effort was made to hold daily phosphorus intakes constant, however daily intakes ranged from 22.10 g/d (LH diet) to 31.76 g/d (LO diet) due to the subsequent variation of feedstuffs (Table 10). Daily urinary excretion of phosphorus via the urine is minimal, but does vary with respect to starch intake. The high starch intake diets resulted in significantly higher daily urinary losses compared with the low starch diets. Fecal phosphorus losses also decreased with the low starch diets. The daily phosphorus balance shows was significantly lower on the high starch intake diets compared to the low starch intake diets. Furthermore, the oat based diet had a higher ($P<.05$) daily phosphorus balance than the corn diets. These data agree with Baker et al. (1993) and Wall et al. (1992) who found the daily urinary phosphorus excretion and balance did not vary with respect to DCAD and tended to mirror intake

Calcium Balance

The effect of treatment on calcium balance is shown in Table 10. Once again as with phosphorus diets were formulated to have constant calcium intake across treatments. However, due to higher than expected levels in the corn and oats that were used to balance the diets, these four diets did tend to result in higher daily calcium intakes over the two hay diets.

Loss of the calcium through the urine was increased ($P<.0001$) on the low DCAD diets. The LC, LO and LH diets resulted in daily losses of 14.44, 14.50, and 22.52 g/d respectively compared to the HC, HO and HH diets losing 9.22, 8.39, and 8.24 g/d respectively. There was no effect of starch intake or source on urinary calcium losses. The effect of DCAD on daily urinary excretion of calcium agrees with many researchers. Baker et al. (1993) found that as the DCAD of the diet decreased the daily urinary calcium loss increased significantly in sedentary horses. Wall et al. (1997) also found that in horses consuming the high DCAD diets; urinary calcium excretion declined. Beck and Webster (1976) also found that acidotic rats experienced an increased urinary calcium loss, possibly due to the increased acid load inhibiting renal tubular reabsorption of calcium.

Fecal calcium loss was significantly higher on the high starch intake diets as well as the corn based diets when compared to the oat based diets. There was no effect of DCAD on fecal calcium losses. The overall calcium balance did not vary with respect to DCAD or starch intake but did with starch source. The HO and LO diets had a higher ($P<.05$) daily balances with values of 104.84 and 90.31 g/d respectively compared to the HC and LC diets which had balances of 65.34 and 73.65 g/d.

CHAPTER V

Summary and Conclusions

Once again this study indicates the strong connection between the dietary cation-anion difference of the diet and acid-base status. Furthermore this trial establishes the correlation between DCAD on acid-base status regardless of starch source or intake. High DCAD diets appear to have a buffering capacity on acid-base parameters regardless of starch source or intakes

Any correlation between treatment and mineral balance on this study is difficult. There was a high degree of variation of daily mineral intakes, that could have confounded the effects of DCAD, starch intake or starch source. However, the link between low DCAD diets and increased urinary calcium excretion was once again solidly established. Therefore, there may have been detrimental losses of Ca^{++} through the urine on the low DCAD diets, possibly due to bone turnover.

To improve the results of the current study some changes need to be made in the experiment. First of all, the diets should be fed on a percent of body weight basis. Secondly the starch intakes may not have been high enough to really elicit any starch effect. This area needs further research to determine of the mineral is that of intake or bone, which would lead to further understanding.

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