

**A Quantitative Analysis of the Effects of Feeding and Daily Variation on
Plasma Acid-Base Status in Resting Horses**

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KERRI JO SMITHURST

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ABSTRACT

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

Kerri Jo Smithurst
University of Guelph, 2003

Advisors: Dr. R Geor
Dr. MI Lindinger

This thesis investigated the main acid-base constituents in equine plasma that exhibit daily variation. A major focus was to determine total carbon dioxide concentration ($[TCO_2]$). Jugular venous blood was sampled every 1 to 2-h over 25-h from 10 resting and either fasted or fed Standardbreds on a 3-wk conditioning program and racehorse diet. The independent variables, strong ion difference ($[SID]$), total concentration of weak acids and bases ($[A_{tot}]$), and partial pressure of carbon dioxide (PCO_2), were assessed to determine factors affecting hydrogen ion concentration ($[H^+]$) and calculated $[TCO_2]$ using the physicochemical approach to acid-base balance. Variations were found in [glucose], haematocrit (Hct), plasma proteins ([PP]), chloride ($[Cl^-]$), bicarbonate ($[HCO_3^-]$), $[H^+]$, $[SID]$, $[A_{tot}]$ and PCO_2 ; all of which appeared to be due to effects of feeding or dehydration. Mean $[TCO_2]$ varied up to 10 mmol/L throughout the day and 70% of the horses had a calculated measurement above 36.0 mmol/L.

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CHAPTER I. INTRODUCTION AND OBJECTIVES

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.

LITERATURE REVIEW

A Summary of the Effects of Feeding and Daily Variation on Acid-Base Status in Resting 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 ($[A_{\text{tot}}]$) 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 alkalization, 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 $[A_{\text{tot}}]$ and K_A . Staempfli et al. (1999) determined K_A as 2.11×10^{-7} Eq/L in equine plasma and $[A_{\text{tot}}]$ 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 alkalization. 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 H_2O 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):

$$[\text{H}^+]^4 + (\text{K}_A + [\text{SID}])[\text{H}^+]^3 + \{\text{K}_A([\text{SID}] - [\text{A}_{\text{tot}}]) - (\text{K}_C \times \text{PCO}_2 + \text{K}_w)\}[\text{H}^+]^2 - \{\text{K}_A \times (\text{K}_C \times \text{PCO}_2 + \text{K}_w) + (\text{K}_3 \times \text{K}_C \times \text{PCO}_2)\}[\text{H}^+] - (\text{K}_A \times \text{K}_3 \times \text{K}_C \times \text{PCO}_2) = 0 \quad (11)$$

Equation 11 is used to calculate the effects of changes of the independent variables (PCO₂, [SID] and [A_{tot}]) on the dependent variables ([H⁺], [HCO₃⁻], [CO₃²⁻] and [OH⁻]) (Stewart 1981, 1983). The equation illustrates that an increase in [H⁺] within a compartment arises as a result of an increase in PCO₂, 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.

	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 PCO_2 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 $Cl^- - HCO_3^-$ 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 $[\text{TCO}_2]$. 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 $[\text{TCO}_2]$ greater than or equal to 37.0 mmol/L in venous blood plasma is considered a positive test. $[\text{TCO}_2]$ 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 $[\text{TCO}_2]$ 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.

CHAPTER II. MATERIALS AND METHODS

Animals

Ten healthy Standardbred horses (8 mares, 2 geldings; body weight: 441-536 kg, age: 5-12 yrs) from the University of Guelph research herd were used for this study. During a 21-day acclimatization period they were housed in individual box stalls overnight and turned out onto pasture during the day with access to water at all times. Horses were fed twice daily and underwent a conditioning program. The first group of horses, numbers 1-5, were tested October 29, 2002 for the feed trial (FT), where horses were fed as usual, and on October 31, 2002 for the daily variation trial (DVT), where food was withheld for the duration of the study. Horse number 3 received veterinary care on the morning of the DVT and was eliminated from that part of the trial. The second group of horses (numbers 6-10) underwent testing for the FT on December 21, 2002 and for the DVT on December 23, 2002 (see table 4 for methodology time line). The animal care and use procedures were approved by the Animal Care Committee at the University of Guelph, in accordance with the *Guide to the Care and Use of Experimental Animals* (CCAC 1993).

Diets

The study diet consisted of grass hay and a pelleted grain concentrate fortified with minerals and vitamins (Purina Request: 14% protein, 6% fat, 10% fiber). The horses were fed at 7:30 am and 6 pm. The proportion of concentrate to feed was increased gradually, beginning with 1.5 kg of grain and 2.5 kg of hay in the first week, 2.0 kg grain and 2.5 kg hay in the second week and 2.5 kg of both hay and grain for a ratio of 50:50 for the third week. (See appendix 1 for feed analysis.) Although horses were

turned out during the day, there was minimal forage available in the paddocks. Water was available ad libitum. Feed DCAD ($\text{Na}^+ + \text{K}^+ - \text{Cl}^-$) was 274 mEq/kg dry matter (DM). The primary rationale for using this diet was to mimic one fed to a typical racehorse.

Conditioning

Horses were exercised 5-6 days per week, either on a mechanical walker, exercised in the paddock or lunged for the 3-week period prior the study. The duration of exercise was increased each week over the adaptation period. During the final week the horses walked for 30-min., trotted for 20-min. and cantered for 5-min.

Table 4: Study sequences time line: methodology.

a. Group 1, October 2002

Day	1-7	8-13	14-21	22	23	24
Period	Adaptation			Testing (FT)	Rest	Testing (DVT)
Feed (2 x day)	1.5 kg grain 2.5 kg hay	2 kg grain 2.5 kg hay	2.5 kg grain 2.5 kg hay	2.5 kg grain 2.5 kg hay	2.5 kg grain 2.5 kg hay	None
Exercise (5-6 days/wk)	20 min walk 15 min trot	20 min walk 20 min trot	30 min walk 20 min trot 3 min canter	None	None	None

b. Group 2, December 2002

Day	1-5	6-11	12-15	16-23	24	25	26
Period	Adaptation				Testing (FT)	Rest	Testing (DVT)
Feed (2 x day)	1.5 kg grain 2.5 kg hay	2 kg grain 2.5 kg hay	No grain 2.5 kg hay	1.5 2.5 kg grain * 2.5 kg hay	2.5 kg grain 2.5 kg hay	2.5 kg grain 2.5 kg hay	None
Exercise (5-6 days/wk)	20 min walk 10 min trot	20 min walk 20 min trot	30 min walk 20 min trot 3 min canter	30 min walk 20 min trot 3 min canter	None	None	None

* Grain for group 2 horses was gradually increased over week 3 from 1.5 to 2.5 kg of grain, as they had not been fed any grain during the prior 3 days.

Sampling

At 0700h on the day of testing the skin over the jugular vein site of catheterisation was clipped, then desensitised with EMLA cream (lidocaine 2.5%, prilocaine 2.5%; AstraZeneca Pharmaceuticals LP). A heparinized blood sample (10 ml.) was taken by jugular venipuncture into a lithium heparin vacutainer (Becton & Dickinson, Mississauga, ON). The site of catheter insertion and suturing to the skin was anaesthetized using a subcutaneous infusion of 2% lidocaine. Subsequently, catheters (Abbocath, 14 g x 5 inch) were inserted into either the left or right jugular vein. The catheters and extension tubing were secured in place by tape and skin sutures. After removal of the saline volume in the catheter and extension line, a 10-ml sample was removed by use of a 10-ml syringe and the blood immediately expelled into a 10-ml vacutainer containing lithium heparin as anticoagulant. Blood samples were taken via the catheter for 25-h. The timing of the blood samples is as shown in the figures in the results section.

Horses had access to water and were kept in the stall throughout the testing period. During the FT they were fed as usual at 7:30 am (0.5-h) and 6 pm (11-h), but did not receive exercise. The horses were accustomed to the sampling procedure and no excitement occurred. Lighting was maintained at the typical 12-h light and 12-h dark cycle.

Blood plasma analysis and calculated parameters

Aliquots of blood were dispensed into two 1.5 ml microcentrifuge tubes and analyzed in duplicate to measure plasma concentrations of sodium ($[Na^+]$), potassium ($[K^+]$), chloride ($[Cl^-]$), glucose ($[Glu]$), pH, the partial pressure of carbon dioxide

(PCO₂) and oxygen (PO₂) and blood hematocrit (Hct) using a NOVA Statprofile 9⁺ analyser (Nova Biomedical, Waltham, Mass.). The NOVA performs all measurements at a temperature of 37°C. These analyses were typically completed within 5-min of blood collection.

The samples were then centrifuged at high speed for 5 min., the plasma removed and total plasma protein concentration ([PP]) determined by use of a clinical refractometer (Atago, Japan). The remaining plasma was transferred into 1.8 ml. screw cap cryovials. Samples from the first study (Oct.) were stored at -80°C and samples from the second study (Dec.) were stored at -20°C. At the time of the on-site analyses, the performance of the Na⁺, K⁺ and Cl⁻ electrodes did not meet laboratory standards (excessive electrode drift). Therefore, the plasma samples were subsequently thawed and re-analyzed to determine the plasma concentrations of Na⁺, K⁺, and Cl⁻ using a Nova Statprofile 5 analyser (Nova Biomedical, Waltham, Mass.).

Calibrations

The Nova analyzers self-calibrated once every 2-h when in use. Each calibration was accompanied by the analysis of quality control solutions and/or known solution concentrations. The NOVA Stat Profile 5, used for plasma analysis of electrolytes, was consistently calibrated and controls were within expected ranges.

Calculations

Bicarbonate concentration ($[\text{HCO}_3^-]$) was calculated using the Henderson-Hasselbalch equation ($\text{pH} = \text{pK} + \log([\text{HCO}_3^-]/\text{PCO}_2)$), which when rearranged gives:

$$\text{Log}_{10} [\text{HCO}_3^-] = \text{pH} + \log_{10}\text{PCO}_2 - 7.604 \quad (\text{mmol/L}) \quad (15)$$

TCO_2 includes both dissolved carbon dioxide (PCO_2) and HCO_3^- and was calculated as follows:

$$[\text{TCO}_2] = [\text{HCO}_3^-] + [\text{PCO}_2] \quad (\text{mmol/L}) \quad (16)$$

Where both pH and PCO_2 were measured,

$$\text{pK} = 6.091,$$

$$(\text{solubility coefficient of plasma CO}_2 \text{ at } 37^\circ\text{C}) = 0.0307 \text{ (mmol CO}_2\text{/L/torr CO}_2\text{)}.$$

Plasma $[\text{H}^+]$ was calculated from the measured pH as the negative antilog (denoted as $[\text{H}^+]_m$). Calculated plasma $[\text{H}^+]$ ($[\text{H}^+]_c$) was derived from the equation describing the mass action equilibria and electroneutrality of solutions (Stewart 1981) employing equation 11:

$$[\text{H}^+]^4 + (\text{K}_A + [\text{SID}])[\text{H}^+]^3 + \{\text{K}_A([\text{SID}] - [\text{A}_{\text{tot}}]) - (\text{K}_C \times \text{PCO}_2 + \text{K}_w)\}[\text{H}^+]^2 - \{\text{K}_A \times (\text{K}_C \times \text{PCO}_2 + \text{K}_w) + (\text{K}_3 \times \text{K}_C \times \text{PCO}_2)\}[\text{H}^+] - (\text{K}_A \times \text{K}_3 \times \text{K}_C \times \text{PCO}_2) = 0$$

Where K_A (equilibrium constant for plasma non volatile weak acids) = 2.11×10^{-7} Eq/L

K_C (CO_2 hydration/dehydration equilibrium dissociation constant) = 2.58×10^{-11} (Eq/L)²

K_3 (equilibrium dissociation constant for HCO_3^-) = 6.0×10^{-11} Eq/L

$$K_w \text{ (dissociation constant of water)} = 4.4 \times 10^{-14} \text{ (Eq/L)}^2.$$

The contributions of each of the independent variables ($[A_{tot}]$, $[SID]$, PCO_2) to the dependent variable $[H^+]$ and $[TCO_2]$ were computed using the ‘ $[H^+]$ Calculator’, an Excel -based software that solves equation 11 for $[H^+]$, $[HCO_3^-]$ and $[TCO_2]$ (Scientific Solutions, Eden Mills, ON). Calculated $[H^+]$ was subtracted from the resulting $[H^+]$ and the difference expressed as a percentage of total $[H^+]$ and compared to the measured $[H^+]$. Each of the independent variables was individually changed, while the remaining two were held constant at $t=0$ h, in order to separate out the contributions of each of the independent variables to the change in the measured $[H^+]$.

Plasma $[A_{tot}]$ and $[SID]$ were calculated as:

$$[A_{tot}] = 2.11 * [PP] \text{ (mEq/L)} \quad (17)$$

$$[SID] = [Na^+] + [K^+] - [Cl^-] \text{ (mEq/L)} \quad (18)$$

Although plasma $[P_i]$ contributes to $[A_{tot}]$ its molar contribution is small and so $[P_i]$ was not used.

Statistical Analyses

Differences between the two groups for both trials (FT: Oct. 29 and Dec. 21, 2002; DVT: Oct. 31 and Dec. 23, 2002) were analyzed using 2-way (time, group) repeated measures analysis of variance (ANOVA) with the Holm-Sidak post-hoc test to determine whether the differences between the means were significant. If they were not, the groups were combined; otherwise they remained as separate groups. Data were then analyzed using 1-way (time) repeated measures ANOVA with a Bonferonni post-hoc test to determine significant differences from the initial time point within each group and between the FT and DVT. The frequency distribution, mean and standard error (SE) of the data were calculated using Sigma Stat software. Data points greater than 2 SE from the mean were discarded. A linear regression analysis between $[H^+]_m$ and $[H^+]_c$ was performed for both trials (see appendix 2). Unless otherwise noted a statistical significance level of $P < 0.05$ was used, power = 80%, and the data expressed as mean \pm SE.

CHAPTER III. RESULTS

1. DAILY VARIATION TRIAL

Glucose: (Figure 1) Plasma glucose concentrations did not change over the 25-h study period when compared to the initial value of 5.1 ± 0.2 mmol/L.

Haematocrit: (Figure 2, top) Haematocrit decreased from $36.1 \pm 2.4\%$ at 0-h to $32.1 \pm 1.9\%$ and $32.6 \pm 2.1\%$ at 13 and 15-h respectively.

Plasma protein: (Figure 2, bottom) Venous [PP] remained unchanged for the first 12-h and then increased and remained elevated from 12 to 25-h. The initial mean [PP] was 6.0 ± 0.4 g/dl and there was an increase of 0.6 g/dl from 14 to 25-h.

Plasma electrolytes: (Figure 3) There were no changes in $[\text{Na}^+]$, $[\text{K}^+]$, or $[\text{Cl}^-]$. The initial mean $[\text{Na}^+]$ was 139.0 ± 0.9 mEq/L, $[\text{K}^+]$ was 3.7 ± 0.2 mEq/L and $[\text{Cl}^-]$ was 99.7 ± 1.7 mEq/L.

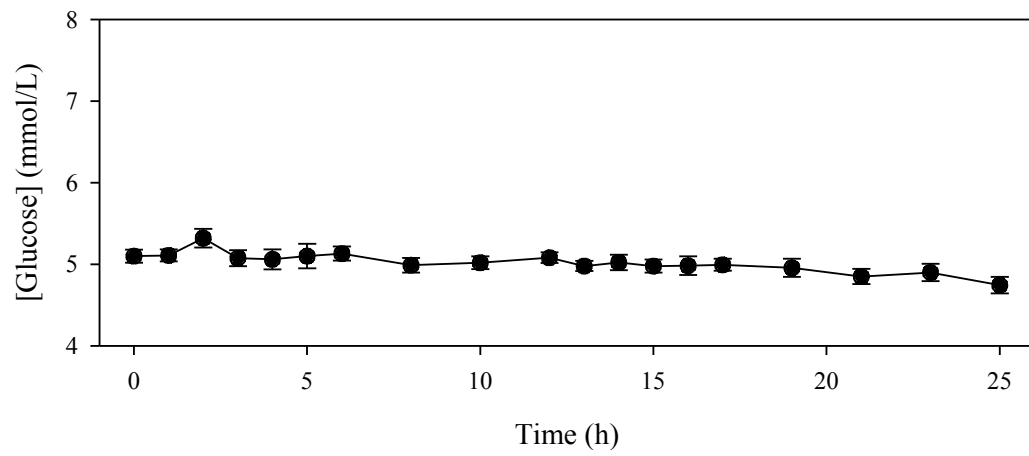


Figure 1. Daily Variation Trial (n=9).
Mean (\pm SE) plasma glucose concentrations over 25-h.

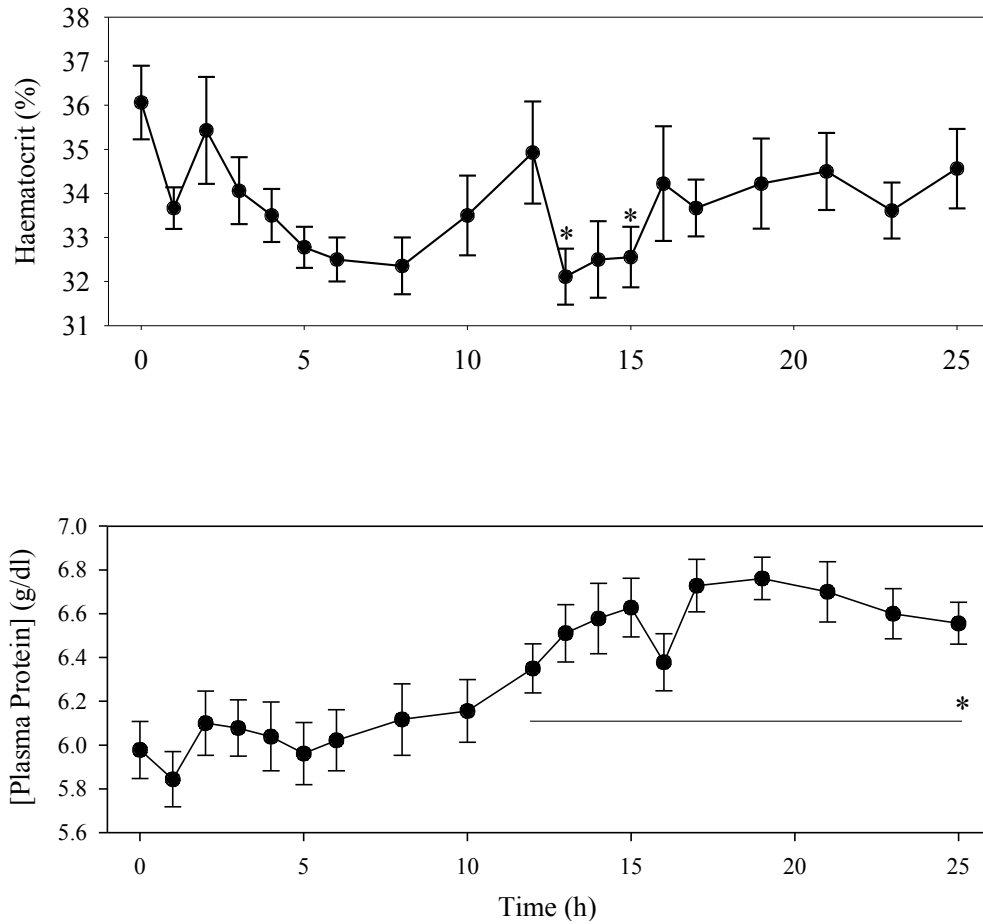


Figure 2. Daily Variation Trial (n=9).
 Mean (\pm SE) haematocrit (top) and plasma protein concentrations (bottom) over 25-h.
 * Indicates significant difference from t = 0-h, p<0.05.

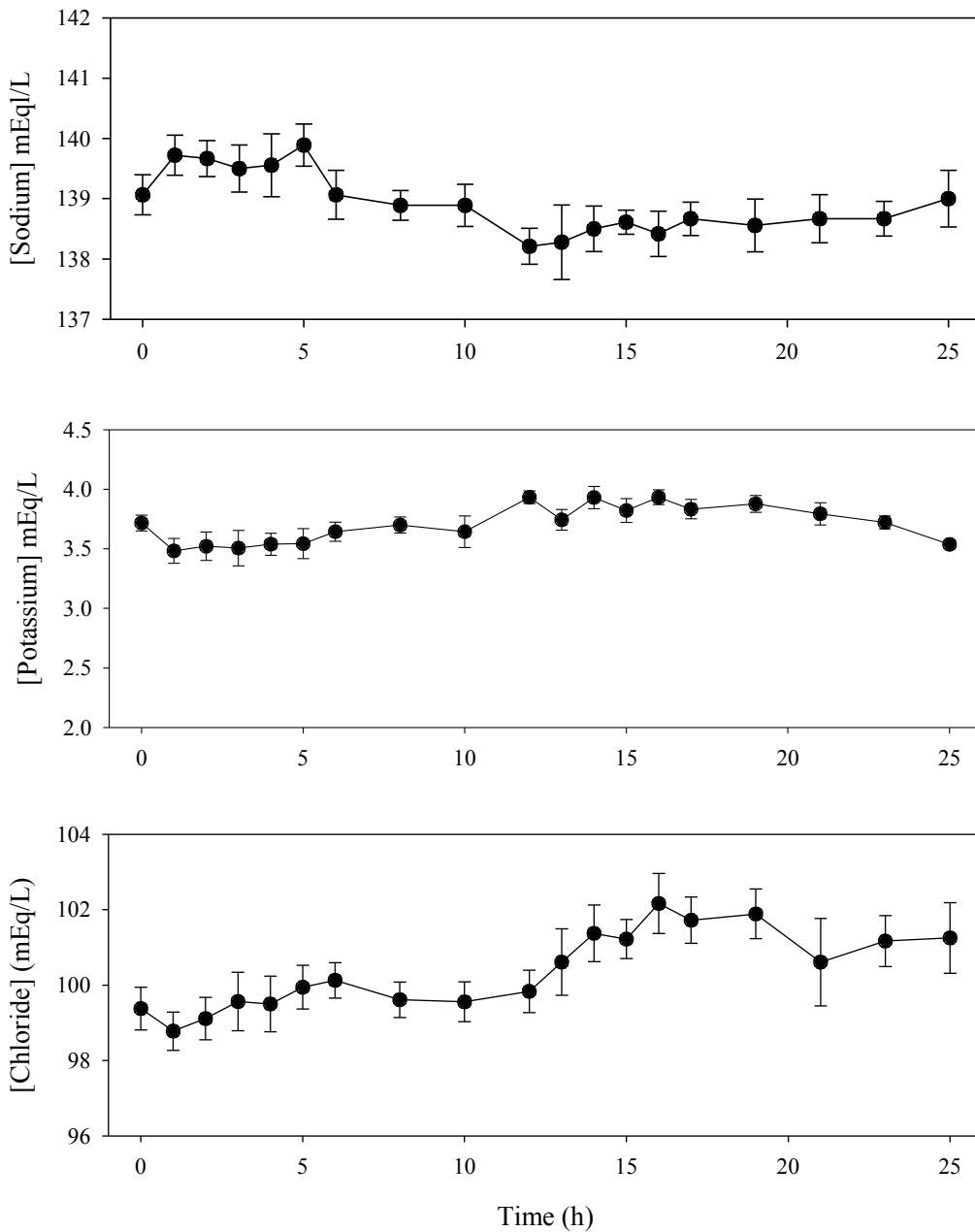


Figure 3. Daily Variation Trial (n=9). Mean (\pm SE) concentrations of plasma sodium (top), potassium (middle), and chloride (bottom) over 25-h.

Independent acid-base variables: (Figure 4)

Strong ion difference (Figure 4 top)

Plasma [SID] decreased between 13 and 18-h and 23 to 25-h when compared to 0-h. The initial [SID] was 43.7 ± 1.5 mEq/L and reached a low of 40.2 ± 1.1 mEq/L at 16-h, a decrease of 3.5 mEq/L.

Total concentration of weak acids and bases (Figure 4 middle)

[A_{tot}] increased from 0-h through 12-25 h. There was an increase of 1.7 mEq/L from the initial mean concentration of 12.6 ± 0.9 mEq/L to a high of 14.3 ± 0.6 mEq/L at 19-h.

Partial pressure of carbon dioxide (figure 4 bottom)

There was a time dependent decrease in PCO₂ between 14 and 17-h and at 25-h as compared to the initial mean of 50.2 ± 1.9 mmHg, which was the highest mean during the 25-h period. The lowest mean was 42.7 ± 3.1 mmHg at 25-h, a difference of 7.5 mmHg.

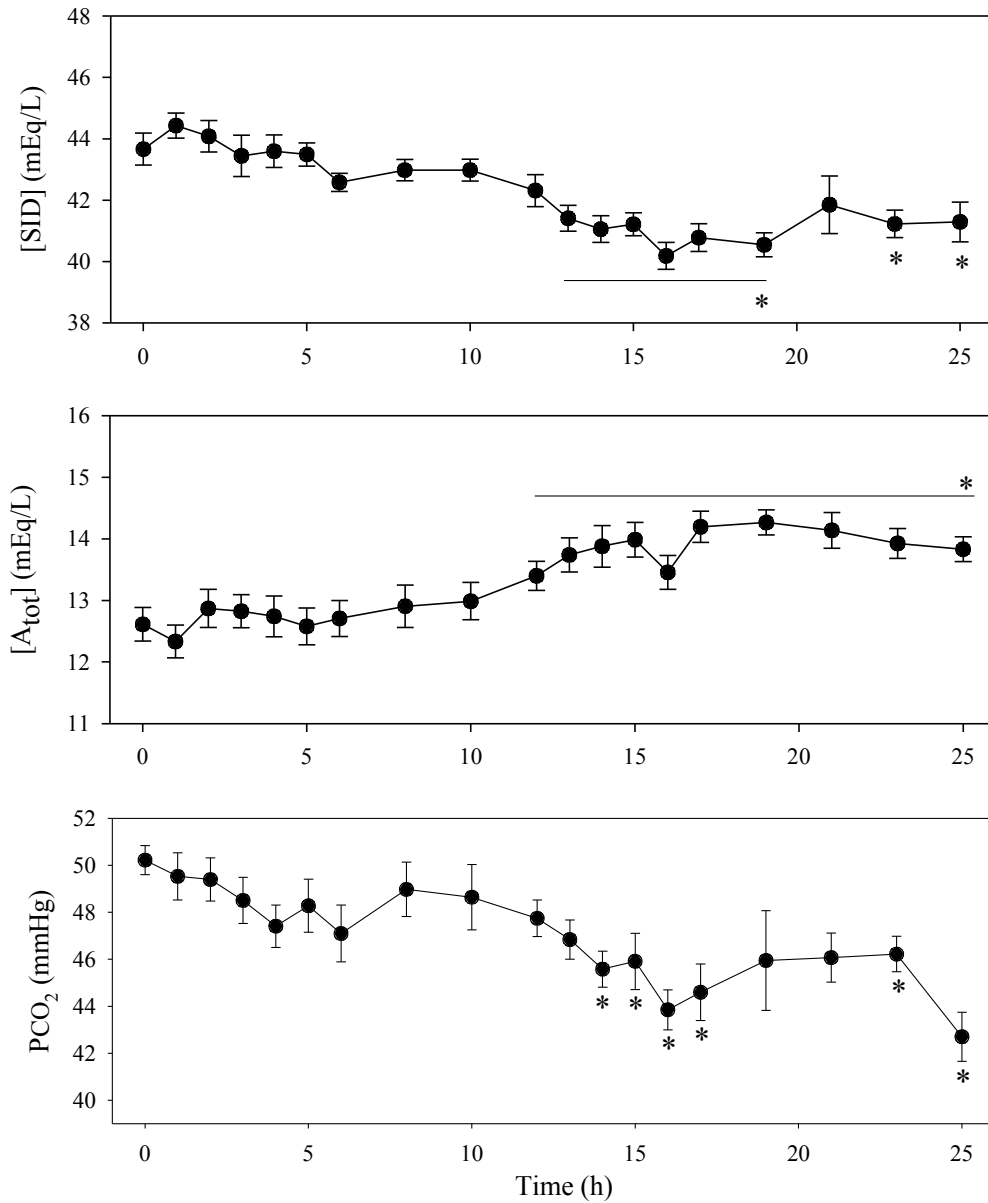


Figure 4. Daily Variation Trial (n=9).

The plasma strong ion difference ($[SID]$) (middle), total concentration of weak acids and bases ($[A_{tot}]$) (top), and partial pressure of carbon dioxide (PCO_2) (bottom) over 25-h. All values are means (\pm SE).

* Indicates significantly different from t = 0-h, p<0.05.

Dependent acid-base variables: (Figure 5)

The daily variation data for pH, $[\text{HCO}_3^-]$, $[\text{H}^+]$ and $[\text{TCO}_2]$ in groups 1 and 2 were not combined into a single data set because the 2-way, repeated measures ANOVA demonstrated significant differences between the two groups.

Plasma pH (Figure 5, top)

Group 1: No variation was detected over the 25-h trial period. The average pH was 7.44 ± 0.03 .

Group 2: There was a time dependent decrease in pH from 7.43 ± 0.02 at 0-h, seen from 3 to 5-h and through 8 to 25-h. Mean pH between 3 to 5-h was 7.39 ± 0.02 . Between 8 and 25-h, means reached lows of 7.35 ± 0.03 at 10-h and 7.33 ± 0.01 at 17-h.

Plasma bicarbonate concentration (Figure 5, bottom)

Group 1: A significant decrease occurred at 15 and 17-h with means of 29.4 ± 2.0 and 29.6 ± 2.1 mEq/L respectively, as compared to the initial mean of 33.7 ± 1.5 mEq/L. The range between high and low $[\text{HCO}_3^-]$ means was 4 mEq/L.

Group 2: There was a significant decrease in mean $[\text{HCO}_3^-]$ between 3 and 25-h when compared to the initial mean of 33.7 ± 1.4 mEq/L. $[\text{HCO}_3^-]$ remained constant between 3 and 13-h with an average of 28.2 ± 0.9 mEq/L and then fluctuated to the end of the study with a low at 16-h of 23.7 ± 1.3 mEq/L, and a subsequent increase to 28.3 ± 1.3 mEq/L at 21-h.

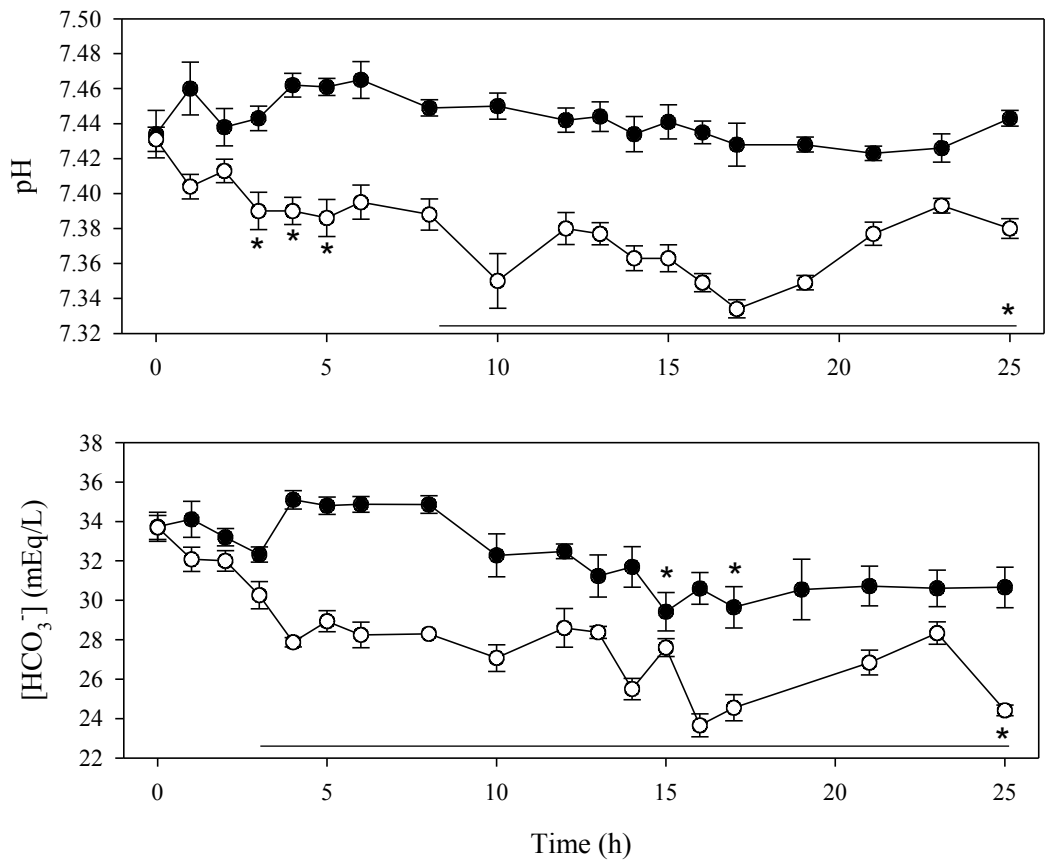


Figure 5. Daily Variation Trial.
 Mean (\pm SE) pH (top) and bicarbonate concentrations ($[HCO_3^-]$) (bottom) over 25-h.
 * Indicates significant difference from t = 0-h, p<0.05.
 Solid circles represent group 1 (n=4) and empty circles represent group 2 (n=5).

Plasma hydrogen ion concentration:

Group 1: (Figure 6) There was no change in $[H^+]$ over the 25-h study period (figure 6 top). The mean over the 25-h study period was 36.1 ± 2.5 nEq/L. The contributions to $[H^+]$ changes (figure 6 bottom) from a decrease in PCO_2 (figure 4 bottom) between 13 and 17-h and at 25-h contributed to a decreased $[H^+]$ that was countered by the increases in $[A_{tot}]$ (figure 4 middle) from 13 to 25-h. The acidifying effect of a decreased $[SID]$ (figure 4 top) was not statistically significant.

Group 2: (Figure 7) There was a significant increase in $[H^+]$ from 3-h onwards, with the exception of 6 and 23-h, when compared to the initial mean of 37.1 ± 1.3 nEq/L (figure 7 top). High means of 44.8 ± 1.2 and 46.3 ± 1.1 nEq/L occurred at 10 and 17-h respectively. The range between the high and low means was 7.7 nEq/L.

Contributions to changes in $[H^+]$ (figure 7 bottom) were from increases in $[A_{tot}]$ (figure 4 middle) between 12 and 25-h and decreases in $[SID]$ (figure 4 top) between 14 to 25-h and PCO_2 (figure 4 bottom) at 8, 14, 16 and 25-h. However, the increase in $[H^+]$ was greater than the combined contributions of $[SID]$, $[A_{tot}]$ and PCO_2 .

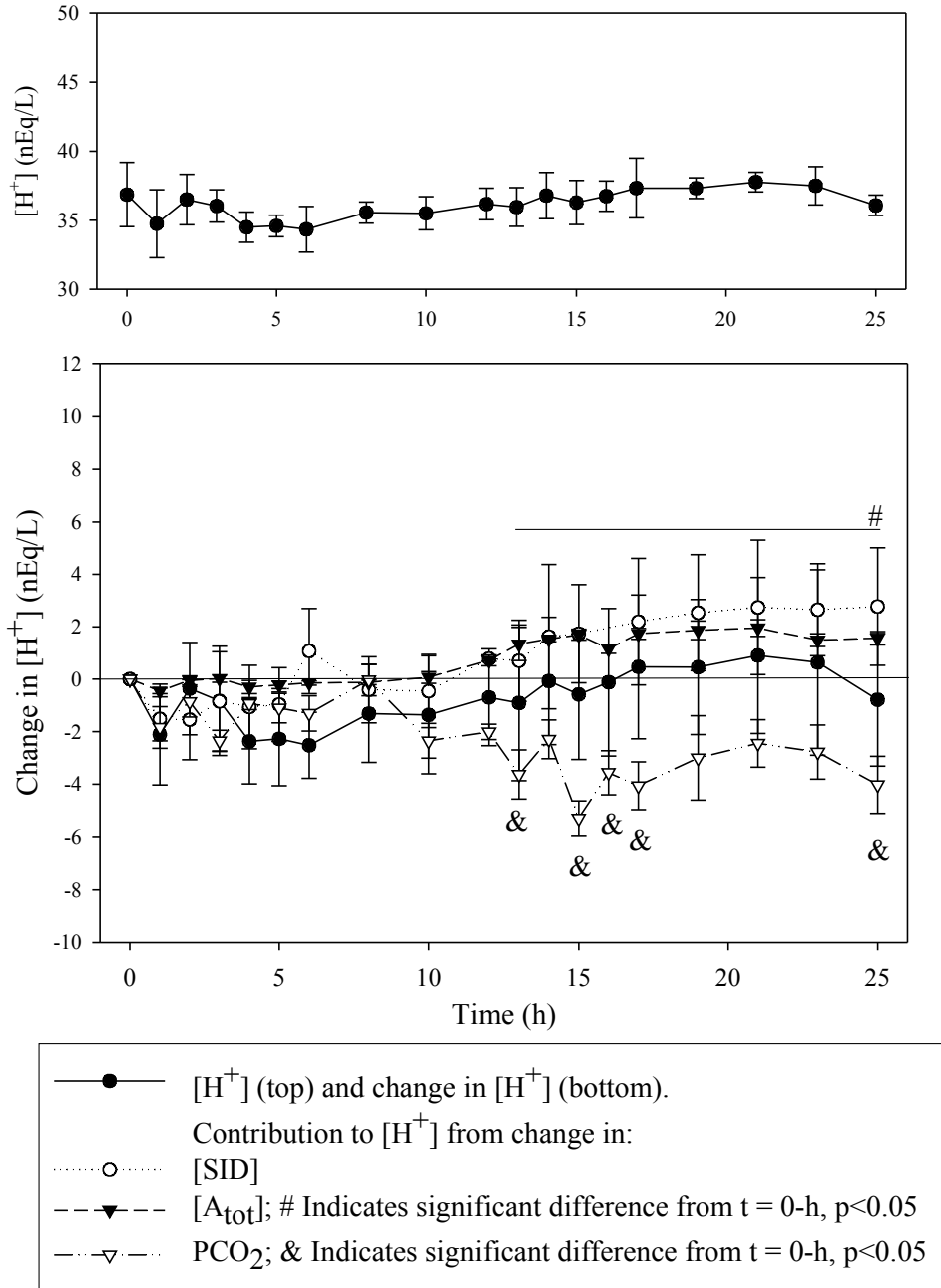


Figure 6. Diurnal Study, Group 1 (n = 4).
 Top: Mean (\pm SE) hydrogen ion concentration variations over 25-h.
 Bottom: Mean (\pm SE) change in hydrogen ion concentration and origins of change in hydrogen ion concentration in each group from changes in the three independent variables, $[A_{tot}]$, [SID] and PCO_2 .

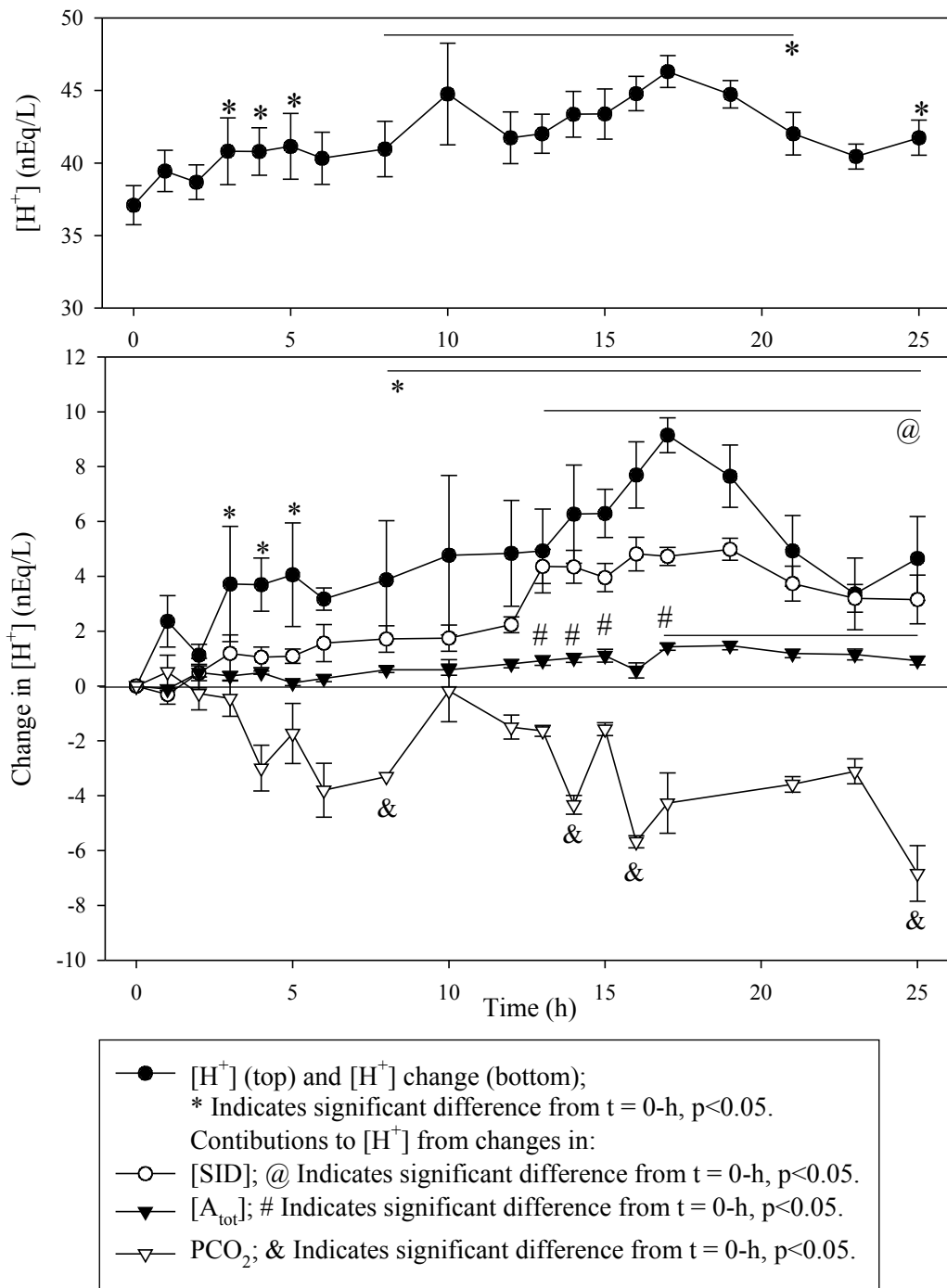


Figure 7. Diurnal Study, Group 2 (n = 5).

Top: Mean (\pm SE) hydrogen ion concentration variations over 25-h.

Bottom: Mean (\pm SE) change in hydrogen ion concentration and origins of change in hydrogen ion concentration in each group from changes in the three independent variables, $[A_{tot}]$, [SID] and PCO_2 .

Total carbon dioxide concentration

Group 1: (Figure 8) There was a decrease in $[\text{TCO}_2]$ from the initial mean of 35.3 mmol/L to 30.8 and 31.0 mmol/L at 15 and 17-h (figure 8 top). The average concentration over the trial was 33.7 ± 3.1 mmol/L.

The increase in $[\text{A}_{\text{tot}}]$ (figure 4 middle) was the only significant variable to account for the decrease in $[\text{TCO}_2]$ (figure 8 bottom) at 15 and 17-h. However, the contributions of decreases in $[\text{SID}]$ (figure 4 top) and PCO_2 (figure 4 bottom) are also required to fully account for the $[\text{TCO}_2]$ decrease.

Group 2: (Figure 9) There was a decrease in $[\text{TCO}_2]$ between 3-h and throughout the rest of the trial period as compared to the initial mean of 35.3 ± 1.4 mmol/L (figure 9 top). $[\text{TCO}_2]$ decreased to a low of 25.0 ± 1.4 mmol/L at 16-h. The range between the high and low mean $[\text{TCO}_2]$ was 10.3 mmol/L.

The magnitude of the decrease in $[\text{TCO}_2]$ from 3-h through the rest of the study (figure 9 bottom) was greater than that of the contributions from $[\text{SID}]$ (figure 4 top), $[\text{A}_{\text{tot}}]$ (figure 4 middle) and PCO_2 (figure 4 bottom). $[\text{SID}]$ provided the greatest decrease in $[\text{H}^+]$ between 13 and 25-h with additional contributions from $[\text{A}_{\text{tot}}]$ between 13 and 25-h and PCO_2 at 8, 16, 18 and 25-h.

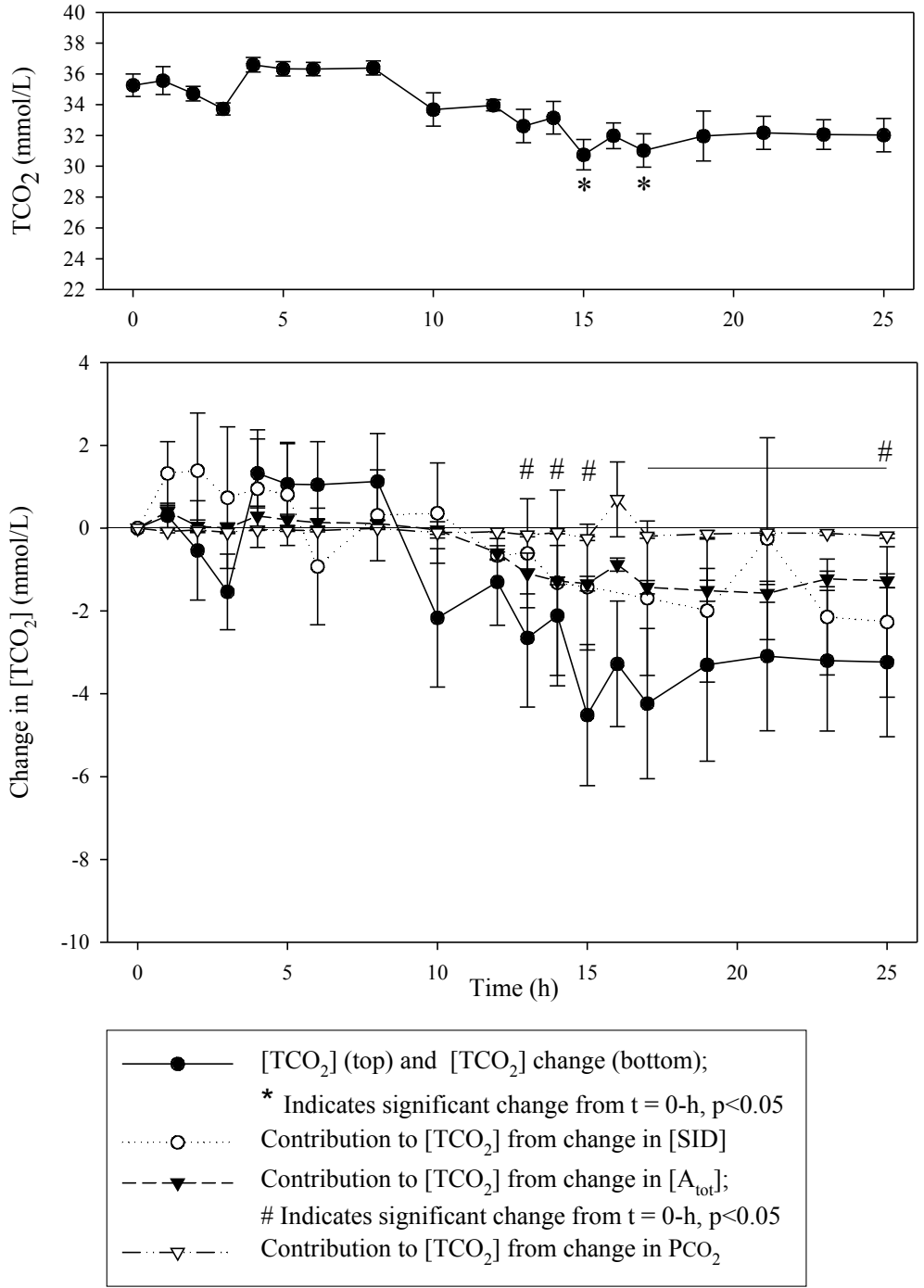


Figure 8. Diurnal Study, Group 1 (n = 4).
 Top: Mean (\pm SE) total carbon dioxide concentration ($[TCO_2]$) over 25-h.
 Bottom: Mean (\pm SE) change in $[TCO_2]$ and origins of change in $[TCO_2]$ in each group from changes in the three independent variables, $[A_{tot}]$, $[SID]$ and PCO_2 .

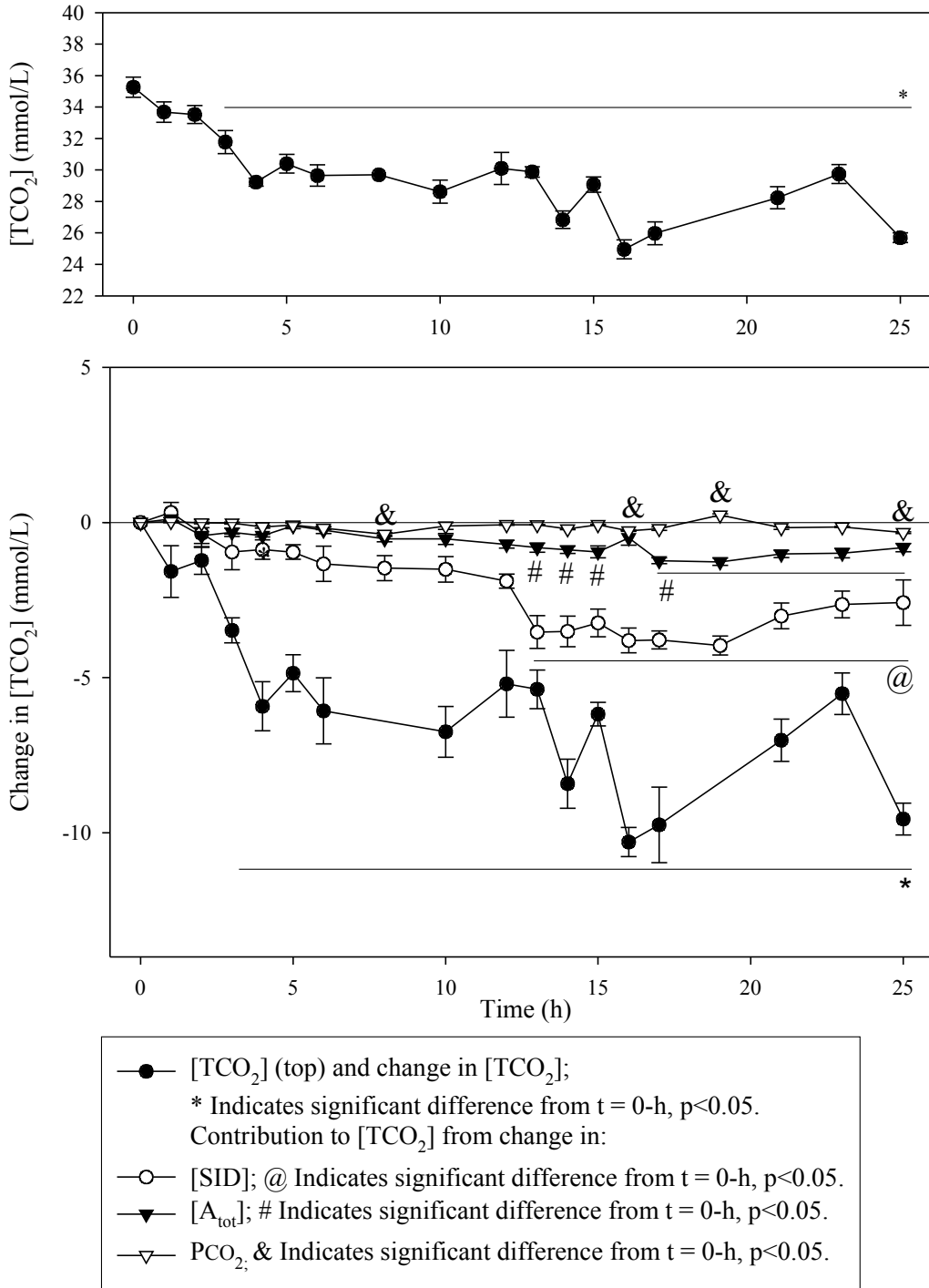


Figure 9. Diurnal Study, Group 2 (n = 5).

Top: Mean (\pm SE) total carbon dioxide concentration ([TCO₂]) over 25-h.

Bottom: Mean (\pm SE) change in [TCO₂] and origins of change in [TCO₂] in each group from changes in the three independent variables, [A_{tot}], [SID] and PCO₂.

2. FEED TRIAL RESULTS

Plasma Glucose: (Figure 10)

[Glucose] increased from an initial mean of 5.3 ± 0.2 mmol/L to 6.7 ± 0.4 mmol/L from 2 to 4 and at 5-h (top). The highest concentration, 7.1 ± 0.9 mmol/L, was reached 3.5-h following feeding. There was no increase in [glucose] following the evening feed. No change over the day was exhibited when the changes in the FT were compared to the DVT (bottom).

Haematocrit: (Figure 11)

Mean Hct at $t = 0$ -h was $34.2 \pm 2.4\%$ and did not change over the 25-h study period (top). There was no difference over the day when the changes from the FT were compared to those of the DVT (bottom).

Plasma protein: (Figure 12)

When compared to the initial mean concentration, [PP] was increased at $t = 2, 8$ and 10-h. The initial increase following the morning feeding was 0.4 g/dl from 6.2 ± 0.3 g/dl at 0-h to 6.6 ± 0.5 g/dl at 2-h. A high of 6.7 ± 0.4 g/dl was reached at 8 and [PP] decreased to 6.1 g/dl at 25-h. When the mean FT [PP] was compared to the DVT mean there was a difference in [PP] with a change of -0.6 g/dl from 0.1 ± 0.1 g/dl at $t = 0$ -h to -0.5 ± 0.2 g/dl at 25-h. There was an overall increase during the day and decrease at night in [PP] compared to the DVT.

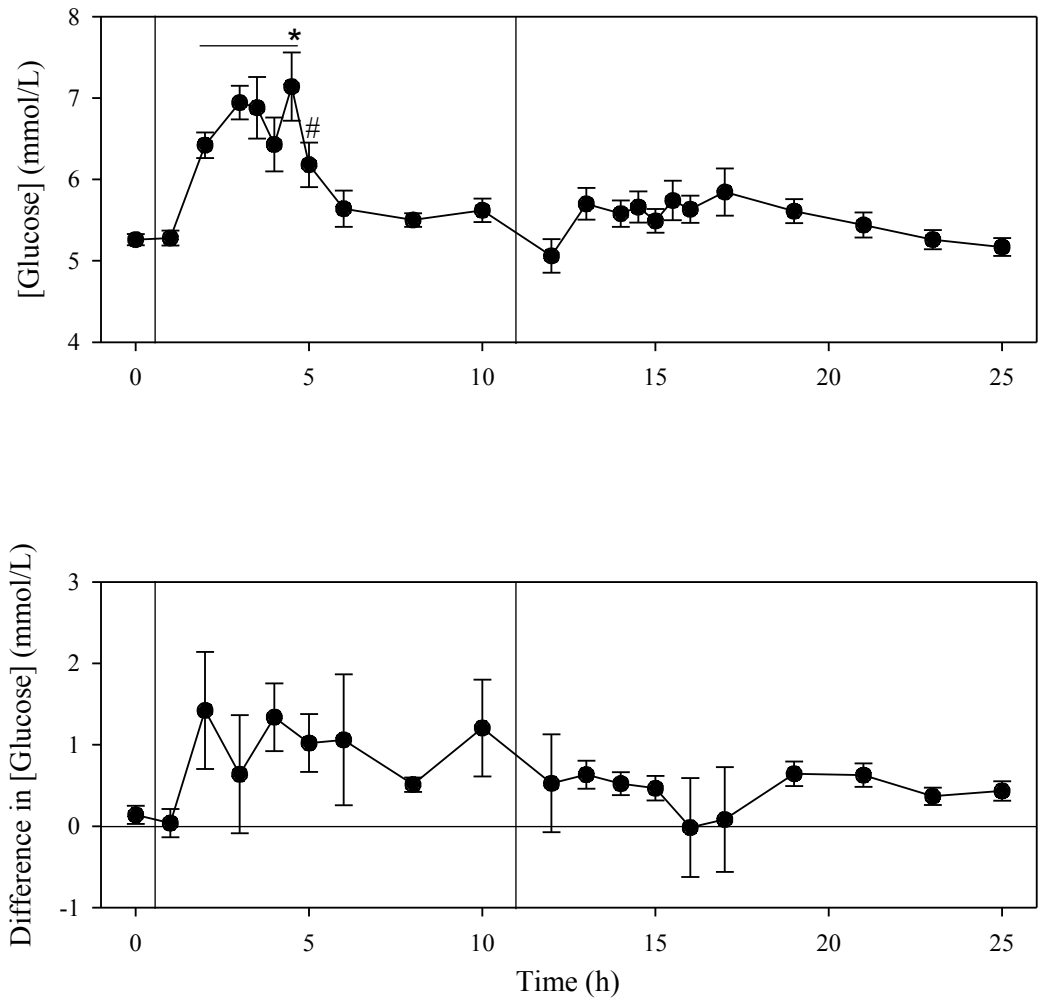


Figure 10. Feed Trial (n=10).

Top: Mean (\pm SE) plasma glucose concentrations over 25-h.

Bottom: Difference in mean (\pm SE) plasma glucose concentrations between FT and DVT.

* Indicates significantly different from t = 0-h, p<0.05.

Lines at t = 0.5-h and 11-h represent feeding times.

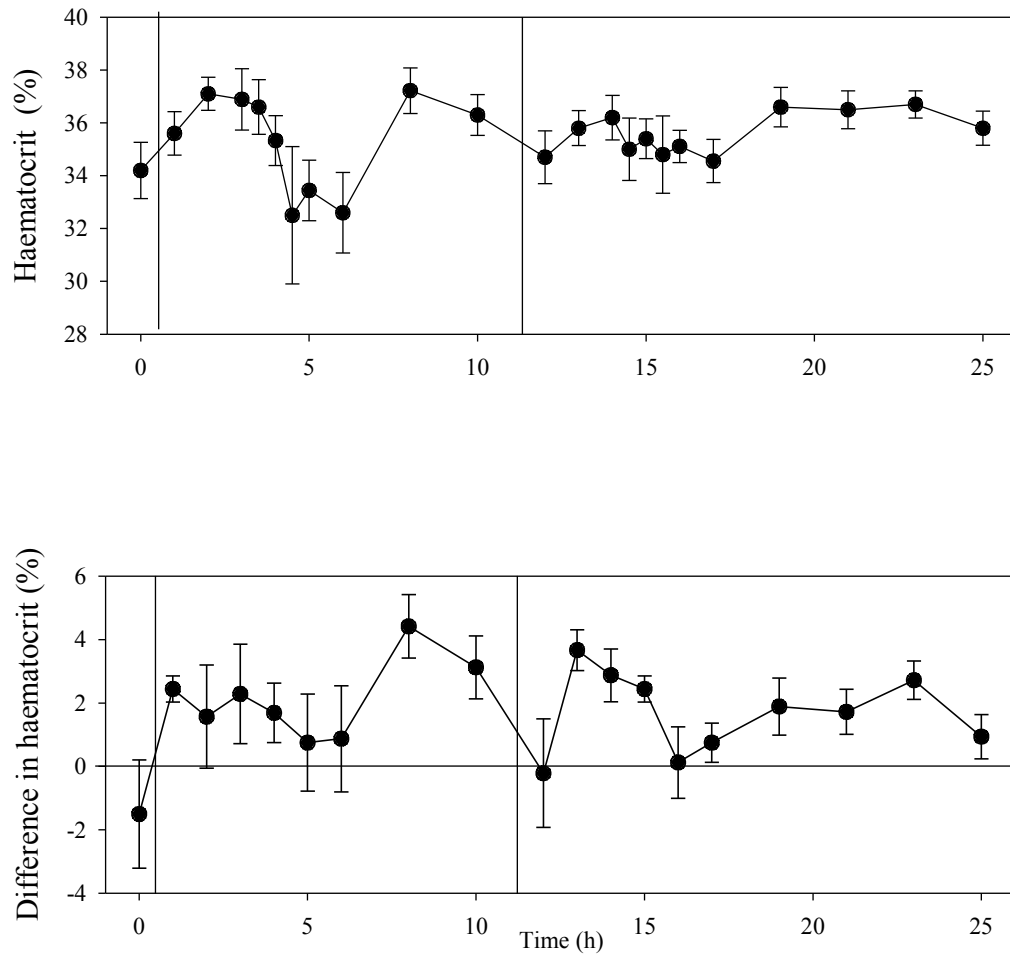


Figure 11. Feed Trial (n=10).

Top: Mean (\pm SE) plasma haematocrit over 25-h.

Bottom: Difference in mean (\pm SE) plasma haematocrit between FT and DVT.

Lines at t = 0.5-h and 11-h represent feeding times.

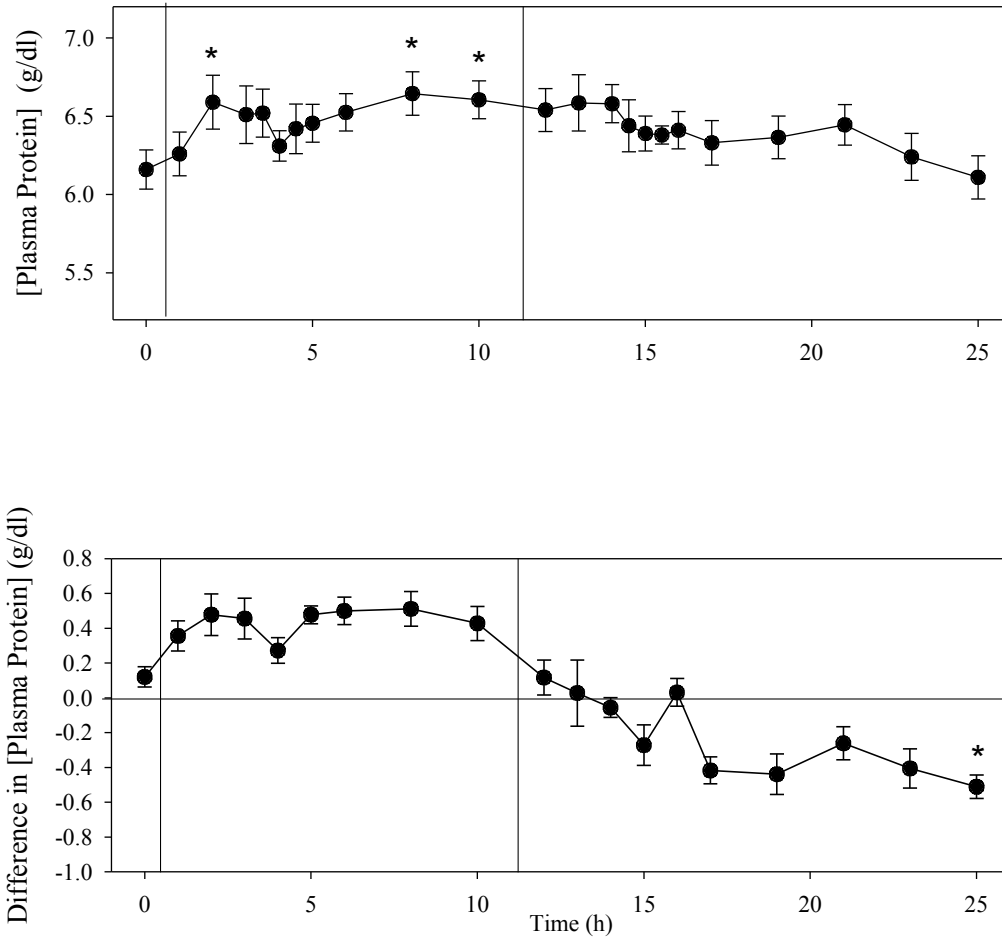


Figure 12. Feed Trial (n=10).

Top: Mean (\pm SE) plasma protein concentration over 25-h.

Bottom: Difference in mean (\pm SE) plasma protein concentrations between FT and DVT.

* Indicates significant different from t = 0-h, p<0.05.

Lines at t = 0.05-h and 11-h represent feeding times.

Plasma electrolytes:

There was no change in $[\text{Na}^+]$ (figure 13), from the initial mean of 140.3 ± 0.5 mEq/L. $[\text{K}^+]$ (figure 14), with an initial mean of 3.6 ± 0.3 mEq/L, also exhibited no change over the trial. $[\text{Cl}^-]$ (figure 15) was increased at 4.5-h with a value of 101.7 ± 0.8 mEq/L compared to the initial value of 98.8 ± 0.5 mEq/L. There were no differences in $[\text{Na}^+]$, $[\text{K}^+]$, and $[\text{Cl}^-]$ over time between the FT and DVT.

Independent acid-base variables:

Strong ion difference (Figure 16) $[\text{SID}]$ decreased from 45.1 ± 1.8 mEq/L at 0-h to 41.6 ± 1.6 mEq/L at 4.5-h. There was no difference in $[\text{SID}]$ over time between the FT and DVT.

Total concentration of weak acids and bases (Figure 17) There was an increase in $[\text{A}_{\text{tot}}]$ from 0-h, with a mean of 13.0 ± 0.6 mEq/L, to 13.9 ± 1.1 mEq/L at 2-h. $[\text{A}_{\text{tot}}]$ was also elevated at 8 and 10-h time points to 14.0 ± 0.9 mEq/L. Means were not different from baseline between 12 and 25-h. For the first 10-h of the trials, FT $[\text{A}_{\text{tot}}]$ means were increased above the DVT, while the FT means were below the DVT means at 17, 19, 23 and 25-h. At 25-h the mean difference between the FT and DVT was different from the initial mean.

Partial pressure of carbon dioxide (Figure 18) PCO_2 decreased from 51.6 ± 1.6 mmHg at 0-h to 46.3 ± 3.9 mmHg at 4.5-h and 45.0 ± 2.2 mmHg at 10-h. The range from the initial to lowest mean was 7.5 mmHg. For most of the trial period the FT PCO_2 remained similar to those of the DVT, however at 23 and 25-h the FT horses PCO_2 was elevated above those of the DVT.

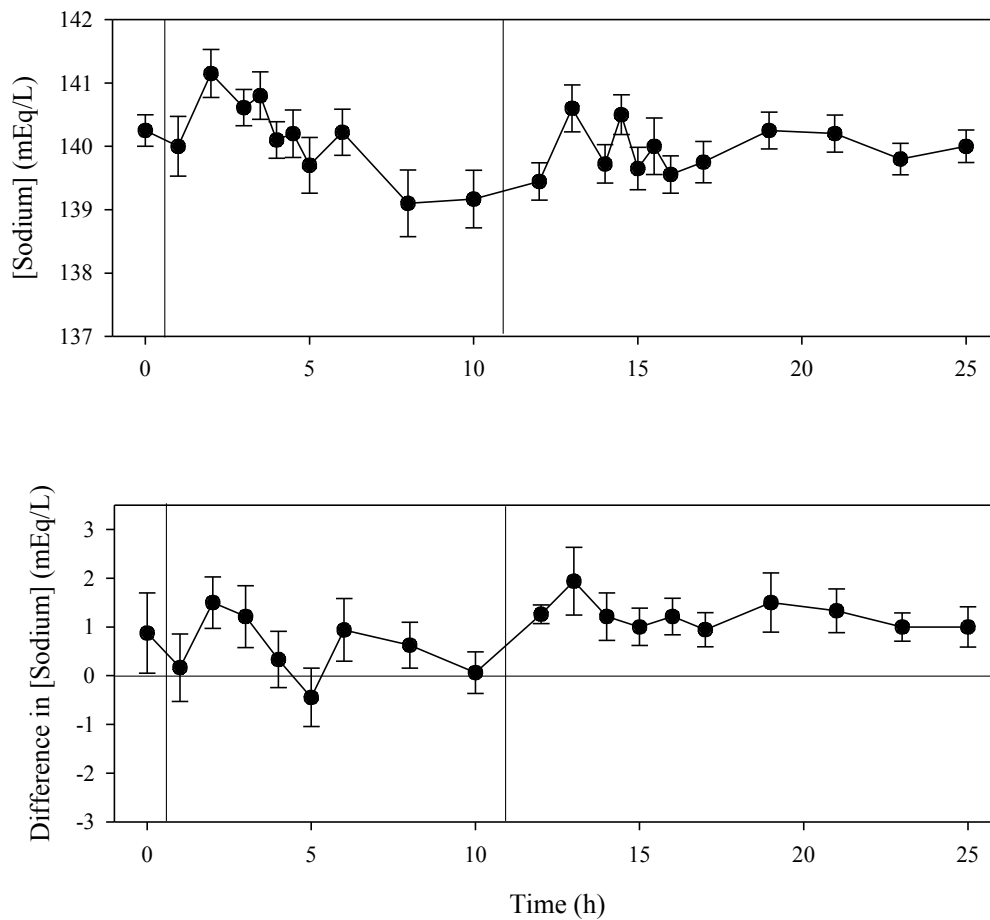


Figure 13. Feed Trial (n=10).

Top: Mean (\pm SE) concentrations of plasma sodium over 25-h.

Bottom: Difference in mean (\pm SE) plasma sodium concentrations between FT and DVT.

Lines at t = 0.5-h and 11-h represent feeding times.

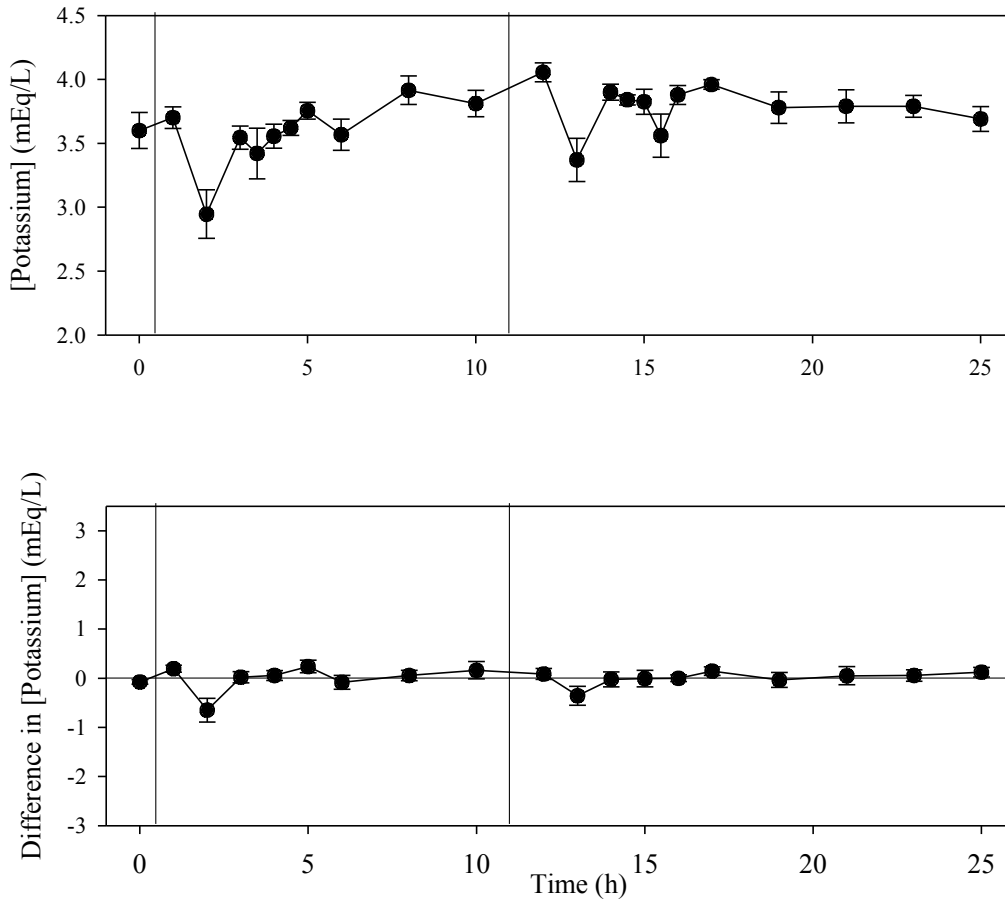


Figure 14. Feed Trial (n=10).

Top: Mean (\pm SE) concentrations of plasma potassium over 25-h.

Bottom: Difference in mean (\pm SE) plasma protein concentrations between FT and DVT studies.

Lines at 0.5 and 11-h represent feeding times.

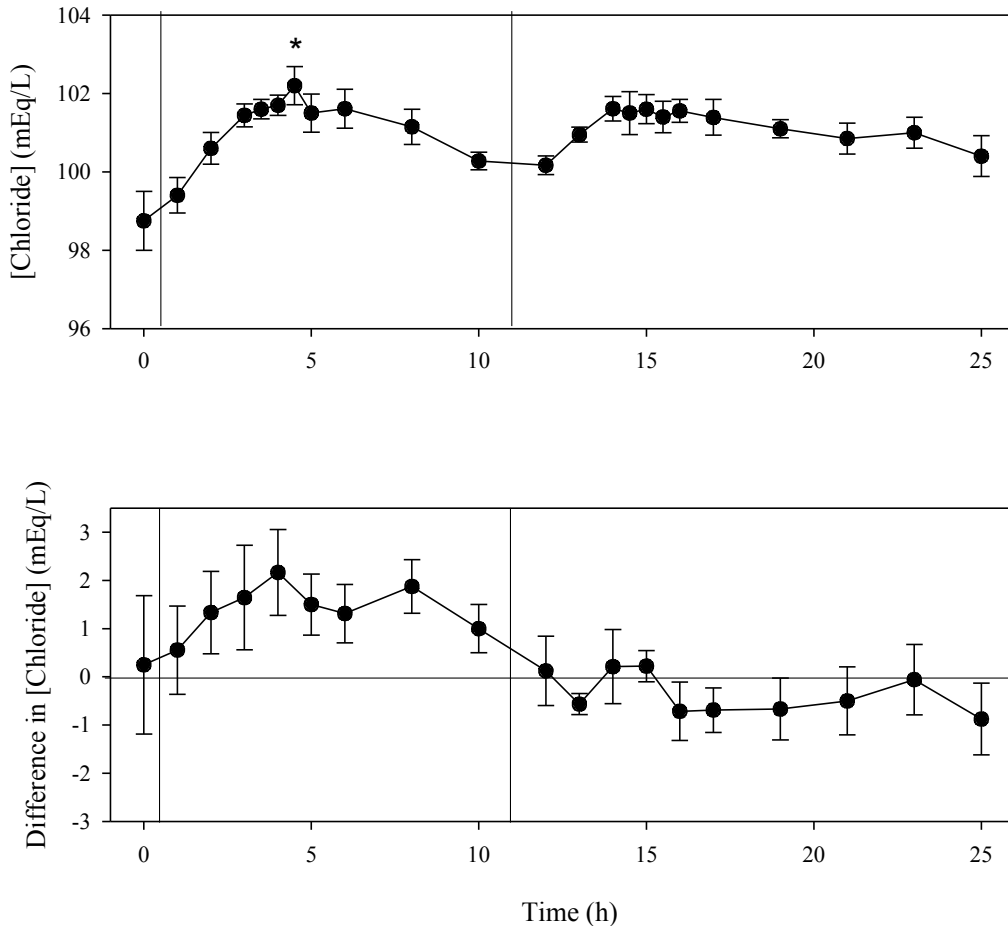


Figure 15. Feed Trial (n=10).

Top: Mean (\pm SE) concentrations of plasma chloride over 25-h.

Bottom: Difference in mean (\pm SE) plasma chloride concentrations between FT and DVT.

* Indicates significantly different from t=0-h, $p < 0.05$.

Lines at 0.5 and 11-h represent feeding times.

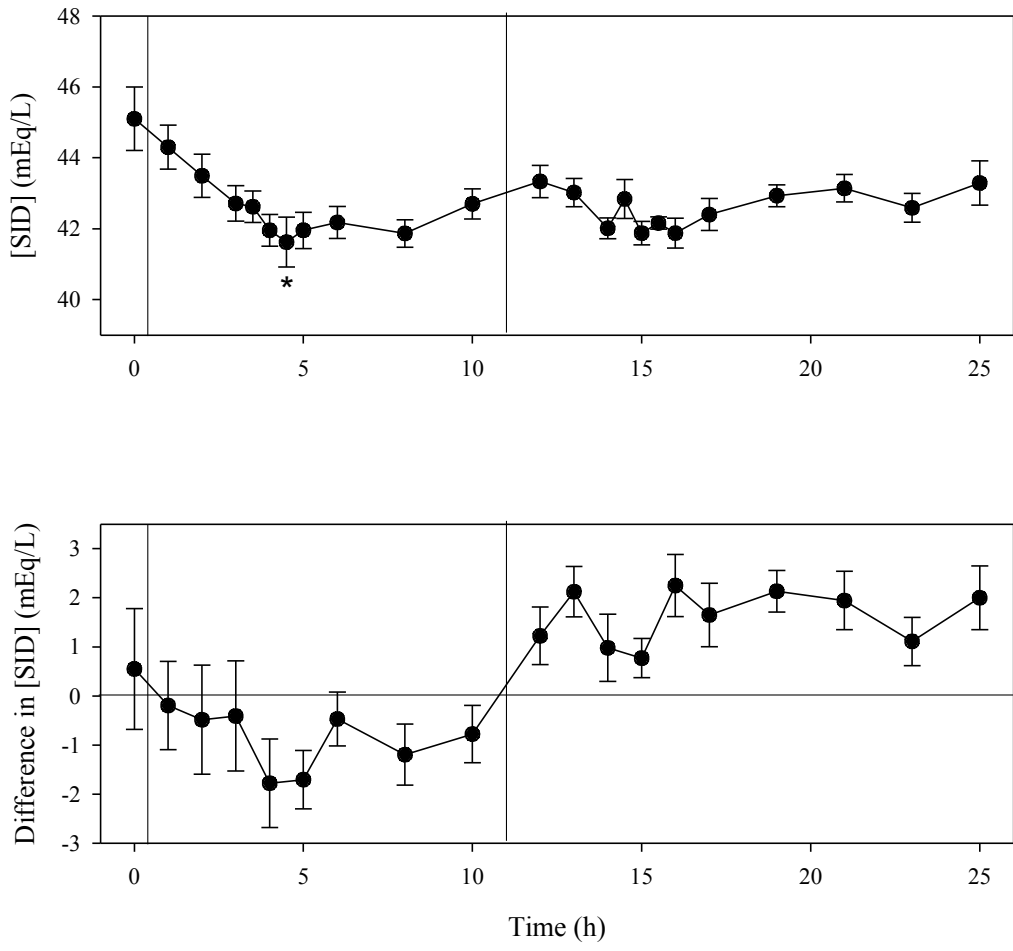


Figure 16. Feed Trial (n=10).
 Top: Mean (\pm SE) concentration of the strong ion difference ([SID]) over 25-h.
 Bottom: Difference in [SID] between FT and DVT.
 *Indicates significantly different from t=0-h, $p < 0.05$.
 Vertical lines at t = 0.5 and 11-h represent feeding times.

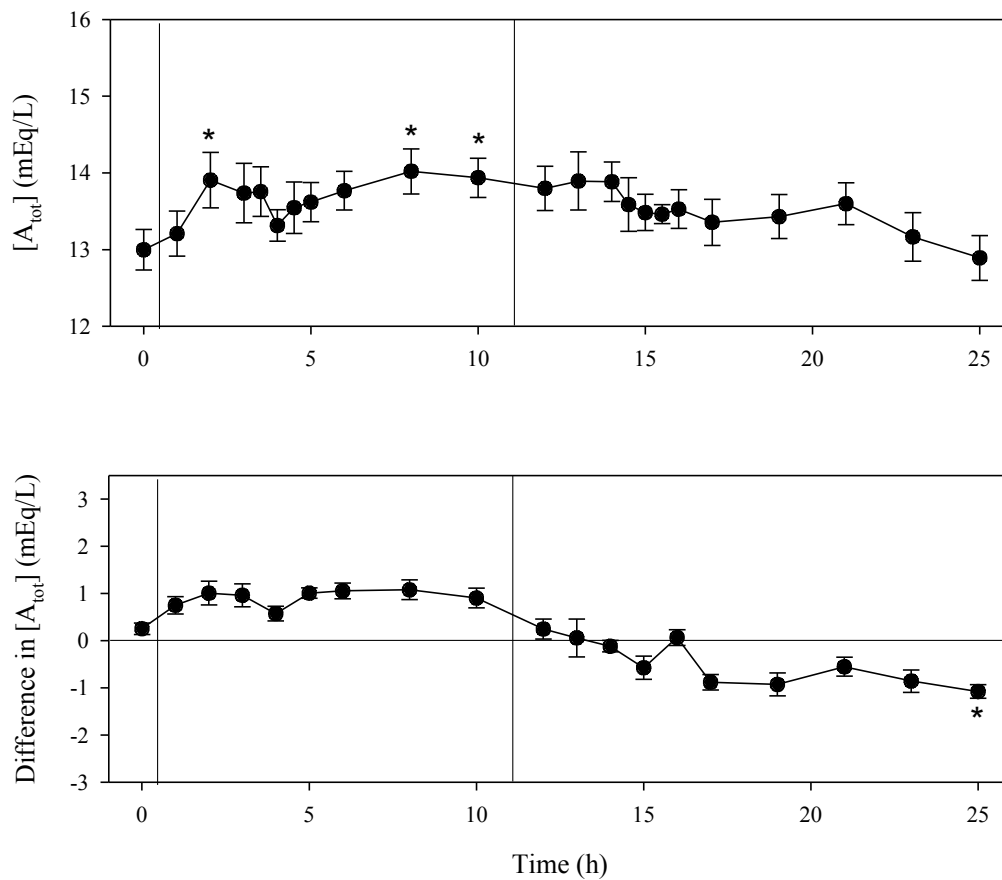


Figure 17. Feed Trial (n=10).

Top: Mean (\pm SE) concentrations of the total concentration of weak acids and bases ($[A_{tot}]$) over 25-h with feed.

Bottom: Difference in mean (\pm SE) total concentration of $[A_{tot}]$ between FT and DVT.

*Indicates significantly different from t = 0-h, p<0.05.

Vertical lines at t = 0.5 and 11-h represent feeding times.

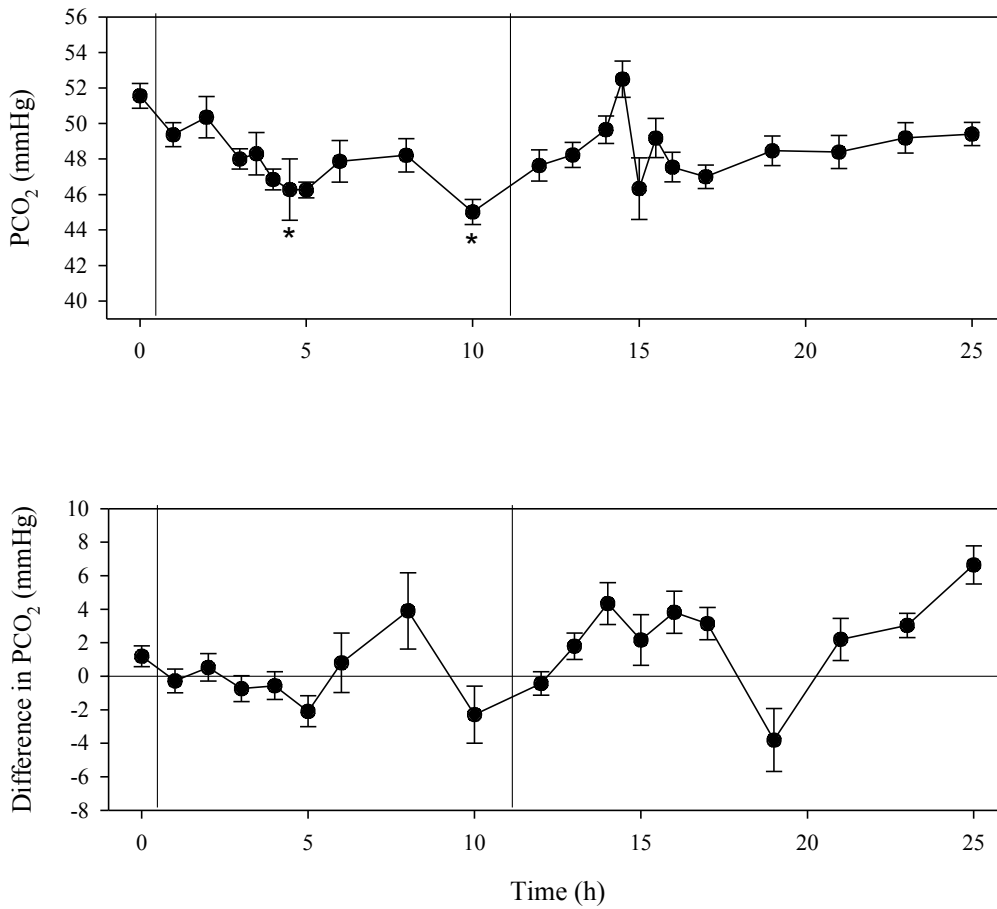


Figure 18. Feed Trial (n=10).

Top: Mean (\pm SE) Partial pressure of carbon dioxide (PCO₂) over 25-h with feed.

Bottom: Mean (\pm SE) difference in partial pressure of carbon dioxide between FT and DVT.

*Indicates significantly different from t=0-h, p<0.05.

Vertical lines at t = 0.5 and 11-h represent feeding times.

Acid-base dependent variables:

The FT data for pH, HCO_3^- , $[\text{H}^+]$ and $[\text{TCO}_2]$ in groups 1 and 2 were not combined into a single group as the two groups were found to be significantly different.

Plasma pH (Figure 19)

Group 1: The initial pH was 7.43 ± 0.02 . At 3 and 4-h the pH decreased to 7.39 ± 0.01 and 7.37 ± 0.01 respectively. There was also a decrease at 3 and 4-h in the FT as compared to the DVT and at 4-h the mean difference between the studies was decreased as compared to the initial value.

Group 2: The initial pH was 7.41 ± 0.01 . pH decreased between 4.5 to 8-h and again at 13 and 14-h. A low of 7.33 ± 0.01 occurred at 8-h. The range between the highest and lowest pH was 0.08. There was a decrease in pH in the FT as compared to the DVT at 6 and 8-h and increases, as well as an increase from the initial mean difference, at 10, 16 and 17-h.

Plasma bicarbonate concentration (Figure 20)

Group 1: There was a decrease at 3 and 4-h in $[\text{HCO}_3^-]$, to 28.5 ± 0.5 mEq/L and 27.0 ± 0.5 mEq/L respectively, from the initial value of 32.2 ± 0.1 mEq/L. The FT $[\text{HCO}_3^-]$ was decreased as compared to the DVT at $t = 3, 4$ and 6-h, but were similar for the rest of the study.

Group 2: There was a significant decrease between 3.5 to 10-h, and at 13, 16, and 17 to 19-h when compared to the initial value of 32.9 ± 0.1 mEq/L. Mean

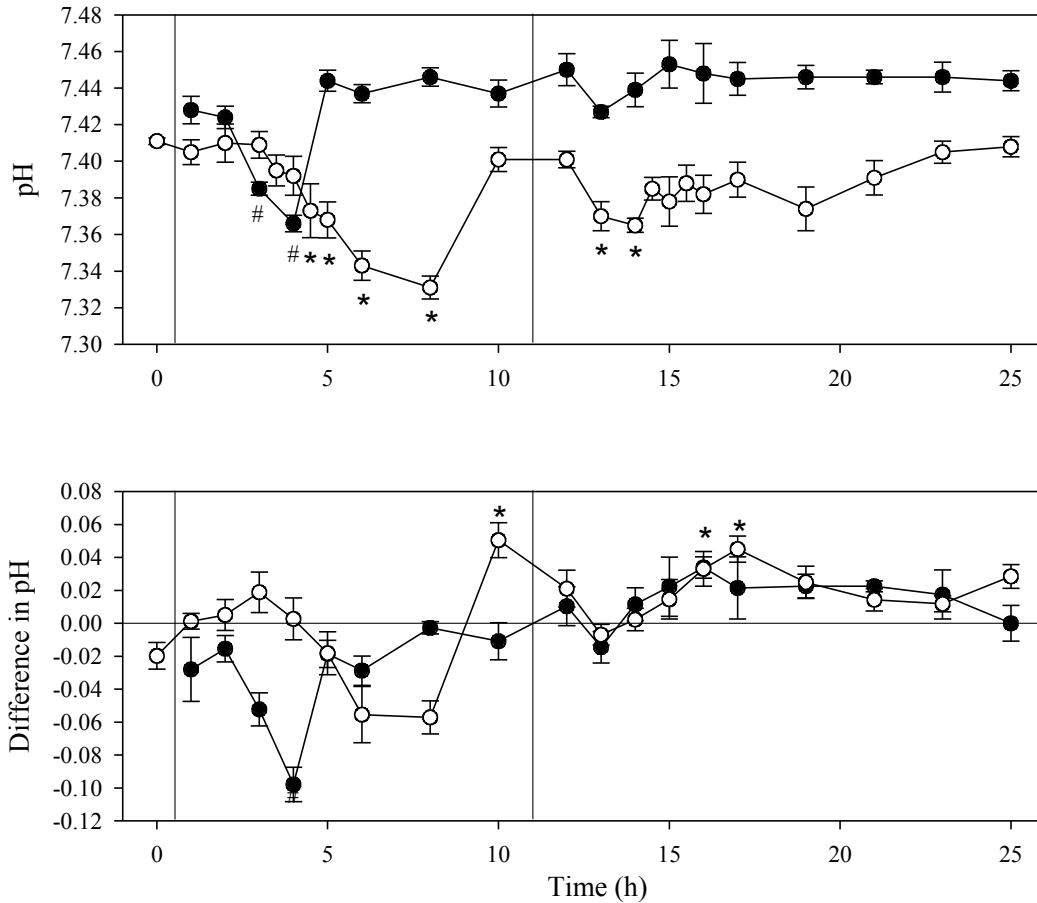


Figure 19. Feed Trial.

Top: Mean (\pm SE) pH, over 25-h with feed.

Bottom: Difference in mean (\pm SE) pH between FT and DVT.

Solid circles represent group 1 (n=5) and empty circles represent group 2 (n=5).

* Indicates a significant difference from t = 0-h, p<0.050.

Indicates a significant difference from t = 1-h, p<0.050.

Vertical lines at t = 0.5 and 11-h represent feeding times.

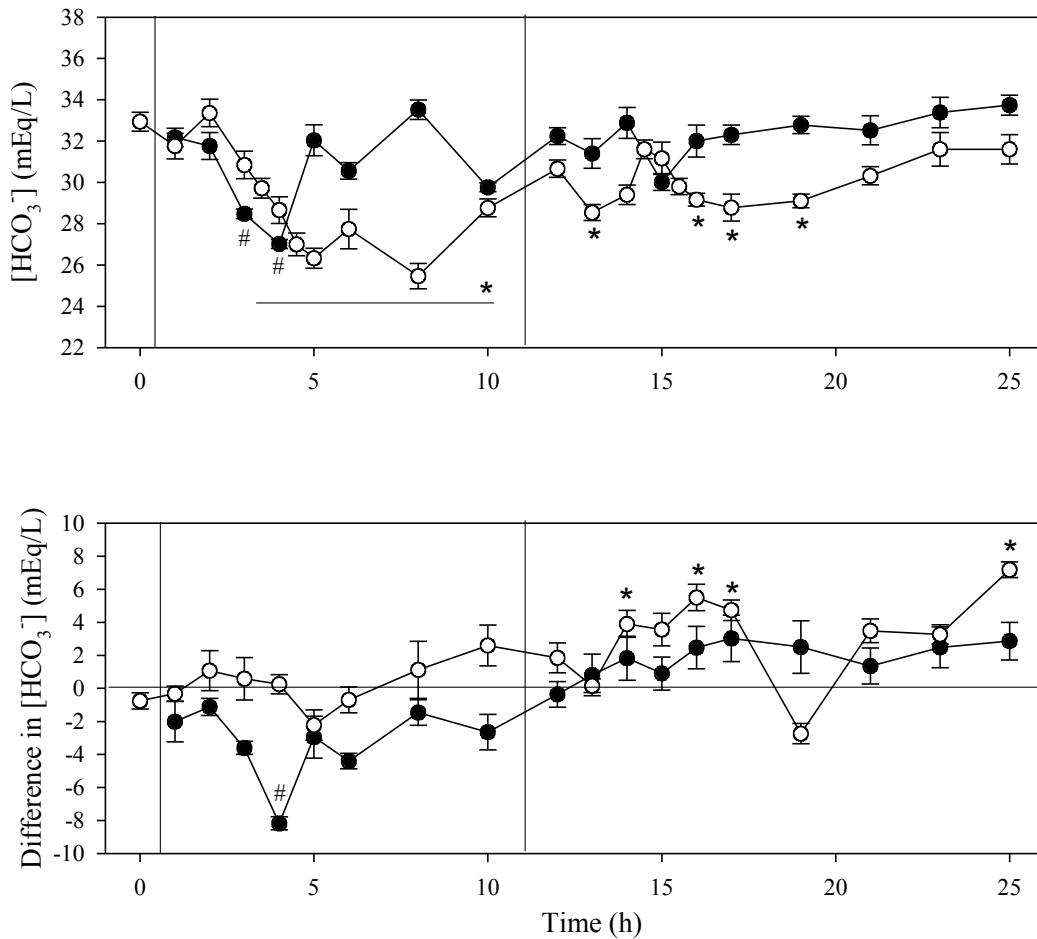


Figure 20. Feed Trial.

Top: Mean (\pm SE) concentration of bicarbonate ($[HCO_3^-]$) over 25-h.

Bottom: Mean (\pm SE) difference in $[HCO_3^-]$ between FT and DVT.

Solid circles represent group 1 (n=5) and empty circles represent group 2 (n=5).

* Indicates significantly different from t = 0-h, p<0.05.

Indicates significantly different from t = 1-h, p<0.05.

Vertical lines at t = 0.5-h and 11-h represent feeding times.

[HCO₃⁻] reached a low of 25.5 ± 1.4 mEq/L at 8-h, a range of 7.4 mEq/L from 0-h. FT [HCO₃⁻] was increased at 14, 16, 17 and 25-h as compared to DVT means and was also increased above the initial mean difference at those times.

Hydrogen ion concentration: (Figure 21)

Group 1: [H⁺] increased from the initial value of 37.3 ± 1.4 nEq/L to 41.2 ± 0.7 and 43.1 ± 1.0 nEq/L at 3 and 4-h respectively. The [H⁺] of the FT horses were also increased at 3 and 4-h above the DVT and showed an increase above the initial mean difference at 4-h as well.

Group 2: There was a significant increase in [H⁺] at 4.5 through 8-h and at 13 and 14-h, compared to the initial value of 38.8 ± 1.4 nEq/L. The high of 46.7 ± 1.5 nEq/L occurred at 8-h. The range between high and low concentrations were 7.9 nEq/L. At 6 and 7-h there was an increase and at 10 and 17-h a decrease in [H⁺] in the FT as compared to the DVT. There was also a decrease below the initial mean difference between the studies at 10 and 17-h.

Contributions to changes in hydrogen ion concentration: (Figure 22)

Group 1: The decrease in [SID] (figure 16 top) had the greatest contribution to the increase in [H⁺] at 3 and 4-h, although its contribution was not considered significant. Neither [A_{tot}] (figure 17 top) nor PCO₂ (figure 18 top) contributed to this increase. The potential contribution to a decreased [H⁺] from a decrease in PCO₂ at 10 and 15-h did not have an effect on [H⁺].

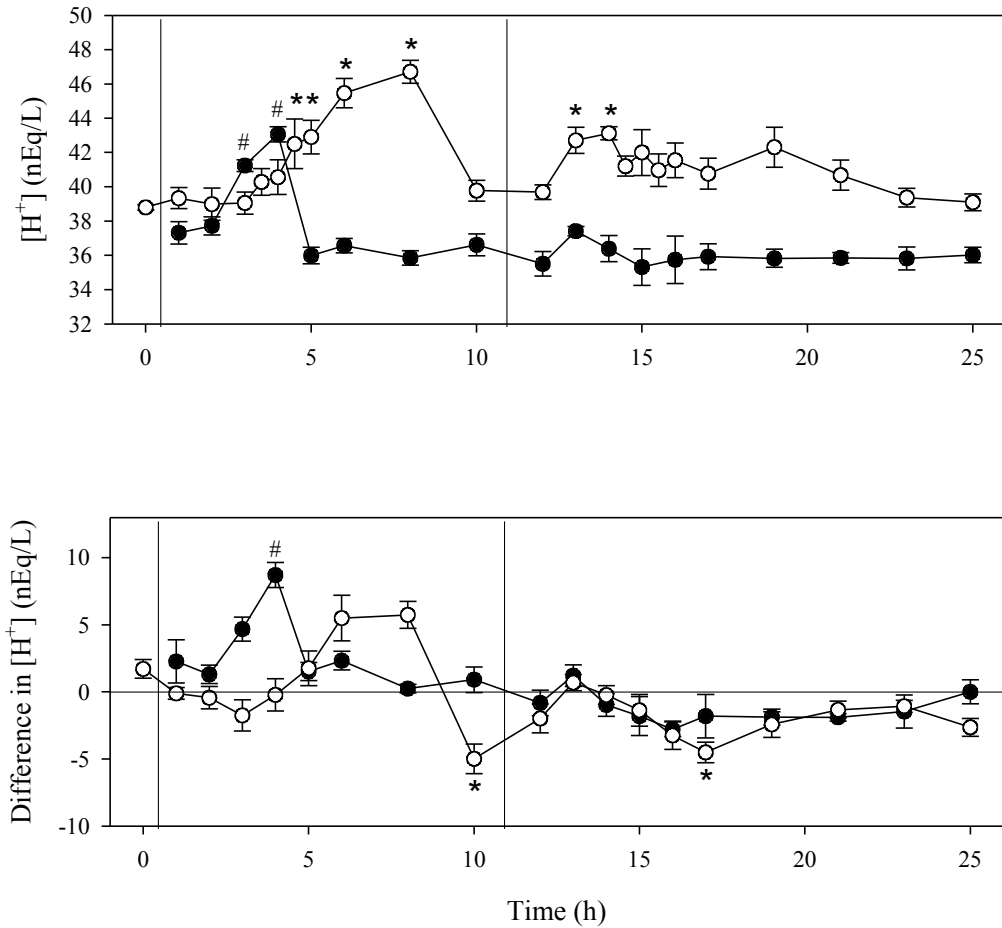


Figure 21. Feed Trial.

Top: Mean (\pm SE) hydrogen ion concentration ($[H^+]$) over 25-h.

Bottom: Difference in mean $[H^+]$ between FT and DVT.

Solid circles represent group 1 (n=5) and empty circles represent group 2 (n=5).

* Indicates significantly different from t = 0-h, p<0.05.

Indicates significantly different from t = 1-h, p<0.05.

Vertical lines at t = 0.5 and 11-h represent feeding times.

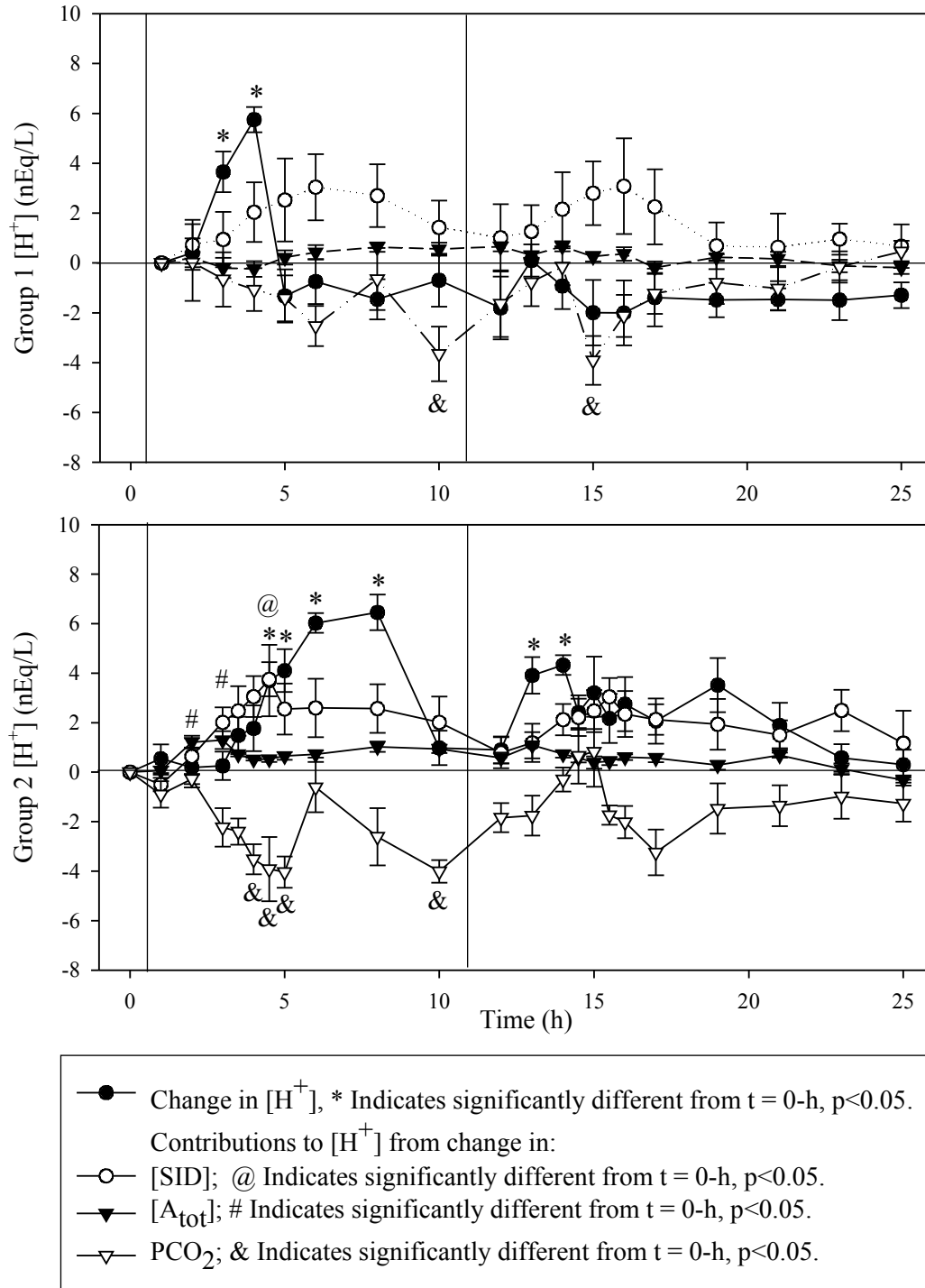


Figure 22. Feed Trial. Change in hydrogen ion concentration ($[H^+]$) and origins in change in $[H^+]$ in each group from changes in the three independent variables, [SID], $[A_{tot}]$ and PCO_2 . Top represents group 1 (n=5) and bottom represents group 2 (n=5). Vertical lines at t = 0.5-h and 11-h represent feeding times.

Group 2: The greatest contribution to the initial increase in $[H^+]$ was $[SID]$ and then $[A_{tot}]$. However, after 12 pm and following the evening feed, the independent variables did not account for the increase in $[H^+]$.

Total carbon dioxide concentration: (Figure 23)

Group 1: The initial $[TCO_2]$ was 33.6 ± 1.0 mmol/L, which decreased to 30.0 ± 0.5 mmol/L at 3-h and 28.5 ± 0.5 mmol/L at 4-h. The remainder of the trial did not differ from 0-h. The FT $[TCO_2]$ decreased when compared to the DVT at 4-h.

Group 2: The initial $[TCO_2]$ was $34.6 + 1.0$ mmol/L, which decreased at 3.5, 4 through 10, 12, 14, 16, 17 and 18-h. The $[TCO_2]$ low of 26.9 ± 1.5 mmol/L was at 8-h decreasing 6.7 mmol/L from the initial value. There was an increase at 14 to 17 and 21 to 25-h in the FT compared to the DVT $[TCO_2]$.

Contributions to changes in total carbon dioxide concentration: (Figure 24)

Group 1. The decrease in $[TCO_2]$ at 3 and 4-h was not accounted for by any changes in $[SID]$ (figure 16), $[A_{tot}]$ (figure 17) or PCO_2 (figure 18). Although it wasn't significant, numerically $[SID]$ provided the greatest contribution to decreased $[TCO_2]$ at 3 and 4-h. Decreases in $[TCO_2]$ from PCO_2 at 10 and 15-h did not significantly affect $[TCO_2]$.

Group 2. The decrease in $[TCO_2]$ was only partially accounted for from the contributions of $[SID]$ (figure 16), $[A_{tot}]$ (figure 17) and PCO_2 (figure 18). $[SID]$, again, provided the greatest contribution numerically, but that was only significant at 4.5-h. $[A_{tot}]$ contributed significantly at 2-h and PCO_2 at 4.5, 5 and 10-h.

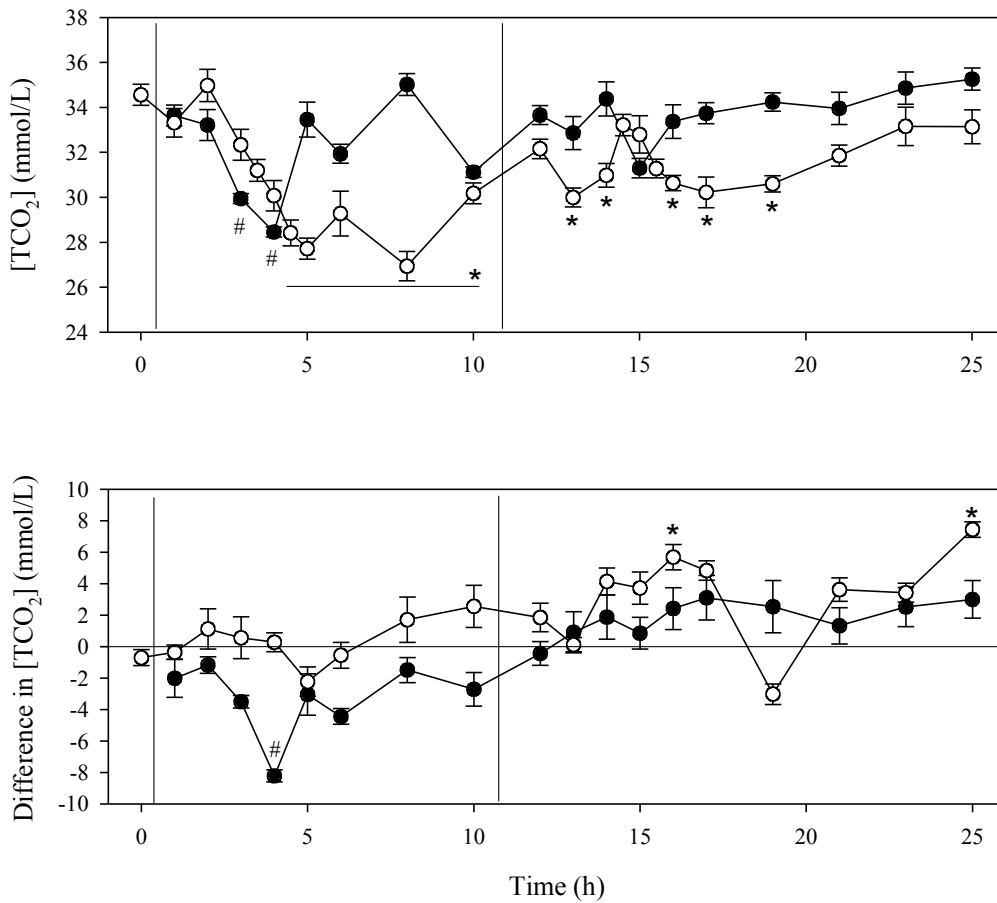


Figure 23. Feed Trial.

Top: Mean (\pm SE) total carbon dioxide concentration ([TCO₂]) over 25-h.

Bottom: Differences in mean (\pm SE) [TCO₂] between FT and DVT.

Solid circles represent group 1 (n=5) and empty circles represent group 2 (n=5)

* Indicates significant change from t = 0-h, p<0.05.

Indicates significant change from t = 1-h, p<0.05.

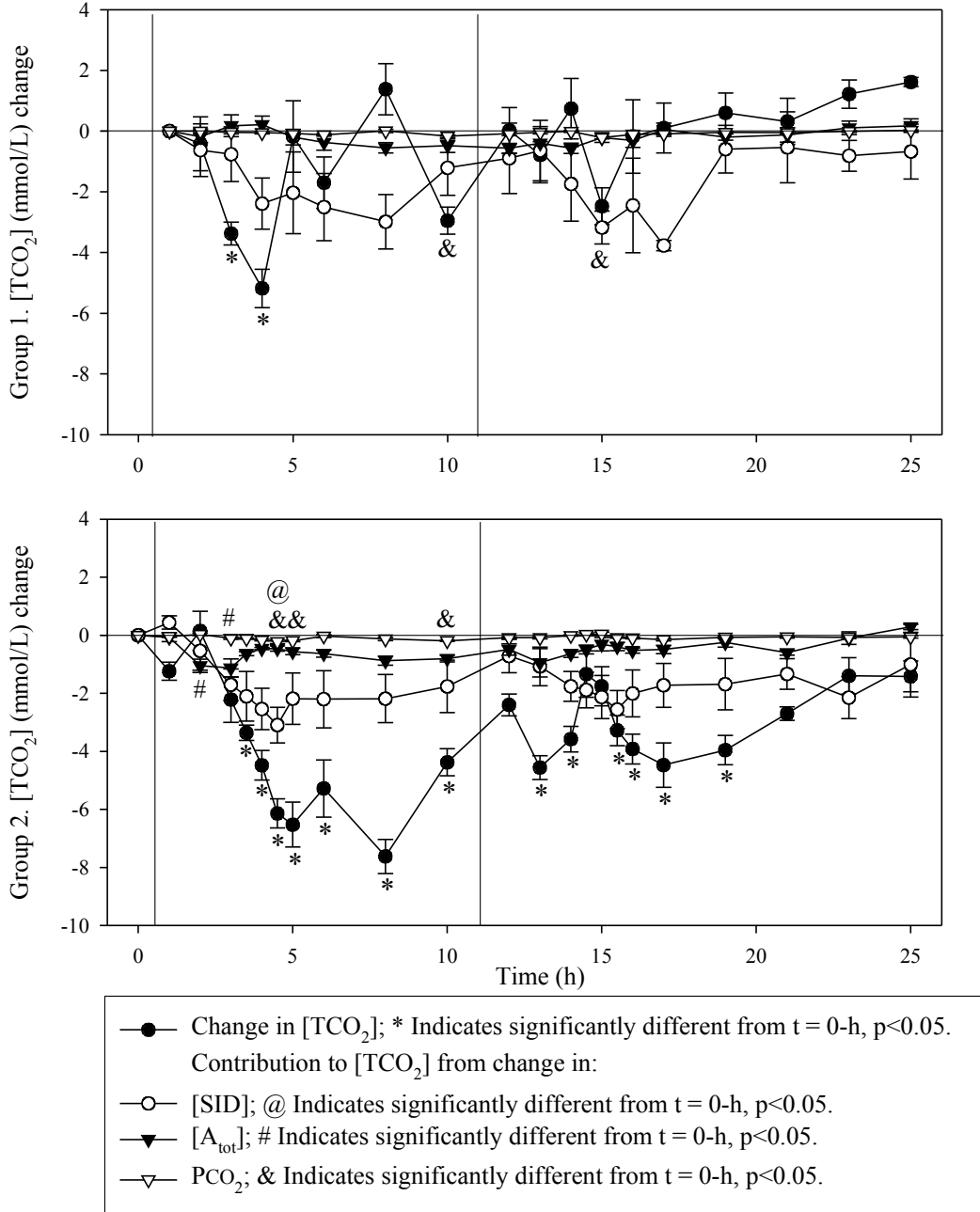


Figure 24. Feed Trial.

Change in total carbon dioxide concentration ([TCO₂]) and origins in change in [TCO₂] in each group from changes in [SID], [A_{tot}] and PCO₂ over 25-h.

Top represents group 1 (n=5) and bottom represents group 2 (n=5).

Vertical lines at t = 0.5-h and 11-h represent feeding times.

CHAPTER IV. DISCUSSION

This study investigated the main electrolyte and acid-base constituents in plasma of fed (FT) and unfed (DVT) resting horses. A physicochemical systems approach was taken to determine the contributions of the acid-base independent variables ($[\text{SID}]$, $[\text{A}_{\text{tot}}]$, PCO_2) to changes in plasma $[\text{H}^+]$ and $[\text{TCO}_2]$ through a 25-h period. The purpose of the research was to investigate effects of feeding and daily variation on equine blood parameters and plasma acid-base status.

Glucose

The mean plasma glucose concentration did not change from 5.2 ± 0.2 mmol/L over the entire 25-h study without feed. These data are similar to Greppi and colleagues (1996) who also found no daily variation of glucose in fasting horses over 24-h. Studies in other animals show that daily variation in plasma glucose concentration is not exhibited in the absence of feed (Hall & Van Ham 1998).

In the FT, the increase in glucose is a response to the morning feeding, but that increase does not occur following the evening feed. The horses first consumed the grain and then ate the hay at a more leisurely pace. The time it took the horses to consume their feed was not monitored, and some hay was still remaining by the evening feed. The lack of a glucose response following the second feed was possibly due to continued consumption of hay throughout the day, decreasing the glucose response from the evening meal. Other studies also report that blood glucose levels typically increase following the initial morning feeding and show lesser increase

following successive feedings (Nielsen et al. 2003; Greppi et al. 1996; Stull & Rodiek 1988).

Fluid Shifts

In the DVT there was an increased $[A_{tot}]$ between 9 pm and midnight, compared to initial values, which may indicate a net loss of fluid from the plasma compartment. This increase in $[A_{tot}]$, resulting from the increase in $[PP]$, over the final 12-h of the study may be indicative of dehydration. Genetzky et al. (1987) conducted a water deprivation test on horses and found $[PP]$ to be a good indicator of dehydration. Although the horses had access to water they consumed less water without feeding than when they ate. They also lost an average mass of 17.6 ± 2.0 kg over the 25-h trial, consisting of about 3.7% of their mean body weight. No measurement of urine or feces was performed, but it is assumed that some weight loss was attributed to both. However, most of the change in body mass was accounted for by the change in extracellular fluid volume (ECFV). There was a decrease of about 10 L, or 10%, of the ECFV, as estimated from the change in $[PP]$.

In the FT following the morning feed, $[A_{tot}]$ increased above pre-feeding levels. Following the evening feeding there was no change from pre-feed values. Jansson and Dahlborn's findings (1999) were in agreement with the 0.6 g/dL increase in $[PP]$ found in this study, creating an increased $[A_{tot}]$ after the morning feed at 9 am. The initial increase 1.5-h post feed was likely due to a fluid shift into the gut due to grain consumption and the later afternoon increase in $[PP]$ was probably due to a fluid shift into the gut from eating hay (Kerr & Snow 1982), which was consumed at a

slower rate than the grain. However, Jansson and Dahlborn's study (1999) also described elevated [PP] following the evening feed, although it was a smaller increase of 0.2 g/dl compared to the morning post-feed increase of 0.4 g/dl.

Electrolytes and the strong ion difference

There were no significant changes over time in $[\text{Na}^+]$, $[\text{K}^+]$ or $[\text{Cl}^-]$ in the DVT, which agrees with Kerr and Snow (1982), who did not find variations in plasma electrolytes in the absence of feed. In the FT $[\text{Cl}^-]$ had a significant change following the morning feed as compared to the initial measurement. Mean $[\text{K}^+]$ decreased post-feedings by ~ 0.7 Eq/L and mean $[\text{Na}^+]$ over the FT fluctuated up to ~ 2 mEq/L, but these variations were not significant.

Studies looking at daily variation of plasma $[\text{Na}^+]$ in horses and other species are not consistent, each exhibiting different levels of variation. Research has shown a daily rhythm with nocturnal increases (Greppi et al. 1996) or decreases (Boning et al. 1974; Lepage et al. 1991), minimal variations (Minematsu et al. 1995; Yashiki et al. 1995) or none at all (Slocombe et al. 1995). Both Kerr and Snow (1982) and Jansson and Dahlborn (1999) reported increased $[\text{Na}^+]$ with the greatest increases following feeding. Studies in various mammals have correlated variations in $[\text{Na}^+]$ to feeding (Greppi et al. 1996; Clarke et al. 1988; Boning et al. 1974; Lepage et al. 1991; Minematsu et al. 1995). Different types of feed, timing of feeding, DCAD levels, age differences, and different breeds and species may also account for these differences between studies.

While plasma $[K^+]$ did not change as compared to 0-h, there was a decreased $[K^+]$ 1.5 to 2-h following both feedings as compared to 0.5 and 1-h post-feed concentrations. At 1.5 to 2-h post-feeding $[K^+]$ was 0.8 (morning) and 0.6 (evening) mEq/L less as compared to their respective 0.5 and 1-h post feeding values. Kerr and Snow (1982) reported a decrease in $[K^+]$ of about 0.4 mEq/L following a large feed. Yashiki and colleagues (1995) showed a decrease of 1.3 mEq/L between 3 am and one hour post-feeding at 7:30 am. Other studies have shown that $[K^+]$ appears to be decreased at night as compared to day (Lepage et al. 1991; Jansson et al. 1999; Yashiki et al. 1995).

Plasma $[Cl^-]$ was increased at 11:30 am, 4-h following the morning, but showed no increase following the evening feed and thus had no further effect on [SID]. Slocombe et al. (1995) found that $[Cl^-]$ peaked in the evening and was lowest in the morning. Other studies have not found an increase in $[Cl^-]$ with feeding or over time (Kerr & Snow 1982; Yashiki et al. 1995). It appears that, in the present study, the $[Cl^-]$ increase could have been response to a morning feeding, as no evening feed response was present. Another possibility is that the $[Cl^-]$ increase may have been related to increased [PP] occurring with a fluid shift due to feeding.

From 7 to 11:30 am during the FT, [SID] fell 3.5 mEq/L due to the decrease in $[Cl^-]$, as $[Na^+]$ and $[K^+]$ showed no variation at this time. Even though no change was found in the individual electrolyte concentrations over time in the DVT, there was a significant decrease in [SID] during the final 12-h period of the trial. The decrease in [SID] indicates that there were underlying changes in electrolyte concentrations that

were not statistically significant. On an individual basis, the decrease in [SID] was due to decreases in [Na⁺] and increases in [Cl⁻] with minimal change in [K⁺] (figure 3).

Partial pressure of carbon dioxide

The decrease in PCO₂ between 9 pm and midnight during the DVT may be accounted for by a respiratory compensation, with increased ventilation, as a response to an acidosis. The increased [H⁺] and accompanying decrease in [HCO₃⁻] in the evening for the group 2 horses, but not group 1 horses, could also be indicative of a mild metabolic acidosis. A common cause of metabolic acidosis in an unfed organism is ketoacidosis (Carlson 1997). Parker and colleagues (2003) found that water and feed deprived steers also had a lower PCO₂ compared to those that were not restricted from water and food. Possible reasons for group differences will be discussed later in the thesis.

During the FT, PCO₂ was decreased at 4-h and 9-h following the morning feed. The initial PCO₂ decrease relates to the digestion of grain and the second decrease to the consumption of hay. This timing follows the fluid shifts from the plasma compartment to the GI tract and it is possible that the acidosis caused by increased [A_{toi}] was compensated by increased ventilation. The initial feeding created a mild acidosis that stimulated a compensatory respiratory response, which then could reduce PCO₂ to observed levels.

In summary, the decrease in PCO₂ and increases in plasma proteins (and thus [A_{toi}]), glucose, and chloride (which decreased [SID]) occurred after the morning feeding. Variation in the independent variables, and the related ions and proteins, was

related to an initial large feed. The extended consumption of hay throughout the day probably mitigated a second response to the large evening meal. In the DVT, variation in the absence of feed may have been linked to dehydration. Although there may have been underlying trends in electrolyte variation, the individual subjects of this study did not appear to share the same cycles. The responses of individual horses would need to be singled out as each horse may have its own daily variation that occurs on its own timing.

Acid Base Analysis

[H⁺]:

The [H⁺] calculated ([H⁺]c) using the physicochemical equation derived by Stewart (1983) was compared to the measured [H⁺] ([H⁺]m) using linear regression analysis (appendix 2.1). Previous exercise studies that have used the physicochemical approach have found agreement between [H⁺]c and [H⁺]m (Putman et al. 2003; Staempfli & Constable 2003; Staempfli et al. 1999; Lindinger et al. 1992; Pieschl et al. 1992). Although this study did not show a strong correlation between the concentrations, they can be taken as a single point from a small change at rest ([H⁺]m = 35-47 nEq/L) as compared to other studies that have a large [H⁺] range due to exercise (33-630 nEq/L). Neither the slopes nor intercepts of the regressions were found to be significantly different.

The regulation of [H⁺] can be quantitatively expressed by the contributions of [SID], [A_{tot}] and PCO₂ (Stewart 1981, 1983), enabling their contributions to [H⁺] to be broken down at each time point in the study (see appendix 3). The independent

variables account for all the variation in $[H^+]$ in the physicochemical approach to acid-base balance. By plotting the contributions of each of the independent variables to the change in $[H^+]$ the relative contributions of $[SID]$, $[A_{tot}]$ and PCO_2 can be illustrated.

For group 1 horses in the DVT there was no change in plasma pH and $[H^+]$ over time (figure 6). While the independent variables were constant for the first 10-h of the trial, from 7 pm onwards there were contributions to $[H^+]$ from the independent variables. Decreased PCO_2 contributed to a decreased $[H^+]$, which countered the increases in $[H^+]$ due to increased $[A_{tot}]$ and decreased $[SID]$, with the result that $[H^+]$ remained unchanged (figure 6 bottom). These data are consistent with the theory that in physiological systems a disturbance to $[H^+]$ caused by an independent variable is usually compensated for by a complementary change in another independent variable (Wilkes 1998). These “complementary changes” in the independent variables work to maintain $[H^+]$ within a normal physiological range (Stewart 1981; Fencl & Leith 1993).

The $[H^+]$ in DVT group 2 horses (figure 7) fluctuated more and were greater as compared to group 1. The initial increases in $[H^+]$ from 10 am to 8 pm were not accompanied by any increase in $[H^+]$ contributed from the independent variables. From 9 pm onwards for the group 2 horses both $[A_{tot}]$ and $[SID]$ contributed to the increased $[H^+]$ but PCO_2 acted to counter the increased $[H^+]$. $[H^+]$ variations were not fully explained by the $[H^+]$ contributions of $[SID]$, $[A_{tot}]$ and PCO_2 . In the FT, group 1 horses had an initial increase in $[H^+]$ following the morning feed that was not supported by the independent variables. Group 2 horses also had increases in $[H^+]$ following both feedings that were not explained by the contributions of $[SID]$, $[A_{tot}]$

and PCO_2 . Decreases in PCO_2 however, did seem to act to decrease the $[H^+]$ back to baseline values in the group 2 horses.

The extent of the increase in $[H^+]$ following feeding does not correspond with Baker and colleagues (1992) who found that horses consuming diets with either a medium or high DCAD experienced a lesser increase in $[H^+]$ 4-h post-feed. They found that $[H^+]$ was higher with the consumption of a low DCAD diet. Baker and colleagues (1993) and McKenzie et al. (2002) also found that plasma $[H^+]$ increased for horses consuming the lower DCAD diets. Stutz and colleagues (1992) found that diets with DCADs from 5 to 327 mEq/L exhibited a maximal increase in $[H^+]$ at 1-h post-feed, with a return to baseline over the following 12-h. The DCAD in this study was considered medium (274 mEq/kg DM), although $[H^+]$ in this study corresponds more closely to the low DCAD results in the Baker and McKenzie studies. It is probable that because the horses consumed the grain first (which had a low DCAD of 73.6 mEq/kg DM) that the initial $[H^+]$ response was due to just eating grain.

[TCO₂]:

The $[TCO_2]$ calculated ($[TCO_2]_c$) using the physicochemical equation derived by Stewart (1983) was compared to the measured $[TCO_2]$ ($[TCO_2]_m$) using linear regression analysis (appendix 2.2). Both the slope ($p=0.003$) and intercept ($p=0.002$) of the regressions were found to be significantly different. The $[TCO_2]_m$ were slightly greater than the $[TCO_2]_c$.

The decrease $[TCO_2]$ at 10 pm and midnight in the DVT group 1 was not accounted for by the independent variables, although $[A_{tot}]$ showed a small contribution (figure

8). The increase in $[A_{\text{tot}}]$ from 8 pm through the rest of the trial (figure 4 middle) also contributed to an apparent decrease in $[\text{TCO}_2]$, although the change in $[\text{TCO}_2]$ did not turn out to be significant. DVT group 2 showed contributions to the decreased $[\text{TCO}_2]$ primarily from $[\text{SID}]$, but $[A_{\text{tot}}]$ and PCO_2 , also contributed (figure 9). Again however, none were of sufficient magnitude to explain the changes in $[\text{TCO}_2]$. In FT group 1 the decrease in $[\text{TCO}_2]$ was not paralleled by any contributions from the independent variables (except a non-significant mathematical contribution from $[\text{SID}]$). The contribution of decreased $[\text{TCO}_2]$ from PCO_2 also did not significantly affect the $[\text{TCO}_2]$ (figure 24). In FT group 2, although $[\text{TCO}_2]$ appears to decrease independently, decreases from 10 am through 5 pm as compared to its initial value, may have been contributed to from an increase in $[A_{\text{tot}}]$ and $[\text{SID}]$ and decrease in PCO_2 . However, the magnitude of its change cannot be explained from the contributions of the independent variables. Decreases in $[\text{TCO}_2]$ from 8 pm through 3 am were not accompanied by any changes in the independent variables. Overall, $[\text{TCO}_2]$ variations could not be fully explained from the contributions of $[\text{SID}]$, $[A_{\text{tot}}]$ and PCO_2 using the physicochemical approach.

The decrease in $[\text{TCO}_2]$ following feeding in the group 2 horses indicates that time of feeding should be taken into account when testing absolute values. TCO_2 consists of about 95% plasma bicarbonate. Baker and colleagues (1993) and McKenzie et al. (2002) also found that plasma $[\text{HCO}_3^-]$ (and thus $[\text{TCO}_2]$) decreased for horses consuming the lower DCAD diets. An interesting component of the study from Stutz et al. (1992) was that the decrease in plasma $[\text{HCO}_3^-]$ seen following both the morning and evening feedings was more pronounced after the morning feed. It is

possible that the increased changes in [TCO₂] following the morning feed may be part of a circadian rhythm. However, it is unknown in this study whether the possibility of continued grazing throughout the day created the decreased response to the evening meal. Both DCAD and timing of feeding are variables that need to be taken into account when looking at the reasons for [TCO₂] variations.

Important [TCO₂] to note are those above 35.0 mmol/L, as a positive TCO₂ test in the Ontario racing jurisdiction is considered at or above 37.0 mmol/L. Group 1 horse means in the DVT were above 35.0 mmol/L for 6-h between 7 am and 3 pm, which then decreased through the evening. Group 2 means started above 35.0 mmol/L but then decreased and showed more variability than group 1. [TCO₂] varied 6.8 mmol/L between 28.5 ± 0.5 to 35.3 ± 1.1 mmol/L in group 1 and 9.1 mmol/L between 26.5 ± 1.5 to 35.6 ± 1.0 mmol/L in group 2 over the study period. Although none of the mean [TCO₂] measurements were above 36.0 mmol/L, 7 of the 10 horses were above 36.0 mmol/L at some point during the study. Although an Australian study by Auer and colleagues (1993) stated that there was less than a 0.001 chance that a horse would have a [TCO₂] greater than 36.0 mmol/L, that was not true of the Standardbred horses in this study. The baseline testing values on horses that were rested and unfed indicate that 37.0 mmol/L may still be inside a “normal” physiological range for [TCO₂] in Standardbred horses in Ontario.

Group Differences:

The reason for different responses between groups in the dependent variables but not in the independent acid-base variables is unknown. One possibility is seasonal differences between the first group tested on October 29 and 31 and the second on December 21 and 23. Although natural light levels were decreased during the second study the internal barn light was kept constant between the studies. Also, the environmental temperature was similar between the studies. Another possibility for the group differences was that there could be a technical and/or methodological error, either from an accumulated measurement error and/or the omission of critical measurements. Alternatively, variations in measurement electrodes on the NOVAstat 9+ may have given imprecise measurements, which were then not accurate enough to determine the contributions of the independent variables (measured on a milliequivalent scale) to the change in $[H^+]$ (expressed on a nanoequivalent scale).

CHAPTER V. CONCLUSIONS AND RECOMMENDATIONS

1. CONCLUSIONS

Daily variation in the measured blood constituents identified in this study was due to either feeding or dehydration. All values were within physiological limits, showing that any circadian rhythm evident in this study was within clinical reference ranges, with the exception of [TCO₂]. Ninety percent of the horses had a [TCO₂] above 35.0 mmol/L at some point during the study and 70% of the horses had a [TCO₂] above 36.0 mmol/L and may have received a positive TCO₂ test result in the Ontario racing industry.

Although no change in electrolytes over the 25-h DVT was found as compared to initial concentrations there was a tendency for the electrolytes to have a nocturnal variation, with decreased [Na⁺] and increased [Cl⁻] as shown by the change in [SID]. The only change in electrolytes when compared to initial concentrations was an increase in [Cl⁻] following the morning feeding. The small change in [Cl⁻] post-feed and the potentially small changes in [Na⁺] and [K⁺] found in this study negate the requirement of time dependent reference ranges for electrolytes for blood testing.

There was also increased [glucose] and [PP] as well as decreased PCO₂ and pH found following the morning feed. The same variations were not present following the evening meal. The discrepancy between morning and evening feeding responses could not be determined to be due to a daily variation as it is possible that the continued digestion of hay over the day attenuated the evening response to feeding.

Theoretically, the independent variables account for all of the changes in $[H^+]$ and $[HCO_3^-]$ (and thus $[TCO_2]$) using the physicochemical approach to acid-base balance. In this study, however, contributions of the independent variables ($[SID]$, $[A_{tot}]$ and PCO_2) were not able to fully account for changes in either $[H^+]$ or $[TCO_2]$ as calculated by the physicochemical equation.

2. RECOMMENDATIONS

There are discrepancies in the literature as to whether equine plasma constituent concentrations vary over a 24-h period. It would be worthwhile to redesign this study to establish whether plasma pH, blood gas and electrolyte concentrations have a daily variation. It would be important to include both fed and unfed horses over the study period as was done in this study. In order to find possible daily variation in electrolytes it would be necessary to accurately measure changes in $[\text{Na}^+]$ and $[\text{Cl}^-]$ of less than 2 mEq/L and $[\text{K}^+]$ to 0.5 mEq/L. As daily rhythms may exist within individuals and each horse may have a rhythm that does not exactly coincide with other horses, this study would require repeated measurements on the same horses over more than one 24-h period. Maintaining hydration of the horses during a non-feed trial is an important consideration. These results could establish reference ranges at specific times of day for blood testing results and increase our understanding of equine physiology.

3. INDUSTRY RELEVANCE

TCO₂ testing is used by the Ontario horse racing community as one of a number of tests to establish whether a horse has been administered an illegal substance. It measures CO₂ and bicarbonate concentrations present in equine plasma. However, as CO₂ occurs naturally in the bloodstream it is difficult to determine whether a horse has naturally high bicarbonate levels or if an enhancer, such as sodium bicarbonate, has been used (called “milkshaking”). A horse on frusemide (lasix or salix) is allowed a [TCO₂] of 39.0 mmol/L, otherwise a positive test is 37.0 mmol/L. Current testing procedures may be prejudiced against horses with naturally high blood bicarbonate concentrations.

It is therefore important to establish correct [TCO₂] reference ranges specific to Ontario racehorses. In this study there were [TCO₂] variations of up to 10 mmol/L over the day, with 9 of the 10 Standardbred horses over 35.0 mmol/L at some point in the study. Current reference ranges used for TCO₂ testing by the Ontario Racing Commission need to be examined.

There is also an increasing amount of evidence that high DCAD diets are beneficial to the overall health of a horse. Studies also show that a high DCAD diet will create a metabolic acidosis. Most racehorses are currently fed high grain diets, typically with a low DCAD. This type of feeding regime may be causing a metabolic acidosis in the racehorses and creating artificially low plasma bicarbonate concentrations. Thus horses being fed a potentially healthier, high DCAD diet may be unfairly penalized.

Possibilities accounting for the differences in [TCO₂] between various studies could be due to breed differences, type of feed and/or timing of feedings and exercise compared to timing of the TCO₂ test. Differences in measurement methodologies also need to be considered. Breed specific testing of [TCO₂] within the geography of each racing jurisdiction on a greater number of horses would also be necessary to generate new reference ranges for [TCO₂]. At this time there is a requirement for more data to develop a more definitive testing procedure to detect “milkshaking”.

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APPENDIX 1.1 Dietary cation/anion difference (DCAD) analysis and forage analysis report on pellet feed used during study.
 Prepared by Central Lab Services, Strathroy, ON. Prepared: 01 Apr 03.

Sample I.D.: FEED SAMPLE (PELLETS)

Parameters	Instrument	Units	As Is Basis	D. M. Basis
Moisture	Calculation	%	13.9	0.0
Dry Matter	F.S. ISOTEMP 655-F	%	86.1	100.0
Crude Protein	Protein - combustion	%	15.1	17.5
Acid Detergent Fiber	Ankom 200	%	9.2	10.7
Neutral Detergent Fiber	Ankom 200	%	19.3	22.4
NDF/ADF	Calculation		2.10	2.10
Calcium	Leeman Labs PS 1000 UV	%	0.50	0.58
Phosphorous	Leeman Labs PS 1000 UV	%	0.50	0.58
Magnesium	Leeman Labs PS 1000 UV	%	0.23	0.27
Potassium	Leeman Labs PS 1000 UV	%	0.75	0.87
Sodium	Leeman Labs PS 1000 UV	%	0.28	0.33
Sulfur	Leeman Labs PS 1000 UV	%	0.2	0.2
Chloride	PC-Titrate	%	0.89	1.03
pH	Fisher Accumet		6.0	6.0

Parameters	Ionic Charge	Units
Dry Matter	--	
Sodium	141.4	mEq/kg
Potassium	223.4	mEq/kg
Chloride	-291.2	mEq/kg
Sulfur	-232.3	mEq/kg
DCAD (Na ⁺ + K ⁺) – (Cl ⁻ + SO ₄ ²⁻)	-158.7	mEq/kg
DCAD (Na ⁺ + K ⁺) – (Cl ⁻)	73.6	mEq/kg

APPENDIX 1.2 Dietary cation/anion difference (DCAD) analysis and forage analysis report on hay forage used during study.
 Details: Mostly Grass. Prepared by Central Lab Services, Strathroy, ON. Prepared: 01 Apr 03

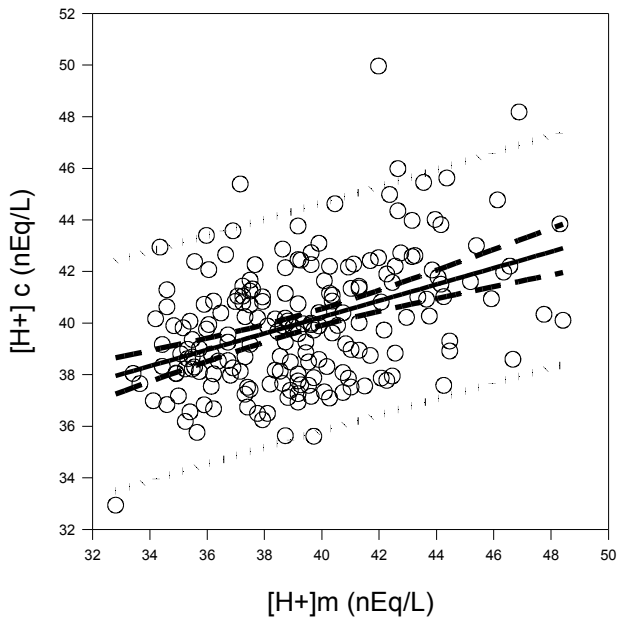
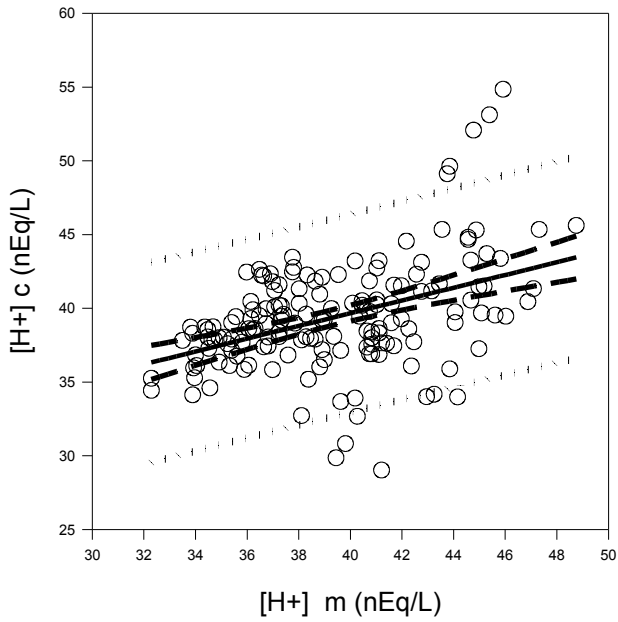
Parameters	Instrument	Units	As Is Basis	D. M. Basis
Moisture	Calculation	%	15.0	0.0
Dry Matter	F.S. ISOTEMP 655-F	%	85.0	100.0
Crude Protein	Protein - combustion	%	15.8	18.6
Acid Detergent Fiber	Ankom 200	%	30.3	35.6
Neutral Detergent Fiber	Ankom 200	%	36.8	43.4
NDF/ADF	Calculation		1.22	1.22
Total Digestible Nutrients	Calculation	%	49.7	58.5
Net Energy of Maintenance	Calculation	MCal/kg	1.07	1.26
Calcium	Leeman Labs PS 1000 UV	%	0.96	1.13
Phosphorous	Leeman Labs PS 1000 UV	%	0.29	0.34
Magnesium	Leeman Labs PS 1000 UV	%	0.25	0.29
Potassium	Leeman Labs PS 1000 UV	%	2.28	2.68
Relative Feed Value	Calculation		131.2	131.2
Sodium	Leeman Labs PS 1000 UV	%	0.04	0.05
Sulfur	Leeman Labs PS 1000 UV	%	0.1	0.1
Chloride	PC-Titrate	%	0.46	0.55
pH	Fisher Accumet		5.6	5.6

Parameters	Ionic Charge	Units
Dry Matter	--	
Sodium	20.5	mEq/kg
Potassium	687.8	mEq/kg
Chloride	-152.4	mEq/kg
Sulfur	-191.2	mEq/kg
DCAD (Na ⁺ + K ⁺) – (Cl ⁻ + SO ₄ ²⁻)	364.6	mEq/kg
DCAD (Na ⁺ + K ⁺) – (Cl ⁻)	555.9	mEq/kg

APPENDIX 1.3 Dietary cation/anion difference (DCAD) analysis and forage analysis report on hay forage used during study.
 Details: Mixed. Prepared by Central Lab Services, Strathroy, ON. Prepared: 01 Apr. 03.

Parameters	Instrument	Units	As Is Basis	D. M. Basis
Moisture	Calculation	%	17.3	0.0
Dry Matter	F.S. ISOTEMP 655-F	%	82.7	100.0
Crude Protein	Protein - combustion	%	14.5	17.5
Acid Detergent Fiber	Ankom 200	%	32.5	39.3
Neutral Detergent Fiber	Ankom 200	%	50.7	61.3
NDF/ADF	Calculation		1.56	1.56
Total Digestible Nutrients	Calculation	%	44.8	54.2
Net Energy of Maintenance	Calculation	MCal/kg	0.92	1.11
Calcium	Leeman Labs PS 1000 UV	%	0.57	0.69
Phosphorous	Leeman Labs PS 1000 UV	%	0.34	0.41
Magnesium	Leeman Labs PS 1000 UV	%	0.23	0.28
Potassium	Leeman Labs PS 1000 UV	%	2.35	2.84
Relative Feed Value	Calculation		88.4	88.4
Sodium	Leeman Labs PS 1000 UV	%	0.05	0.06
Sulfur	Leeman Labs PS 1000 UV	%	0.1	0.2
Chloride	PC-Titrate	%	1.06	1.29
pH	Fisher Accumet		6.6	6.6

Parameters	Ionic Charge	Units
Dry Matter	--	
Sodium	141.4	mEq/kg
Potassium	223.4	mEq/kg
Chloride	-291.2	mEq/kg
Sulfur	-232.3	mEq/kg
DCAD (Na ⁺ + K ⁺) – (Cl ⁻ + SO ₄ ⁻)	-158.7	mEq/kg
DCAD (Na ⁺ + K ⁺) – (Cl ⁻)	73.6	mEq/kg



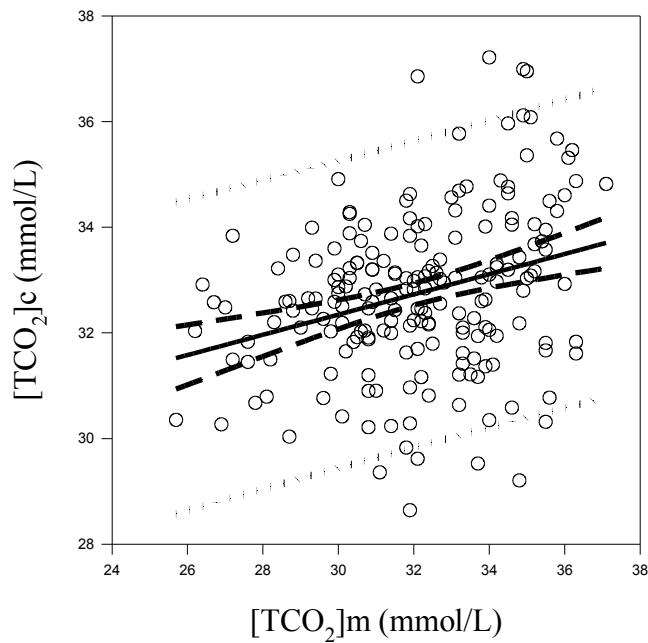
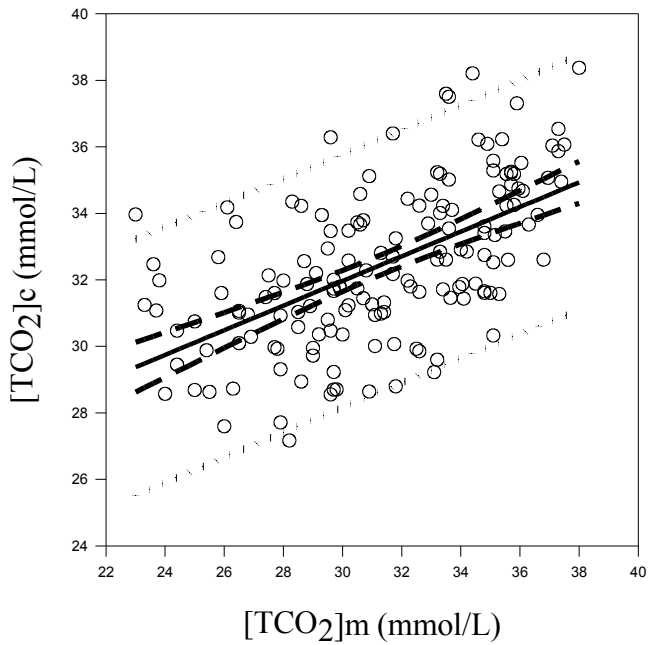
APPENDIX 2.1 Measured $[H^+]$ ($[H^+]_m$) versus calculated $[H^+]$ ($[H^+]_c$) linear regression, with mean (—), 95% confidence intervals (---) and standard deviation (.....) results.

Top: Daily Variation Trial.

$$[H^+]_c = (22.39 \pm 2.90) + ((0.43 \pm 0.07) * [H^+]_m), r^2 = 0.18, p < 0.05$$

Bottom: Feed Trial.

$$[H^+]_c = (27.56 \pm 1.93) + ((0.32 \pm 0.04) * [H^+]_m), r^2 = 0.18, p < 0.05$$



APPENDIX 2.2 Measured $[\text{TCO}_2]$ ($[\text{TCO}_2]_m$) versus calculated $[\text{TCO}_2]$ ($[\text{TCO}_2]_c$) linear regression, with mean (—), 95% confidence intervals (--) and standard deviation (····) results.
 Top: Daily Variation Trial.

$$[\text{TCO}_2]_c = (20.85 \pm 1.32) + ((0.37 \pm 0.04) * [\text{TCO}_2]_m), r^2 = 0.33, p < 0.05$$

Bottom: Feed Trial.

$$[\text{TCO}_2]_c = (26.61 \pm 1.40) + ((0.19 \pm 0.19) * [\text{TCO}_2]_m), r^2 = 0.09, p < 0.05$$

Daily Variation Trial: Group 1				Daily Variation Trial: Group 2				Feed Trial: Group 1				Feed Trial: Group 2			
Time (h)	[H ⁺ sum]	[H ⁺]m	Difference	Time (h)	[H ⁺ sum]	[H ⁺]m	Difference	Time (h)	[H ⁺ sum]	[H ⁺]m	Difference	Time (h)	[H ⁺ sum]	[H ⁺]m	Difference
0	0	0	0	0	0	0	0	0	--	--	--	0	0	0	0
1	-3.81	-2.11	1.70	1	0.09	2.36	2.27	1	0	0	0	1	-1.35	0.54	1.88
2	-2.44	-0.36	2.09	2	0.67	1.12	0.44	2	0.95	0.41	-0.54	2	1.57	0.19	-1.38
3	-3.18	-0.83	2.35	3	1.10	3.72	2.62	3	0.08	3.66	3.58	3	1.07	0.25	-0.81
4	-2.29	-2.37	-0.08	4	-1.46	3.70	5.16	4	0.72	5.75	5.03	3.5	0.79	1.49	0.70
5	-2.28	-2.28	0	5	-0.52	4.06	4.58	5	1.32	-1.33	-2.64	4	0.05	1.76	1.72
6	-0.39	2.52	-2.14	6	-1.96	3.17	5.13	6	0.93	-0.74	-1.68	4.5	0.35	3.70	3.32
8	-0.56	-1.31	-0.75	8	-0.10	3.87	4.87	8	2.69	-1.46	-4.15	5	-0.84	4.10	4.92
10	-2.73	-1.36	1.37	10	2.17	4.77	2.60	10	-1.67	-0.70	0.97	6	2.71	6.03	3.32
12	-0.51	-0.69	-0.18	12	1.55	4.84	3.29	12	0.03	-1.80	-1.83	8	0.98	6.45	5.48
13	-1.58	-0.90	0.68	13	3.66	4.93	1.27	13	0.80	0.10	-0.69	10	-1.07	0.98	2.04
14	0.86	-0.07	-0.93	14	1.03	6.27	5.23	14	2.67	-0.92	-3.59	12	-0.49	0.89	1.38
15	-1.87	-0.58	1.30	15	3.49	6.29	2.80	15	-0.86	-2.00	-1.13	13	0.52	3.91	3.39
16	--	-0.12	--	16	-0.30	7.70	7.99	16	1.32	-2.01	-3.32	14	2.54	4.32	1.78
17	-0.13	0.47	0.60	17	1.89	9.15	7.26	17	0.84	-1.39	-2.23	14.5	3.44	2.41	-1.03
19	1.40	0.46	-0.94	19	--	7.65	--	19	0.13	-1.49	-1.61	15	3.68	3.20	-0.48
21	2.25	0.91	-1.34	21	1.34	4.93	3.59	21	-0.24	-1.47	-1.23	15.5	1.74	2.17	0.43
23	1.36	0.64	-0.73	23	1.23	3.36	2.13	23	0.67	-1.50	-2.17	16	0.93	2.75	1.82
25	0.31	-0.78	-1.09	25	-2.75	4.65	7.41	25	0.94	-1.29	-2.24	17	-0.55	2.06	2.61
												19	0.74	3.51	2.77
												21	0.83	1.88	1.06
												23	1.63	0.57	-1.06
												25	-0.42	0.30	0.72

APPENDIX 3.1 Difference in hydrogen ion concentration ([H⁺]) (nEq/L) between the measured [H⁺] ([H⁺]m) (nEq/L) and the [H⁺] calculated from the means of the independent variables, [SID], [A_{tot}] and PCO₂ ([H⁺ sum]) (nEq/L) for the DVT and FT.

Daily Variation Trial: Group 1			Daily Variation Trial: Group 2			Feed Trial: Group 1			Feed Trial: Group 2		
Time	[TCO ₂]	Differ-	Time	[TCO ₂]	Differ-	Time	[TCO ₂]	Differ-	Time	[TCO ₂]	Differ-

(h)	sum	m	ence	(h)	sum	m	ence	(h)	sum	m	ence	(h)	sum	m	ence
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1.64	0.3	-1.34	1	0.45	-1.58	-2.03	1	0	0	0	1	0.32	-1.24	-1.56
2	1.39	-0.54	-1.93	2	-0.79	-1.23	-0.43	2	-0.81	-0.42	0.39	2	-1.56	0.15	1.71
3	0.63	-1.54	-2.17	3	-1.29	-3.48	-2.19	3	-0.62	-3.38	-2.76	3	-2.94	-2.22	0.72
4	1.20	1.33	0.12	4	-1.44	-5.93	-4.48	4	-2.23	-5.18	-2.95	3.5	-2.85	-3.36	-0.51
5	0.96	1.06	0.10	5	-1.15	-4.86	-3.71	5	-2.30	-0.18	2.12	4	-3.17	-4.48	-1.31
6	-0.85	1.05	1.90	6	-1.77	-6.08	-4.31	6	-3.00	-1.7	1.30	4.5	-3.78	-6.14	-2.36
8	0.42	1.13	0.70	8	-2.39			8	-3.53	1.38	4.91	5	-2.94	-6.525	-3.58
10	0.21	-2.17	-2.38	10	-2.16	-6.75	-4.59	10	-1.85	-2.95	-1.10	6	-2.87	-5.28	-2.41
12	-1.35	-1.3	0.05	12	-2.67	-5.2	-2.53	12	-1.54	0.02	1.56	8	-3.19	-7.62	-4.43
13	-1.87	-2.65	-0.78	13	-4.42	-5.38	-0.96	13	-1.08	-0.78	0.30	10	-2.76	-4.38	-1.62
14	-2.70	-2.11	0.59	14	-4.60	-8.42	-3.82	14	-2.31	0.74	3.05	12	-1.30	-2.4	-1.10
15	-3.02	-4.51	-1.50	15	-4.26	-6.18	-1.92	15	-3.60	-2.48	1.13	13	-2.11	-4.56	-2.45
16	--	-3.28		16	-4.57	-10.3	-5.73	16	-2.86	-0.18	2.69	14	-2.41	-3.58	-1.17
17	-3.31	-4.24	-0.93	17	-5.22	-9.75	-4.53	17	-3.82	0.1	3.92	14.5	-2.35	-1.34	1.01
19	-3.65	-3.3	0.35	19	-5.00			19	-0.83	0.6	1.43	15	-2.40	-1.76	0.64
21	-1.94	-3.09	-1.15	21	-4.21	-7.02	-2.81	21	-0.71	0.32	1.03	15.5	-3.03	-3.28	-0.25
23	-3.50	-3.2	0.30	23	-3.78	-5.52	-1.74	23	-0.72	1.22	1.94	16	-2.63	-3.92	-1.29
25	-3.72	-3.24	1.49	25	-3.73	-9.56	-5.83	25	-0.47	1.62	2.09	17	-2.37	-4.48	-2.10
												19	-2.00	-3.96	-1.96
												21	-2.00	-2.7	-0.70
												23	-2.33	-1.4	0.92
												25	-0.79	-1.42	-0.63

APPENDIX 3.2 Difference in total carbon dioxide concentration ([TCO₂]) (mmol/L) between the measured [TCO₂] ([TCO₂]_m) (mmol/L) and the [TCO₂] calculated from the means of the independent variables, [SID], [A_{tot}] and PCO₂, ([TCO₂] sum) (mmol/L) for the DVT and FT.