

LITERATURE REVIEW

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A Summary of the Effects of Feeding and Daily Variation on Acid-Base Status in Resting Horses

INTRODUCTION

Plasma acid-base state affects, and may also be a reflection of, the health of equine athletes. The physicochemical model, as developed by Stewart (1981), defines the blood constituents that effect or determine acid-base state. These constituents, the partial pressure of carbon dioxide (PCO_2), the strong ion difference ($[SID]$), and the total concentration of weak acids and bases ($[A_{tot}]$), are the independent variables in the physicochemical equation. These independent variables are affected by external and internal influences throughout the day. The acid-base state in equine plasma can be completely described by the equilibrium between the independent variables and quantified using the physicochemical approach to acid-base balance.

By monitoring plasma acid-base parameters the occurrence and origins of daily variations can be understood. Variations in plasma result from diurnal influences such as activity and feeding, nocturnal influences such as sleeping, and the underlying circadian rhythm of an organism. Results from blood analysis affect the interpretation of a horse's biochemistry, which leads to conclusions about health and affect drug-testing results.

The purposes of this literature review are to provide an introduction to acid-base assessment in clinically normal horses at rest and outline changes observed with feeding over a 24-h period. The literature on diet, feeding and daily variation of blood acid-base status of horses at rest is summarized. The purpose of the research described in this thesis

is to investigate effects of feeding and daily variation of equine blood parameters on plasma acid-base status. Therefore, the presented physiological information should improve our basic knowledge of the daily changes in plasma acid-base status and set the stage for further research on acid-base status in horses.

1. A quantitative approach to acid-base chemistry

The strength of the physicochemical approach to the regulation of acid-base balance lies in its quantitative assessment of acid-base status (Constable 1999). It evaluates a large range of acid-base disorders and permits a greater understanding of acid-base physiology when compared to the traditional Henderson-Hasselbach approach (Aguilera-Tejero 2000). The physicochemical approach quantifies the contributions of the independent variables (the strong ion difference ($[SID]$), weak electrolyte concentrations ($[A_{tot}]$) and the partial pressure of carbon dioxide (PCO_2)) to changes in variables whose concentrations are dependent on the equilibrium of all the systems (pH , $[H^+]$, $[OH^-]$, $[HCO_3^-]$, $[CO_3^{2-}]$, $[HA]$ and $[A^-]$) (Stewart 1981, 1983).

Stewart based his foundation for the physicochemical approach on three fundamental physical laws governing $[H^+]$ in physiological fluid. The interactions between the independent and dependent physicochemical variables recognise the constraints imposed by the law of conservation of mass, where the total mass of all of the reactants is equal to the total mass of products in a chemical reaction (see below: equations 2, 4 & 7), the maintenance of electrical neutrality, where the sum of the positive ions (cations) is equal to the negative ions (anions) (see below: equations 1 & 10), and the equilibrium constraints on dissociation reactions (see below: equations 3, 5 & 6) (Stewart 1981, 1983). All electrolytes exist in aqueous solution as charged particles and behave

chemically as acids and bases. Cations are positively charged electrolytes and are considered to be bases while anions are negatively charged and are considered to be acids (Singer & Hastings 1948). The law of maintenance of electrical neutrality requires that there be an equivalent number of cations and anions in solution (Stewart 1983). The acid-base state can be described by the equilibrium of the independent variables in any physiological solution.

Strong Ions Strong ions are electrolytes that, based on their effective dissociation constant (K_A), are completely dissociated, or nearly so, in solution. The net strong ion charge is represented by what Stewart labelled the Strong Ion Difference ([SID]). In mammalian plasma the sum of the concentration of the completely dissociated cations is usually greater than the sum of the concentrations of completely dissociated anions, so [SID] has a positive charge:

$$[\text{SID}] = [\text{strong cations}] - [\text{strong anions}] \quad (\text{mEq/L}) \quad (1)$$

The principal strong ions in plasma are sodium (Na^+), potassium (K^+) and chloride (Cl^-). Calcium (Ca^{2+}), magnesium (Mg^{2+}), lactate (Lac^-) and sulphate (SO_4^{2-}) have low concentrations in plasma and, because the sum of their concentrations ($([\text{Ca}^{2+}] + [\text{Mg}^{2+}] - ([\text{Lac}^-] \text{ and } [\text{SO}_4^{2-}])))$ is close to zero at rest in healthy horses, their concentrations can effectually be negated (Lindinger et al. 1994). Increases in plasma [SID] contribute to plasma alkalinization, while decreases contribute to plasma acidification.

Weak Ions Another independent variable in physiological fluids is the total concentration of weak electrolytes, represented by $[A_{tot}]$. Plasma protein concentration ($[PP]$), including the concentrations of albumin and globulin, provide the major contribution to $[A_{tot}]$ and therefore independently affect acid-base balance (Constable 1997). Inorganic phosphate concentration ($[Pi]$) is a minor component of $[A_{tot}]$ and changes are insignificant with respect to changes in $[PP]$ (Figge et al. 1991; Stewart 1981). However, there may be fluctuations with influences such as feeding and exercise (Watson 1999; Waters et al. 1995).

Weak electrolytes are not fully dissociated in solution. The extent of the dissociation is represented by the pK (Stewart 1983). An increase in $[A_{tot}]$ contributes to plasma acidification, as the pK of most weak acids is slightly acidic. $[A_{tot}]$ is used to represent the total available anionic charge of the weak electrolytes, which consist of associated (HA), and dissociated (A^-) forms, and is described as:

$$[A_{tot}] = [HA] + [A^-] \text{ (mEq/L)} \quad (2)$$

At equilibrium, the apparent weak acid dissociation constant (K_A) can be calculated from the equation defining the law of mass action. HA only partially dissociates into H^+ and A^- ions, so K_A is defined as:

$$K_A = ([H^+] \times [A^-]) / [HA] \quad \text{(mEq/L)} \quad (3)$$

A requirement of the physicochemical approach to acid-base balance is the need for fluid-specific values for $[A_{tot}]$ and K_A . Staempfli et al. (1999) determined K_A as 2.11×10^{-7} Eq/L in equine plasma and $[A_{tot}]$ to be 0.21 mEq/L of plasma protein. These values

compare closely with 0.24 mEq/L protein determined by van Slyke and colleagues (1928) and 0.22 mEq/L protein determined by Constable (1997) for equine plasma.

Carbon Dioxide The third independent variable in acid-base chemistry is the $[\text{CO}_2]$, a product of cellular metabolism. CO_2 is moderately soluble in water at physiological temperature, pressure and $[\text{H}^+]$. It also reacts with H_2O to form several other solutes, all of whose concentrations are thus dependent variables.

The contribution of CO_2 to the change in $[\text{H}^+]$ is usually calculated from the PCO_2 . CO_2 acts as an acid in physiological solution and so increases in PCO_2 contribute to acidification and decreases contribute to alkalinization. The addition of CO_2 to an aqueous solution and its removal can be summarised by:



Since the amount of dissolved CO_2 ($[\text{S}_{\text{CO}_2}]$) can be derived from its solubility constant and the PCO_2 , the equations are:

$$K_c = ([\text{H}^+] [\text{HCO}_3^-]) / [\text{PCO}_2] \quad ((\text{Eq/L})^2) \quad (5)$$

$$K_3 = ([\text{H}^+] [\text{CO}_3^{2-}]) / [\text{HCO}_3^-] \quad (\text{Eq/L}) \quad (6)$$

Where K_3 is the equilibrium dissociation constant for HCO_3^- , and

$$[\text{PCO}_2] = [\text{S}_{\text{CO}_2}(\text{PCO}_2)].$$

Water The specific properties of water are important for this approach because H₂O has both a high dielectric constant and an extraordinarily high molar concentration in physiological solution at 55.5 M. These properties cause the electrostatic bonds between molecules to dissociate when they are placed in aqueous solutions. The dissociation of water is described by the reaction:



The water dissociation reaction reaches equilibrium rapidly, so the dissociation of water:

$$[\text{H}^+] \times [\text{OH}^-] = K_w \times [\text{H}_2\text{O}] \quad (8)$$

has a negligible effect on the water concentration. $K_w \times [\text{H}_2\text{O}]$ can be considered a constant, K_w :

$$K_w = [\text{H}^+] \times [\text{OH}^-] \quad ((\text{Eq/L})^2) \quad (9)$$

Where K_w becomes the ion product for water.

Interaction Among Systems

Based on the information given above, water interacts with both weak electrolytes (Equation 2) and the CO₂ system (Equations 5 & 6). Water also interacts with strong electrolytes:

$$[\text{SID}] + [\text{H}^+] - [\text{HCO}_3^-] - [\text{A}^-] - [\text{CO}_3^{2-}] - [\text{OH}^-] = 0 \quad (10)$$

When the above equations (3, 5, 6, 9 & 10) are combined, they can be rearranged to form a single quadratic equation for $[H^+]$, in terms of the independent variables and the equilibrium constants of each system that interacts in solution (Stewart 1981, 1983):

$$[H^+]^4 + (K_A + [SID])[H^+]^3 + \{K_A([SID] - [A_{tot}]) - (K_C \times PCO_2 + K_w)\}[H^+]^2 - \{K_A \times (K_C \times PCO_2 + K_w) + (K_3 \times K_C \times PCO_2)\}[H^+] - (K_A \times K_3 \times K_C \times PCO_2) = 0 \quad (11)$$

Equation 11 is used to calculate the effects of changes of the independent variables (PCO_2 , $[SID]$ and $[A_{tot}]$) on the dependent variables ($[H^+]$, $[HCO_3^-]$, $[CO_3^{2-}]$ and $[OH^-]$) (Stewart 1981, 1983). The equation illustrates that an increase in $[H^+]$ within a compartment arises as a result of an increase in PCO_2 , a decrease in $[SID]$, an increase in $[A_{tot}]$, or a combination of these changes (Kowalchuk & Scheuermann 1995).

2. Daily Variation of factors affecting acid-base balance

Although there have been many studies on equine haematological parameters, not many have looked at chronohaematology. There are reference ranges for equine blood constituents (table 1) used as a baseline for comparative testing, but the ranges encompass any variations due to ‘typical’ influences such as daily variation and feeding. It is hoped that chronobiological analysis of equine blood parameters that affect acid-base status will provide more specific threshold parameters for testing blood samples, including for diagnostic, therapeutic, medical, and drug testing, at various times of the day and night, and even between seasons.

There are many factors influencing variation in blood constituents, including daily variations resulting from exercise and feeding, and circadian rhythm. Daily variations, or those changes taking place over a 24-h period, can be further broken down into diurnal variation, or changes occurring during the daylight hours, and nocturnal variation, or changes occurring during the dark hours. Endogenous circadian clocks with periods of about 24-h control daily rhythms (Davidson et al. 2002). Circadian rhythm is variation according to time of day, and is thought to be in part a response to daylight and darkness, as well as to feeding and activity. This variation allows biological systems to predict changes in their environment instead of just reacting to them (Davidson et al. 2002). Although daily variations in blood variables have been investigated to determine baseline values to be used for evaluation of physiological parameters, those variations have not been looked at in terms of their effects on acid-base balance nor in terms of what type of variation they represent.

Table 1. Physiologically important acid-base variables, and their concentrations, in arterial plasma of horses at rest. (Data from Lindinger 2004 and Robinson 2003.)

	plasma	
Dependent variables	reference value	reference range
[H ⁺] nEq/L	40	33-45
pH	7.40	7.35-7.48
[HCO ₃ ⁻] mEq/L	28	22-34
Independent variables		
pCO ₂ mmHg	40	35-50
[TCO ₂] mmol/L	30	23-36
Strong Ions		
[SID] mEq/L	40	37-43
[Na ⁺] mEq/L	140	132-146
[K ⁺] mEq/L	3.7	2.7-4.7
[Cl ⁻] mEq/L	105	96-109
Weak Ions		
[A _{tot}] mEq/L	12	11-13
[plasma protein] g/dl		5.4-7.5
Metabolites		
Glucose (mmol/L)		3.5-5.6

Studies on horses and other mammals have found daily variation in many physiological parameters, including body temperature (T), heart rate (HR), respiratory rate (RR), blood pressure (BP), sympathetic nervous system (SNS) activity, hormone levels, and plasma electrolyte and protein concentrations (Clarke et al. 1988; Slocombe et al. 1995; Piccione et al. 2002; Carrington et al. 2003; Yashiki et al. 1995). Variation in T and SNS activity is related to circadian influences, BP variance largely due to sleep onset, while HR variation is due to both sleep onset and a circadian influence (Carrington et al. 2003; Piccione et al. 2002). Other factors to consider include effects of the environment (seasonal and temperature) as well as overlapping feeding and/or exercise responses in plasma, to whether a response is due to a metabolic influence or a circadian rhythm.

Research shows various strong ions exhibit a circadian rhythm in equine plasma. Lepage and colleagues (1991), Jansson and colleagues (1999), Slocombe and colleagues (1995) and Yashiki and colleagues (1995) have shown that plasma $[K^+]$ has an evening increase, or nocturnal variation, thought to be a result of circadian rhythm. $[Na^+]$ and $[Ca^{2+}]$ have also been shown in many studies to have a daily rhythm, however the pattern is inconsistent. Both nocturnal increases (Greppi et al. 1996) and decreases (Boning et al. 1974; Lepage et al. 1991) have been found with other studies showing only minimal variations (Minematsu et al. 1995) or none (Slocombe et al. 1995). Slocombe and colleagues (1995) also found $[Cl^-]$ to have a daily variation, peaking at 1500-h and reaching a low at 1800 h. Although these researchers reported variations in a wide variety of blood constituents, at some point in all these studies the animals were fed during the 24-h study period and some were exercised.

Feeding and activity creates a daily variation that affects circadian rhythm (Sultzman et al. 1977; Stephan 1986; Davidson et al. 2002). A problem in the equine

research literature is often that limited measurements were taken and/or that parameters were not looked at over a full 24-h period. However, information that is available does suggest that numerous physiological parameters exhibit daily variation in horses. It is important to establish baseline parameters for a true analysis of circadian rhythm. A summary of studies on horses indicating whether variation was present, where horses were fed but not exercised before and/or during measurements, is presented in table 2.

Table 2. Studies indicating whether circadian rhythm or daily variation was present.
 NR: Times not reported.

Physiological Variable	Low time	Peak time	Change	Authors
Calcium	NR	NR	Yes	Yashiki et al 1995
	NR	NR	Yes	Greppi et al. 1996
Potassium	1815, 1000 h	1330, 2400 h	Yes	Jansson & Dahlborn 1999a
	0700 h	1800 h	Yes	Slocombe et al. 1995
	1130, 1930 h	0730, 1530, 2330, 0330 h	Yes	Yashiki et al. 1995
Sodium	--	--	No	Jansson & Dahlborn 1999a
	--	--	No	Slocombe et al. 1995
	NR	NR	Yes	Yashiki et al. 1995
Chloride	--	--	No	Yashiki et al. 1995
	0600 h	1500 h	Yes	Slocombe et al. 1995
Glucose	1200, 2000 h	0730 h	Yes	Yashiki et al. 1995
	NR	NR	Yes	Greppi et al. 1996
	--	--	No	Stull et al. 1988
Total plasma protein	0530 h	1800 h	Yes	Jansson and Dahlborn 1999a
	1200 - 1600 h	2400 h	Yes	Yashiki et al. 1995
	NR	NR	Yes	Greppi et al. 1996
Haemoglobin	--	--	No	Greppi et al. 1996
	0400, 2000 h	1200 h	Yes	Piccione et al. 2001
Total carbon dioxide	1600 h	1000 h	Yes	Slocombe et al. 1995

3. Feeding influence on acid-base status

Nutrition and diet strongly influence acid-base status and play an important role in equine health. The effect of diet depends both on the type of feed and the timing of feeding. Usually a combination diet of forage and grain rations is fed to performance horses, but feeding can vary from 24 hours a day access to pasture forage with a small amount of grain supplement to two feedings a day with a high grain to forage ratio. Individual large meals have an immediate impact on acid-base balance over periods of 6 to 8-h via fluid shifts, while metabolic/respiratory effects appear to be the main influence over 24-h (Kronfeld 2001; Mongin 1981).

Dietary cation-anion difference

The dietary cation-anion difference (DCAD) of a feed can be used to characterize the mineral content of diets. DCAD (also known as dietary cation-anion balance or DCAB) is a major determinant of plasma [SID] as the strong ions enter the blood from the digestive tract (Riond 2001). DCAD affects systemic acid-base balance because it defines the overall net cation to anion content of the feed. The potential benefits associated with managing the acid-base balance in food are affected by the dietary component of anions and cations by creating overall net acidic or basic environments in body compartments. DCAD can be calculated as follows:

$$\text{DCAD} = (\text{Na}^+ + \text{K}^+) - \text{Cl}^- \text{ (mEq/kg dry matter (DM))} \quad (12)$$

This DCAD equation takes into account the most readily absorbed ions with the greatest metabolic impact on acid-base balance (Baker 1992, 1998). It includes only monovalent

dietary electrolytes and ions with a higher valence are ignored. Some studies include SO_4^{2-} in the equation (Baker et al. 1998; Cooper et al 1998; Popplewell et al. 1993):

$$\text{DCAD} = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{2-}) \text{ mEq/kg DM} \quad (13)$$

The contents of Ca^{2+} , Mg^{2+} and SO_4^{2-} in feed act to neutralize each other and have a variable and incomplete intestinal absorption. Baker and colleagues (1998) suggested that if SO_4^{2-} was to be included in the DCAD equation it would need a modifying coefficient as it is not as acidogenic as Cl^- . They also confirmed that Na^+ and K^+ have similar alkalogenic properties. H_2PO_4^- is also left out of the equation, as it is a weak acid and is present in plasma in low concentrations.

The chemical components of the diet affecting acid-base status include the amount of weak acids, including protein and P, and the strong acids, Na^+ , K^+ , Ca^{2+} , and Mg^{2+} , and bases Cl^- and SO_4^{2-} (Riond 2001; Kronfeld 2001). Riond (2001) corrected for the absorption rate (assuming 38% absorption of Ca^{2+} , 30% from Mg^{2+} , 60% from both SO_4^{2-} and H_2PO_4^- and 100% absorption rate for Na^+ , K^+ , and Cl^- (Riond 2001; Kronfeld 2001)) and obtained the following DCAD equation:

$$(\text{Na}^+ + \text{K}^+ + 0.38 \text{ Ca}^{2+} + 0.30 \text{ Mg}^{2+}) - (\text{Cl}^- + 0.60 \text{ SO}_4^{2-} + 0.60 \text{ H}_2\text{PO}_4^-) \quad (14)$$

Actual absorption values may be higher for Ca^{2+} (50-70%) and Mg^{2+} (60-70%), and lower for Na^+ (75-95%) depending on the mineral content of the diet (Schryver et al. 1987). However, currently equation 13 is the most commonly used DCAD equation.

DCAD and acid-base status.

A medium DCAD is between 250-300 mEq/kg of feed dry matter (DM). A DCAD of greater than 300 mEq/kg may result in an increased cation content of extracellular fluids, generating a systemic alkalosis characterized by increased plasma pH, $[\text{Na}^+]$, $[\text{HCO}_3^-]$, $[\text{Ca}^{2+}]$, and PCO_2 as well as decreased plasma $[\text{Cl}^-]$ (Baker 1993; Popplewell 1993). A DCAD of less than 250 mEq/kg may result in a metabolic acidosis, decreasing plasma pH and $[\text{Na}^+]$, $[\text{K}^+]$, $[\text{Mg}^{2+}]$, $[\text{HCO}_3^-]$ and PCO_2 and increasing plasma $[\text{Cl}^-]$, as well as increasing the urinary excretion of K^+ , Na^+ , Cl^- and Ca^{2+} (Topliff et al. 1989; Baker et al. 1992, 1993, 1998; Mueller et al. 1999; McKenzie et al. 2002, 2003). Plasma pH and $[\text{HCO}_3^-]$ and urine pH have been shown to increase in proportion to DCAD over a range of 0 to 407 mEq/Kg (Baker et al. 1992, 1998; Topliff et al. 1989).

McKenzie and colleagues (2002, 2003) found that a high DCAD diet resulted in higher plasma $[\text{Pi}]$ and lower $[\text{K}^+]$ compared to a neutral diet, but plasma $[\text{Na}^+]$, $[\text{Cl}^-]$ and $[\text{Mg}^{2+}]$ did not differ between horses consuming the neutral and high DCAD diets. Popplewell and colleagues (1993) found that horses on a high DCAD diet had faster times in a standard anaerobic test (1.64 km) when compared to those on a lower DCAD diet. Graham-Thiers et al. (2001) also found that horses on higher DCAD diets were faster than those on a low DCAD diet (20 mEq/kg DM) and found no difference between the medium and high DCAD diets (125 – 350 mEq/kg). Although there is no consensus on whether a high DCAD diet will enhance performance in horses, the expectation is that it will help to offset or delay the acidic component of fatigue (Graham-Thiers et al. 2001).

A low DCAD diet produces a systemic acidosis which may lead to a negative calcium balance from increased Ca^{2+} loss through the urine and an overall weakening of the skeletal system (Wall et al. 1991; Fressetto et al. 2001; Sebastian et al. 1994; Baker et

al. 1998). However, Cooper and colleagues (2000) found that weanling horses consuming highly anionic diets were able to make up for an increased urinary excretion of Ca^{2+} , and growth performance was not affected by DCAD. More research is needed to look into the effect of DCAD and Ca^{2+} with respect to growth and performance specifically in horses.

Feed Components

In general, grains have low cation content ($\text{Na}^+ + \text{K}^+ + \text{Ca}^{2+}$) and high anion content (Cl^-), which results in a low DCAD, while forages generally have higher cation contents with an increased DCAD. The NRC (1989) rated corn with a DCAD ($\text{Na}^+ + \text{K}^+ - \text{Cl}^-$), at about 58 mEq/kg dry matter (DM) and oats at 73 mEq/kg DM, compared to alfalfa at 323 mEq/kg DM and Bermuda grass hay at 427 mEq/kg DM. Dietary protein and fat have also been found to affect acid-base status. However, with fat, a change is exhibited only during exercise. Table 3 provides a description of the feed and DCAD used in various studies.

Grain. Before DCAD was considered it was thought that the metabolic acidosis following ingestion of a high grain meal was due to lactic acid production from the digestion of starch (Ralston 1994). The current thinking, however, is that the acidosis is due to the low levels of cations typically found in cereal grains contributing to a low DCAD (Mueller 1999). Mueller (1999) found no significant differences in plasma pH between both starch sources and starch intake.

When grains are fed in high concentration they tend to cause a metabolic acidosis (Roby et al. 1987; Abu Damir et al. 1990; Ralston 1994). Many foals and performance horses are fed a high grain ration, which has a low DCAD (<100 mEq/L), consisting of equal to or greater than 50% of their total intake. A chronic metabolic acidosis may

increase the incidence of developmental orthopaedic diseases, including stress fractures from a decreased bone mineral content (Jones 1990; Frassetto et al. 2001). A systemic acidosis can be corrected by increasing the DCAD of a high grain diet as the DCAD, and not the actual food source, is responsible for acid-base changes. (Mueller 2001).

Forages. A diet consisting of only forage seems to have a decreased immediate effect on acid-base balance when compared to eating grain rations. Ralston (1993) fed hay only and found its digestion had minimal effects on plasma pH during the first hour of feeding. In contrast, Kerr and Snow (1982) found an increase in [PP] and a decrease in plasma [K⁺] within the first hour following feeding with a large meal of hay (5.5 kg). However, they found no change in [PP] or [K⁺] after a morning feed of 1.8 kg of a commercial cube diet (composed of high fiber, low starch, no cereal grain) and it was not until following a second feed of the same diet at noon and during a feeding of 2.7 kg cubes with 5.5 kg of hay at 1700-h that there was an increase in [PP] and a decrease in

Table 3. Description of the feed and DCAD measurement of various studies.

Authors	Feed	Concentrate: Forage	DCAD (mEq/kg DM)	DCAD equation
Baker et al. 1992	2 / day	60:40	21, 125, 231, 350	$(\text{Na}^+ + \text{K}^+) - \text{Cl}^-$
Baker et al. 1993	2 / day	60:40	24, 127, 227, 352	$(\text{Na}^+ + \text{K}^+) - \text{Cl}^-$
Baker et al. 1998	2 / day	60:40	0, 53, 360, 405	$(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{2-})$
Cooper et al. 1998	5 / day	60:40	86, 110, 307	$(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{2-})$
Cooper et al. 2000	5 / day	70:30	-52, 325	$(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{2-})$
McKenzie et al. 2002	2 / day	45:55	85, 190, 380	$(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{2-})$
Mueller et al. 1999, 2001	2 / day	1. 70:30 2. 50:50	1. 3<155 2. 3>300	$(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{2-})$
Popplewell et al. 1993	2 / day	60:40	10, 95, 165, 295	$(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{2-})$
Ralston et al. 1997		50:50		$(\text{Na}^+ + \text{K}^+) - \text{Cl}^-$
Stutz et al. 1992	2 / day	60:40	5, 107, 201, 327	$(\text{Na}^+ + \text{K}^+) - \text{Cl}^-$
Topliff et al. 1989			-50, 50, 150, 250	$(\text{Na}^+ + \text{K}^+) - \text{Cl}^-$
Wall et al. 1991	2 / day	60:40	5, 107, 201, 327	$(\text{Na}^+ + \text{K}^+) - \text{Cl}^-$

[K⁺]. However, studies suggest that hay appears to be healthier for the horse overall (Pagan & Harris 1999; Meyer et al. 1985).

Combination Diets. Most athletic horses are fed some combination of a hay and grain diet and many studies have looked at the effects of mixed diets and DCAD. Studies show that DCAD affects the plasma acid-base status. When the DCAD is kept constant research can show how the amount and proportion of grains to forage in the diet can affect the horse. By separating the feed variables affecting acid-base status other potential contributors, such as starch, can be investigated.

Protein. Protein is acidogenic as it contains sulphur and phosphorus that oxidize to sulphate (SO₄²⁻) and phosphate (Pi), which become elevated in plasma. Graham-Thiers and colleagues (1999, 2001) found plasma [SID] and pH were higher and PCO₂ was lower in horses that were fed less protein. However, the effects on acid-base balance may have been due to the low DCAD of the high protein diet. Greppi et al. (1996) found no differences in plasma biochemistry between feeding 3 different levels of protein over 27 days (713g crude protein (CP) (7.4% of diet), 824g CP (8.2%), and 962g CP (9.8%)). Graham-Thiers and colleagues (1999, 2001) also used diets at 7.5% CP and 14.5% CP so perhaps Greppi et al. (1996) did not sufficiently vary the amount of CP or it is the overall percentage of the CP in the diet that elicits a response. Although these studies suggest that a low protein diet may have an alkalizing acid-base response, those effects are so small that they are of questionable physiological significance.

Fat. With high fat diet supplementation (increased to 10-12% of diet intake compared to a control diet of 3.5-5% fat), high intensity exercise increased plasma

[Lac⁻] and decreased acidosis (Custalow et al. 1993; Taylor et al. 1993; Ferrante et al. 1994). An increased reliance on fatty acid metabolism with exercise may spare protein during energy demanding states, for example, fat adaptation decreased the acidotic response to repeated sprints (Graham-Thiers et al. 2001). This effect is thought to be largely due to limiting the increase in PCO₂ in venous blood (Kronfeld et al. 1998). Although supplementation may be beneficial to exercise performance, there appears to be no influence of up to 12% dietary fat supplementation on acid-base status at rest (Graham-Thiers et al. 2001, Kronfeld 2001).

Fluid Shifts

The equid's voluminous saliva production required with feeding, and their gastric, intestinal, and bile secretions require extracellular fluid (ECF) from the plasma and interstitial fluids. The number of osmotically active metabolites and elements mainly determine the intercompartmental distribution and moderate the fluid movement between compartments (Hyypä et al. 1988). The Na⁺ content of the ECF and the K⁺ content of the intracellular fluid (ICF) are the main determinants of fluid volume (Carlson 1997). This fluid movement creates transient fluid shifts during feeding (Clarke et al. 1990). The majority of NaCl transport between the blood and small intestine (SI) appears to be regulated by an electrically neutral transport mechanism, which probably creates parallel Na⁺-H⁺ and Cl⁻-HCO₃⁻ exchanges (Argenzio et al. 1977). These fluid shifts also cause changes in both [SID], by altering the concentrations of strong ions, and [A_{tot}], by changing the [PP], thus creating a transition in acid-base status as a result of feeding.

Fluid shifts from the ECF to compartments involved with feeding and digestion are dependent on the type and amount of feed. A single large feeding

initiated a 15% ECF loss to the small intestine (Clarke et al. 1988), while multiple small feedings did not disturb the fluid balance (Argenzio & Clarke 1989; Clarke et al. 1988, 1990). When feeding frequent small meals acid-base balance was not greatly affected, as plasma volume remained the same (Argenzio & Clarke 1989; Clarke et al. 1988, 1990).

Clarke and colleagues (1990) also found that the rapid ingestion of a concentrated meal caused increased fluid shifts between the blood and small intestine. In contrast, Pagan and Harris (1999) found that feeding hay created a greater decrease in plasma volume than feeding grain. The latter concluded that the increased fluid shift was also because a grain diet produces half as much saliva as either a hay or grass diet, as shown by Meyer et al (1985). Feeding effects of a diet on acid-base status is the result of the size and frequency, as well as the constituents, of feeding during the day.

Varying DCAD

Various studies have investigated the effects of different DCAD feeds on equine plasma acid-base variables. Stutz and colleagues (1992) found that diets with DCAD from 5 to 327 mEq/L exhibited a maximal decrease in pH and increased PCO_2 at 1-h post feed, with a return to baseline over the following 12-h. Plasma $[HCO_3^-]$ decreased for the first 3-h following feeding and then also returned to baseline over the next 5 to 9-h. Baker and colleagues (1993) and McKenzie et al. (2002) found that plasma pH and $[HCO_3^-]$ was decreased and plasma $[Cl^-]$ increased for horses consuming the lower DCAD diets. An interesting component of the study from Stutz and colleagues (1992) was that the decrease in plasma $[HCO_3^-]$ seen following both

the morning and evening feedings was more pronounced after the morning feed, which suggests that this response may be part of a circadian rhythm.

Ralston and colleagues (1997) manipulated the feed DCAD with the addition of 1% NaHCO₃ to a 50:50 ratio grain and alfalfa diet. This reduced the decrease in the resultant post-feeding plasma pH and increased [HCO₃⁻]. Baker et al. (1998) also found that feeding additional strong cations (Na⁺ and K⁺), in bicarbonate or citrate form to increase DCAD, increased both urine and plasma pH and [HCO₃⁻] levels. Both Sebastian (1994) and Frassetto and colleagues (2001) also found they were able to induce a low grade metabolic acidosis (which they believe to be the optimal acid-base state) by adding 60-120 millimoles of potassium bicarbonate (KHCO₃) or other exogenous base to the diet daily for humans.

Ralston and colleagues (1994) compared two meals of differing grain: forage ratios that were controlled for DCAD, protein and caloric content. The first was at 60% grain: 40% forage, and the second was with 10% grain: 90% forage. A decrease in pH was seen consistently by 60 min. after a meal of grain, which was also found by Stutz et al. (1992). Both studies found that pH remained depressed for up to 2 to 3-h if no other feed was available. Ralston et al. (1994) also found a decrease in urine pH 3-4 hours later and that fecal pH was lower in horses fed higher grain versus those fed hay only. They concluded that it was the amount of starch in the diet, and not the DCAD, which caused the different acid-base responses following ingestion. However, Mueller and colleagues (1999) found that high starch diets had no effect on plasma acid-base balance, regardless of source (corn, oats or alfalfa). They used 3 high DCAD and 3 low DCAD diets, with starch comprising 45-49% of each diet. They

concluded that the acidogenic effects of a high starch source were overcome by increasing the DCAD of that feed source.

In summary, these studies show that although feeding a large amount of starch can create a metabolic acidosis, it is possible to minimize this effect by manipulating the feed's DCAD. Using an exogenous base can also increase the low DCAD level of feeds, such as those with high grain rations. When a high DCAD feed is consumed, there is a proportional increase in the cation content of extracellular fluids from absorption by the small intestine. It is generally thought that feeding a higher DCAD diet is of greater benefit to the overall health of a horse.

CONCLUSION, RELEVANCE AND HYPOTHESIS

The acid-base state can be described by the equilibrium between the independent variables, [SID], $[A_{\text{tot}}]$, and PCO_2 , and quantified using the physicochemical approach to acid-base balance. Acid-base status is affected by daily variations due to feeding factors (including DCAD, amount, composition and timing of meals), confounding the ability to establish baseline values for plasma constituents. The influence of feeding and exercise as well as incomplete sampling over a full 24-h period have confounded research looking at daily variations in equine plasma constituents.

Besides the importance of establishing baseline values for plasma constituents, acid-base variables are important to the horse racing industry for drug testing. Alkalinizing agents are used to enhance performance. Drugs can be used to manipulate plasma $[TCO_2]$. A TCO_2 blood test is performed in Ontario prior to both Standardbred and Thoroughbred races to determine whether an alkalinizing substance (usually in bicarbonate form) has been administered (colloquially known as “milkshaking”). A $[TCO_2]$ greater than or equal to 37.0 mmol/L in venous blood plasma is considered a positive test. $[TCO_2]$ is a measure of the total carbon dioxide concentration in blood, which is primarily made up of HCO_3^- and CO_2 in solution. However, CO_2 occurs naturally in the blood, therefore controversy exists over the reliability of the TCO_2 test. TCO_2 status is also affected by Hct, Hb, total [PP], Na^+ , K^+ , Cl^- , Ca^{2+} , Lac^- and Pi concentrations. By quantifying the plasma acid-base variables under minimal outside influences the daily variation of $[TCO_2]$ can be assessed.

We hypothesized that equine plasma acid-base parameters exhibit daily variation independent of feeding and exercise. We examined variation in plasma

[TCO₂] and other plasma constituents throughout the day, without the effects of feeding, to identify the main factors in blood that determine the daily acid-base state of the horse. The purpose of the first trial was to identify the main electrolyte and acid-base constituents in blood plasma that exhibit daily variation. The second trial's purpose was to determine the effect of feeding on plasma TCO₂ and 19 blood constituents describing the acid-base and electrolyte state of horses. Blood constituents were assessed to allow definitive determination of factors affecting [TCO₂] and other acid-base variables.