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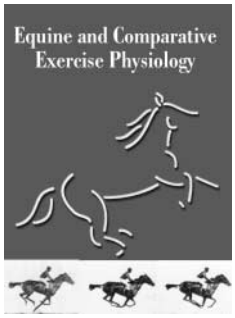

Effect of seven common supplements on plasma electrolyte and total carbon dioxide concentration and strong ion difference in Standardbred horses subjected to a simulated race test

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Abstract

This study used a randomized crossover design, with investigators blind to the treatment given, to test the hypothesis that seven commercially available electrolyte supplements would alter plasma concentrations of Na^+, K^+, Cl^-, lactate, total protein (TP) and total carbon dioxide (tCO_2) as well as plasma strong ion difference (SID) and haematocrit (HCT). Ten unfit Standardbred mares (~450 kg, 4–9 years) completed a series of simulated race exercise tests (SRT) during which venous blood was collected at five sampling intervals (prior to receiving electrolyte treatment, prior to the SRT, immediately following exercise and at 60 and 90 min post-SRT). Plasma electrolyte and tCO_2 concentrations were measured in duplicate using a Beckman EL-ISE electrolyte analyser. No difference ($P > 0.05$) between treatments was detected at any of the five sampling intervals for plasma [Na^+], [K^+], [Cl^-] or [tCO_2]. Similarly, no significant difference was detected between treatments across each of the five sampling intervals for plasma SID, HCT or TP concentration. There were differences ($P < 0.05$) in plasma [Na^+], [K^+] and [tCO_2] (as well as plasma SID, HCT, and TP concentration) in the immediately post-SRT samples that were attributable to the physiological pressures associated with acute exercise. No differences ($P > 0.05$) were detected between treatments across the pre-electrolyte and pre-SRT sampling intervals for plasma lactate concentration. There was, however, a significant time by treatment interaction during the 0, 60 and 90 min post-SRT sampling intervals for this parameter. The electrolyte supplements featured in this investigation did not affect either plasma tCO_2 concentration or SID; however, this result does not rule out the potential for other supplements, especially those containing alkalinizing ingredients, to exert an effect that could push a horse towards threshold values.

Keywords: Electrolyte; exercise; supplements; tCO_2; strong ion difference

Introduction

In recent years, some professional trainers have sought methods to accelerate the commencement of bicarbonate buffering in order to improve the performance of athletic horses[1–7]. The nasogastric administration of sodium bicarbonate to horses, 'milkshaking' as the practice is termed in industry vernacular, is just one of several alkalinization procedures forbidden by racing commissions throughout the world[1–7]. Not only does the use of such agents jeopardize the physical health and welfare of athletic horses[7–9], but also threatens the integrity of the sports in which they participate[2–7].

One major concern for the racing industry has been the impact of substances that have the potential to exert an effect upon strong ion difference and thus acid–base status[10,11]. Such substances include a large number of electrolyte supplements given to horses to counter the loss of key salts in sweat following intense exercise[10,11]. Electrolyte supplements are mixtures of essential physiological salts commonly administered to horses for the purpose of replacing inorganic sweat losses[10]. Due to the fact that electrolyte supplements contain strong ions naturally present in horse plasma (namely, sodium, potassium and

chloride), the question exists (New Jersey Racing Commission *vs* Campbell, 1997) as to whether or not such products significantly affect internal acid–base balance in accordance with Stewart's quantitative theory[12,13]. The ionic character of electrolyte supplements suggests their potential to alter plasma SID and thus bicarbonate concentration as a result of the variation in charge equilibrium that may be precipitated via electrolyte administration[10,11,14].

Animals transport more than 90% of all CO_2 produced in metabolically active tissue to the lungs in the form of carbonic acid[15,16]. This chemical species rapidly dissociates into bicarbonate and hydrogen ions in the presence of an aqueous environment and thus renders tCO_2 an effective measure of bicarbonate concentration within blood plasma[15–21]. The purpose of this investigation was to determine the effect(s) of seven commercially available electrolyte supplements upon plasma tCO_2 levels and SID in Standardbred horses at rest and following exercise. It was hypothesized that the provision of electrolyte supplements would elicit an effect upon plasma SID and tCO_2 concentrations. Empirical tCO_2 measurements were compared to calculated values of SID in order to correlate (or substantiate the absence of correlation between) any significant variation in tCO_2 and the administration of electrolyte supplements. Concomitant changes in tCO_2 and SID would suggest a significant effect of electrolyte supplements upon the acid–base milieu of the performance horse, a finding to wield considerable impact not only upon Standardbred racing but also upon other equine athletic industries worldwide.

Materials and methods

Animals

Ten clinically healthy, unfit Standardbred mares (~450 kg, 4–9 years old) were used in this study. All horses were trained to run on a high-speed equine treadmill (Sato I, Equine Dynamics, Lexington, KY) and familiarized with equipment present in the Equine Science Center's exercise physiology laboratory prior to beginning the experiment. The mares were housed as a group on pasture, fed approximately 12 kg horse^{-1} day^{-1} of mixed alfalfa–grass hay and provided with 6 kg horse^{-1} day^{-1} of a commercially available grain diet divided into two feedings per 24-h period. Water and trace-mineral blocks were available *ad libitum*. The Rutgers University Institutional Board for Animal Care and Use approved all methods and procedures employed in this investigation.

General experiment protocol

A crossover design was instituted to ensure that each mare performed a simulated race exercise test (SRT) after receiving either the control treatment or each of the seven electrolyte supplements selected for this investigation. On the day of each test, either the control treatment (60 ml oral dose of water) or one of seven commercially available electrolyte supplements was administered 4 h prior to conducting the SRT. This time period was chosen because it is a similar period to that used by trainers and clinicians because of other regulatory concerns. Electrolyte supplements were administered per manufacturer's instruction for a moderate level of activity (Table 1).

Blood samples (14 ml) were collected at five intervals throughout the course of each mare's SRT via jugular venipuncture and were stored in two 7 ml pre-chilled tubes (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ) containing lithium heparin to prevent coagulation of erythrocytes. Sampling intervals were defined as *pre-electrolyte* (prior to administering treatment), *pre-exercise* (10 min before beginning the SRT), *post-exercise* (immediately following the conclusion of the SRT), *post-exercise 60* (60 min following completion of the SRT) and *post-exercise 90* (90 min following the conclusion of the SRT). With the exception of the post-exercise interval, all blood samples were obtained using a 20-gauge needle (Becton-Dickinson). The post-exercise sample was obtained through the use of an 18-gauge needle (Monoject, Sherwood Medical Corporation, St. Louis, MO) due to the greater viscosity of post-exercise blood resulting from splenic contraction in the horse. All blood samples were stored on ice prior to instrumental analysis.

Exercise test protocol

Each horse's SRT was tailored to its individual exercise capacity using data from a previously performed incremental exercise test (GXT) to determine maximal oxygen consumption ($\dot{V}O_{2max}$) and the oxygen consumption *vs* speed relationship. All GXT tests were performed no less than 1 month prior to beginning the SRT series and these conformed to methods established in previous equine exercise physiology investigations[22–24]. Prior to beginning the SRT, horses stood quietly for a period of 10 min. The pre-exercise blood sample was obtained at the conclusion of this interval. Horses then ran on the treadmill (fixed 6% incline) for 2 min at a warm-up speed of 4 ms^{-1}, 2 min at a speed calculated to be 110% of the speed required to elicit $\dot{V}O_{2max}$ and 2 min at a cool-down speed of 4 ms^{-1}.

Assay procedures

One 7 ml tube per sampling interval was spun in a refrigerated centrifuge (TJA-6, Beckman-Coulter, Fullerton, CA) at a rate of 2000 rpm and a temperature of 4°C for a period of 10 min. The plasma fraction was

Table 1 Electrolyte supplement treatment specifications for moderate exercise with ingredients as listed on manufacturer's label

Product name	Route of administration	Physical description	Dose
Stress-Dex (Squire Laboratories, Revere, MA)	Oral (60 ml syringe)	Powder: Dextrose (73%), sodium chloride (6%), potassium chloride (3%), dicalcium phosphate, hydrophobic silica, saccharin sodium, artificial colouring, orange flavour	42.5 g + 60 ml H_2O (slurry)
Acculytes (Vita Flex Nutrition, Omaha, NE)	Oral (60 ml syringe)	Powder: Dextrose, salt (NaCl), sodium citrate, glycine, magnesium amino acid chelate (aspartate), calcium amino acid chelate, calcium silicate, calcium carbonate, magnesium carbonate, silicon dioxide, monosodium phosphate, natural and artificial flavourings, *Lactobacillus acidophilus, Lactobacillus plantarum*, *Enterococcus faecium, Bifidobacterium hermophilum* and *Bifidobacterium longum*	42.5 g + 60 ml H_2O (slurry)
Electroplex (Oralyx, Ogden, UT)	Oral (pre-loaded syringe)	Paste: Water, isolated soy protein, dextrose (7.5 g), potassium (500 mg), amino acid complex, magnesium amino acid chelate (500 mg), calcium amino acid chelate (375 mg), sodium chloride (625 mg), niacinamide (375 mg), thiamin HCl (25 mg), calcium pantothenate (7.5 mg), riboflavin (10 mg), cyanocobalamin (7.5 μg), xanthan gum, sorbic acid, apple flavouring	34 g (full syringe)
Lyte-Now (Pro-Formula Laboratories, Davie, FL)	Oral (pre-loaded syringe)	Paste: corn syrup, water, iron (28 mg), zinc (4 mg), copper (2 mg), cobalt (0.02 mg), sodium chloride (7.2 g), calcium chloride (5.2 mg), potassium chloride (7.2 g), magnesium chloride (5.2 g), manganese ascorbate (20 mg), natural and artificial flavour, sodium benzoate	30 ml (full syringe)
Summer Games (Kentucky Equine Research, Versailles, KY)	Oral (60 ml syringe)	Powder: sodium (6.5 g), potassium (3.5 g) chloride (13 g), magnesium (160 mg), calcium (170 mg), copper (10 mg), iron (52 mg), manganese (4 mg), zinc (25 mg).	56.7 g + 60 ml H_2O (slurry)
EnduraMAX (Kentucky Equine Research, Versailles, KY)	Oral (60 ml syringe)	Powder: sodium (5.5 g), potassium (3.7 g), chloride (11.9 g), magnesium (153 mg), calcium (754 mg).	56.7 g + 60 ml H_2O (slurry)
Perform'N Win (Buckeye Feed Mills, Dalton, OH)	Oral (60 ml syringe)	Powder: NaCl and KCl (sodium: 1.1 g chloride: 2.6 g potassium: 910 mg), magnesium sulphate, dextrose, sucrose, calcium citrate, fumaric acid, citric acid anhydrous, silicon dioxide, natural and artificial flavour	28.4 g + 60 ml H_2O (slurry)

then decanted and analysed using an ion-sensitive electrolyte analyser (Synchron EL-ISE, Beckman-Coulter, Fullerton, CA) to determine tCO_2 and the concentrations of sodium, potassium and chloride ions ($mEq\,l^{-1}$), respectively. The concentration of lactate ($mmol\,l^{-1}$) in plasma samples was determined using a conventional lactate analyser (1500 Sport, Yellow Springs Instrument Company, Yellow Springs, OH). Plasma SID was calculated as SID ($mEq\,l^{-1}$) = $[Na^+] + [K^+] - [Cl^-] - [Lactate^-]$[12,13]. The second 7 ml tube of blood collected during each sampling interval was used to determine haematocrit and total protein concentration ($mg\,dl^{-1}$; via refractometry) of each sample. Haematocrit and total protein concentrations were monitored to ensure that electrolyte supplements did not alter the hydration status of horses; however, these parameters were not considered in the final assessment of the effects of the electrolytes on tCO_2 status. All assays were performed in duplicate to reduce the occurrence of random error.

Statistical analysis

Two-way analysis of variance for repeated measures was employed to analyse data. When significant, means were separated using the Student–Newman Keuls and Dunnets *post hoc* tests. The null hypothesis was rejected when $P < 0.05$. Results are reported as mean value ± standard error.

Results

Figs. 1a and 1b illustrate that there were no significant differences ($P > 0.05$) between treatments across each of the five sampling intervals for haematocrit and plasma total protein concentration, respectively. However, significant increases in haematocrit and plasma total protein concentration were demonstrated for all eight treatments in the post-exercise sampling interval. Both parameters decreased to pre-exercise levels by 60 min post-exercise. Figs 2, 3a and 4a illustrate that there were no differences ($P > 0.05$) between

treatments across each of the five sampling intervals for plasma concentrations of sodium, potassium, chloride and tCO_2, respectively. Significant increases in plasma potassium and sodium concentrations were demonstrated for all eight treatments in the post-SRT sampling interval, whereas all eight treatments demonstrated a significant decrease in plasma tCO_2 concentration in the post-exercise sampling interval. Plasma concentrations of sodium, potassium and tCO_2 returned to pre-exercise levels by 60 min post-exercise. None of the eight treatments demonstrated a significant effect upon plasma chloride concentration across the five sampling intervals. Plasma chloride concentration remained constant throughout the duration of the experiment. Fig. 3b illustrates no significant difference in plasma lactate concentration between treatments across the pre-electrolyte and pre-exercise sampling intervals. Plasma lactate concentration increased ($P < 0.05$) from pre- to post-exercise. At 60 min post-exercise, plasma lactate levels

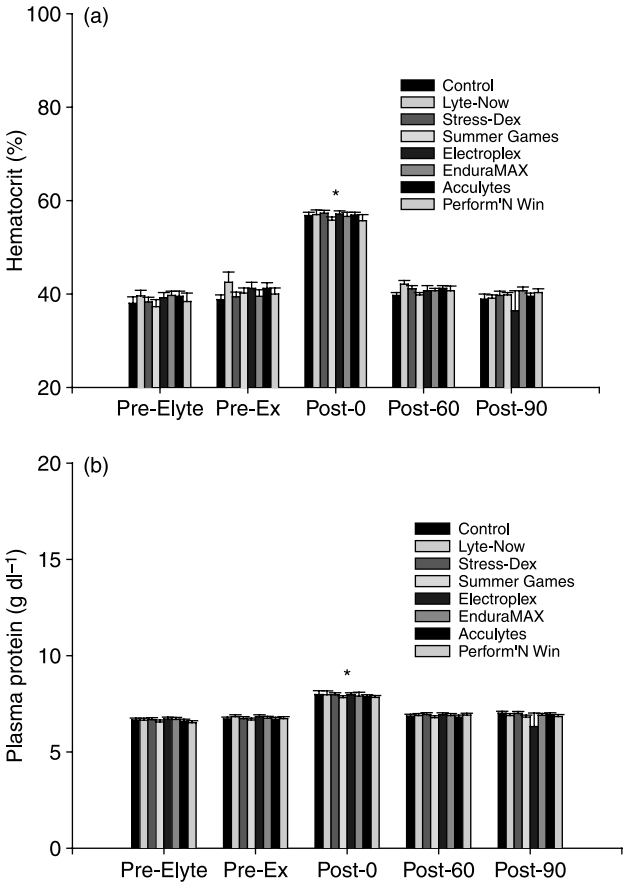

Fig. 1 Data present the effect of electrolyte supplements and exercise on a) haematocrit and b) plasma total protein concentration (means ± SE). No significant difference ($P > 0.05$) was detected between treatments across each of the five sampling intervals. An asterisk (*) indicates that HCT and plasma [TP] increased ($P < 0.05$) for all treatment groups from the pre-exercise to post-exercise interval and returned to pre-exercise levels by 60 min post-exercise

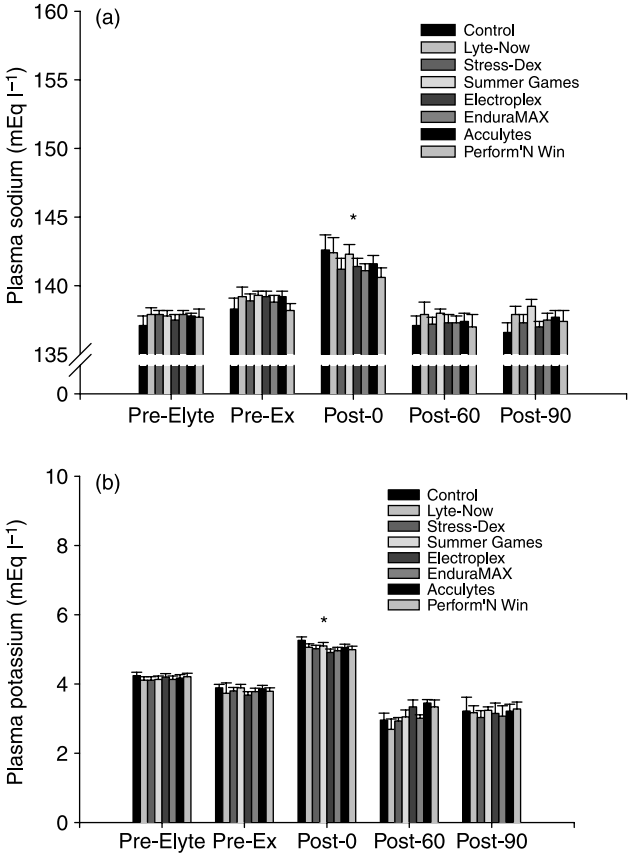

Fig. 2 Data present the effect of electrolyte supplements and exercise on a) plasma [Na^+] and b) plasma [K^+] as means ± SE. No significant difference ($P > 0.05$) was detected between treatments across each of the five sampling intervals. An asterisk (*) indicates that plasma [Na^+] and [K^+] increased ($P < 0.05$) for all treatment groups from the pre-exercise to post-exercise interval and returned to pre-exercise levels by 60 min post-exercise

decreased but remained significantly higher than pre-exercise concentrations through 90 min post-SRT. There was also a significant time by treatment interaction ($P < 0.05$) for the post-exercise, post-exercise 60 and post-exercise 90 sampling intervals. *Post hoc* analysis revealed that horses treated with EnduraMAX had slightly lower ($P < 0.05$) plasma lactate concentrations compared to the runs where the horses were treated with either the control or the other supplements. Fig. 4b illustrates that there was no significant difference in plasma SID between treatments across each of the five sampling intervals. Plasma SID, significantly decreased in the post-exercise sampling interval, however, returned to pre-exercise levels by 60 min post-exercise.

Discussion

The major finding of the present investigation was that administration of the seven chosen electrolyte supplement products did not alter the plasma

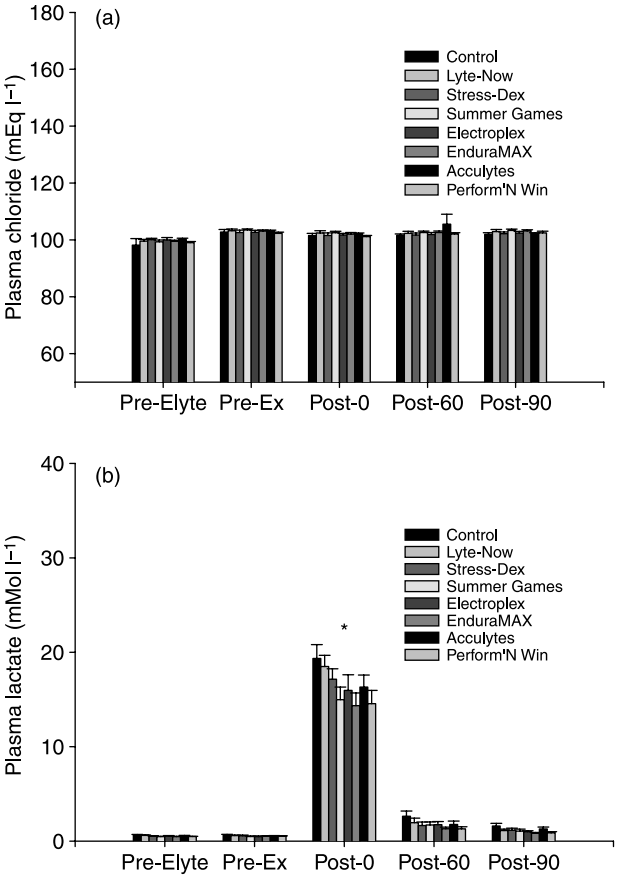

FIG. 3 Data present the effect of electrolyte supplement and exercise on a) plasma chloride and b) lactate concentrations (mean ± SE). Treatment did not alter plasma [Cl^-] ($P > 0.05$) at any of the five sampling intervals examined in this investigation. Exercise did not alter plasma [Cl^-]. An asterisk (*) indicates that plasma [lactate] increased ($P < 0.05$) for all treatment groups from the pre-exercise to post-exercise interval and returned to pre-exercise levels by 60 min post-exercise

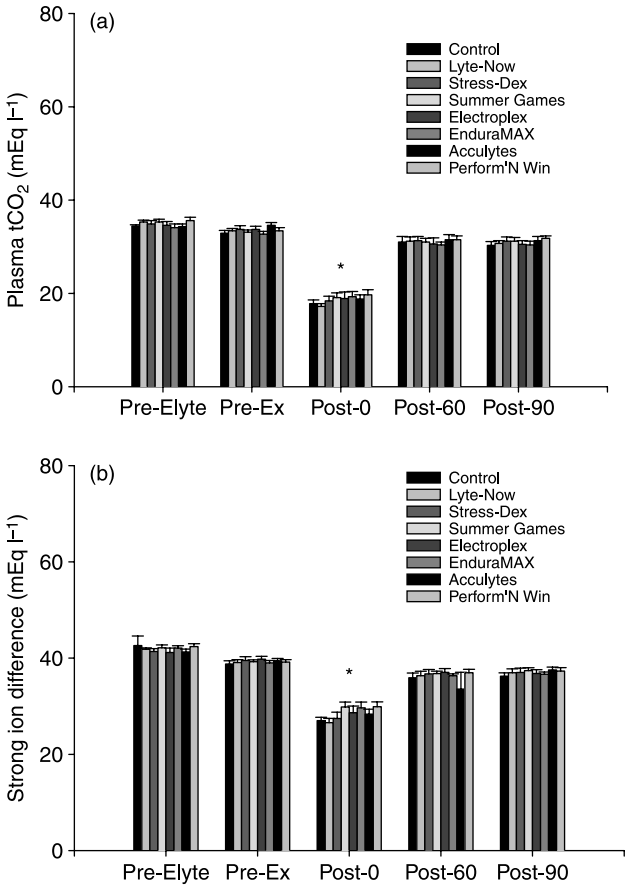

FIG. 4 Data present the effect of electrolyte supplements and exercise on a) plasma tCO_2 concentration and b) plasma SID (mean ± SE). No difference ($P > 0.05$) was detected between treatments across each of the five sampling intervals. An asterisk (*) indicates plasma tCO_2 concentration and SID decreased ($P < 0.05$) for all treatment groups from pre- to post-exercise intervals and returned to pre-exercise levels by 60 min post-exercise

concentrations of tCO_2, the major electrolytes or the plasma strong ion difference. This answers the speculation made in key enforcement litigation that the administration of electrolyte supplements or failure to provide electrolyte supplements would have an influence on plasma tCO_2 concentrations. Such an effect is a concern because the administration of alkalinizing agents ('milkshaking') has become a problem for those interested in maintaining the integrity of competitive equine athletic events throughout the world[1–7,17–21]. Equine milkshakes and associated alkalinization procedures are designed to accentuate a horse's natural ability to buffer changes in internal acid–base balance[1–7]. Data are mixed on the ergogenic and ergolytic effects of alkalinizing practices[8,19,25–30]. However, the confusion is more a factor of study design as most have used a variety of 'field type' experiments that are hard to control. Many studies suggested that there was a distinct lack of performance-enhancing effect[19] or a negative effect due to gastrointestinal disturbances[8]. However, more recent controlled treadmill studies have demonstrated an increase in indices of performance (name-specific markers, run time to fatigue, aerobic capacity, etc.) in horses given various alkalinizing agents[22,23,29,30].

Independent of the question regarding any effect on performance, substances like sodium bicarbonate can also act as masking agents through their effect on urine pH and the pronounced diuresis that follows their administration. The negative impact of the use of alkalinizing agents also affects the welfare of the equine athlete, with the potential for severe gastrointestinal problems[8] as well as the potential for inadvertent infusion into the lungs via improper use of a nasogastric tube. Thus, milkshaking is viewed by many as a threat to the welfare of horses and to the credibility of equine sports industries on a global scale, a threat that has led to the imposition of severe legal sanctions upon those individuals found guilty of alkalinizing performance horses[1–8]. To that end, many racing jurisdictions test for the administration of alkalinizing agents by measuring changes in

either blood bicarbonate concentrations or by measuring plasma total carbon dioxide concentration[1,6,14,17–20]. Plasma tCO_2 concentration is an important physiological factor that is controlled so as to insure the tight regulation of blood pH[15,16]. Animals transport more than 90–95% of all CO_2 produced in metabolically active tissue to the lungs in the form of carbonic acid[15,16]. This chemical species rapidly dissociates to bicarbonate and hydrogen ions in the presence of an aqueous environment and thus renders tCO_2 an effective measure of bicarbonate concentration within blood plasma[14–16]. This strong association with bicarbonate status and the fact that it can be measured directly rather than indirectly using ion-sensitive electrode technology, has led to use of plasma tCO_2 concentration as a reliable physiological marker for milkshake testing. Multiple investigations have reported that the average plasma tCO_2 concentration for racing horses is $\sim$30 mmol/l[1–3,6,17,18,20,31]. Thus, to avoid false positives, most racing jurisdictions have set the threshold for a positive test at 37 mmol/l[1–3,6,17,18,20,31], approximately four standard deviations from the mean. However, one should caution that this threshold is based on a natural physiological marker and the administration of any substance (legitimate or illicit) that alters acid–base status can push a horse towards the threshold recognized as an actionable positive.

Of concern to racing officials and horsemen who wish to avoid a positive test is the fact that, while a normal healthy horse will not have an elevated plasma tCO_2 concentration, some legitimate medications (furosemide, dexamethazone, etc.) can elevate plasma tCO_2 concentration[31–33]. Plasma tCO_2 concentration can be elevated inadvertently by feeding pellets, dietary supplements and electrolyte mixtures that contain alkalinizing ingredients (sodium bicarbonate, calcium carbonate, etc.). Unfortunately, until now no scientific information has been reported on electrolyte supplements and their effect on plasma tCO_2 concentration. Thus, it is an important finding of the present investigation that the seven electrolyte supplements chosen did not affect plasma tCO_2 concentrations or SID in Standardbred horses at rest or following exercise. Whereas plasma tCO_2 levels decreased significantly from pre-exercise concentrations in the post-exercise sampling interval, plasma sodium and potassium concentrations increased significantly from pre-exercise to post-exercise levels. Since such magnitudes of change were consistent between treatments, this effect was most likely not due to the provision of electrolyte supplements but rather attributable to the physiological pressures associated with acute exercise[34–37]. Strong speculation has been made in court cases that electrolyte supplements would alter plasma strong ion difference and in doing so would have the potential to affect acid–base status and, subsequently, plasma tCO_2 concentration (NJ Racing Commission *vs* Campbell). Unfortunately, until now there have been no data to support or rebuke this speculation. The present study fills that void; however, equine clinicians, trainers and owners should be cautioned to read the labels of the products they use for the welfare of the equine athlete as any incremental increase in plasma tCO_2 concentration cannot be distinguished from the unscrupulous administration of large amounts of performance-enhancing buffering agents.

Schott and Hinchcliff[11] have recently demonstrated how the ingestion of large amounts of sodium bicarbonate can induce metabolic acidosis both through the change in plasma bicarbonate concentration and through changes in the strong ion difference. The former can be viewed through calculations using the Henderson–Hasselbach equation, a method classified by many as qualitative in its approach to understanding the cause of a metabolic alkalosis. The Stewart, or quantitative, approach to acid–base physiology utilizes many physiological variables measured in a simultaneous fashion to understand the cause of a metabolic acidosis[11–13]. Simplified theory presented by Stewart[12] and others[11,13] utilizes the changes in the P_{CO2}, the plasma concentrations of weak ions (primarily protein) and the plasma concentrations of strong ions (primarily sodium, potassium, chloride and lactate) to determine the physiological source of any acidosis or alkalosis. With the exception of plasma lactate concentration, no differences were detected between electrolyte treatments across the five sampling intervals for any of the other parameters monitored in this investigation. Stress-Dex, Acculytes, Electroplex, Lyte-Now, Summer Games, EnduraMAX and Perform'N Win failed to impact plasma sodium, potassium, chloride and tCO_2 concentrations as well as SID. As expected, exercise did affect plasma sodium, potassium, lactate and tCO_2 concentrations as well as SID. Importantly, fluid and electrolyte were only affected by exertion and not by electrolyte treatment. One could question why there was no increase in plasma electrolyte concentration due to ingestion of the supplements. This can be explained by two factors. First, the recommended dose of the electrolytes given is very small and, secondly, the horses had access to water. Thus, while ingestion would increase total body content of sodium, potassium, etc. ingestion of water to maintain tonicity would prevent any resultant change in plasma concentration. Secondarily, as at the track, the horses were allowed access to water and could urinate during the 4 h period between administration and the exercise test.

Another expected finding of the present study was that haematocrit and plasma total protein concentration increased significantly from pre- to post-exercise levels. These data are consistent with the results of other investigations conducted in the resting and

exercising horse[34–37]. Because there were no differences in haematocrit or plasma total protein concentration detected between treatments across the five sampling intervals examined in this study, it was concluded that none of the seven featured electrolyte supplements appeared to exert an effect upon these markers of hydration status.

The overall lack of statistical significance between treatments across the five sampling intervals examined in this investigation suggests an absence of effect of the seven electrolyte supplements upon plasma SID. Plasma SID did decrease significantly from pre- to post-exercise levels and this effect is most likely attributable to the shifts in plasma ionic composition described above associated with the physiological pressures of acute exercise. The absence of significant effect of electrolyte supplements upon plasma tCO_2 and SID suggests that the seven electrolyte supplements featured in this experiment did not affect equine plasma bicarbonate concentration. As such, these products are most likely not the cause of inadvertent alkalinization in performance horses.

The results of the present study indicate that the seven representative electrolyte supplements examined in this investigation do not affect plasma bicarbonate concentrations significantly in the resting and exercising horse. Data appear to discredit the claim that electrolyte supplements elevate plasma bicarbonate concentrations to levels commensurate with those associated with illegal alkalinization. However, one must strongly caution that the list of supplements analysed is not exhaustive. For products not tested, one must read the label to see if there are any ingredients that could elevate plasma total carbon dioxide concentrations to levels that would yield a positive.

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References

1 Auer DE, Skelton KV, Tay S and Baldock FC (1993). Detection of bicarbonate administration (milkshake) in Standardbred horses. *Australian Veterinary Journal* **70**: 338–340.

2 Irvine CHG (1992). Control of administration of sodium bicarbonate and other alkalis: the New Zealand experience. In: Short CR (ed.), *Proceedings of the 9th International Conference of Racing Analysts and Veterinarians*. Newmarket, UK: R&W Press, pp. 139–143.

3 Irvine CHG (1996). An international bicarbonate threshold – is it feasible? *Proceedings of the 12th International Conference of Racing Analysts and Veterinarians*. Newmarket, UK: R&W Press, pp. 27–31.

4 Lloyd DR and Rose RJ (1992). Issues relating to use of products that can produce metabolic alkalosis prior to racing. *Australian Equine Veterinarian* **10**: 27–28.

5 Rose RJ and Lloyd DR (1992). Sodium bicarbonate: more than just a milkshake? *Equine Veterinary Journal* **24**: 75–76.

6 Vine JH (1998). *Proceedings of the 12th International Conference of Racing Analysts and Veterinarians*. Newmarket, UK: R&W Publications, pp. 32–36.

7 Williams RB (1995). Sodium bicarbonate loading and the rules of racing. *British Veterinary Journal* **151**: 473–475.

8 Frey LP, Kline KH, Foreman JH, Brady AH and Cooper SR (1995). Effects of warming up, racing and sodium bicarbonate in Standardbred horses. *Equine Veterinary Journal Supplement* **18**: 310–313.

9 Rivas LJ and Hinchcliff KW (1996). Sodium bicarbonate and performance horses: effects on blood and urine constituents and a review of beneficial and adverse effects. *Proceedings of the American Association of Equine Practitioners* **42**: 90–92.

10 Schott HC and Hinchcliff KW (1993). Fluids, electrolytes, and bicarbonate. *Veterinary Clinics of North America: Equine Practice; Drug Use in Performance Horses* **9**: 557–600.

11 Schott HC 2nd and Hinchcliff KW (1998). Treatments affecting fluid and electrolyte status during exercise. *Veterinary Clinics of North America: Equine Practice* **4**: 175–204.

12 Stewart PA (1978). Independent and dependent variables of acid–base control. *Respiration Physiology* **33**: 9–26.

13 Constable PD (1997). A simplified strong ion model for acid–base equilibria: application to horse plasma. *Journal of Applied Physiology* **83**: 297–311.

14 Lloyd DR and Rose RJ (1996). The effects of several alkalinizing agents on acid:base balance in horses. In: Auer DE & Houghton E (eds), *Proceedings of the 11th International Conference of Racing Analysts and Veterinarians*. Newmarket, UK: R&W Publications, pp. 104–110.

15 Guyton AC (1981). Transport of oxygen and carbon dioxide in the blood and body fluids. In: Guyton AC (ed.), *Textbook of Medical Physiology*, 6th edn. Philadelphia: Saunders, pp. 512–515.

16 Martin DW (1981). The chemistry of respiration. In: Martin DW, Mayes PA & Rodwell VW (eds), *Harper's Review of BioChemistry*, Vol. 37, 18th edn. Los Altos, CA: Lange Medical Publications, pp. 516–526.

17 Lloyd DR, Reilly P and Rose RJ (1992). The detection and performance effects of sodium bicarbonate administration in the racehorse. In: Short CR (ed.), *Proceedings of the 9th International Conference of Racing Analysts and Veterinarians*. Newmarket, UK: R&W Press, pp. 131–138.

18 Reilly P, Duffield A, Suann CJ, Vine J, Batty, Auer D, Skelton K, Tay S, Stenhouse A and Ralston J (1996). The analysis of plasma total carbon dioxide in racehorses. In: Auer DE & Houghton E (eds), *Proceedings of the 11th International Conference of Racing Analysts and Veterinarians*. Newmarket, UK: R&W Publications, pp. 238–240.

19 Schuback K, Kallings P, Bonderson U, Essen-Gustavsson B and Persson S (1998). Effect of sodium bicarbonate treatment on anaerobic metabolism and total carbon dioxide in plasma

during post exercise recovery. *Proceedings of the 12th International Conference of Racing Analysts and Veterinarians*. Newmarket, UK: R&W Press, pp. 14–19.

20 Slocombe RF, Huntington PJ, Lind KL and Vine JH (1995). Plasma total CO_2 and electrolytes: diurnal changes and effects of adrenaline, doxapram, rebreathing, and transport. *Equine Veterinary Journal Supplement* **18**: 331–336.

21 Roelofson R (1992). Sucrose and bicarbonate overloading in Standardbred horses in Ontario. *Proceedings of the 9th International Conference of Racing Analysts and Veterinarians*. Newmarket, UK: R&W Publications, pp. 45–147.

22 Hinchcliff KW and McKeever KH (1996). Furosemide reduces accumulated oxygen deficit and rate of lactate production in horses during brief intense exertion. *Journal of Applied Physiology* **81**: 1550–1554.

23 Hinchcliff KW and McKeever KH (1996). Furosemide and weight carriage alter the acid:base responses of horses to incremental and brief intense exertion. *Equine Veterinary Journal Supplement* **30**: 375–379.

24 McKeever K, Malinowski K, Christensen R and Hafs H (1998). Chronic equine somatotropin administration does not affect aerobic capacity or indices of exercise performance in geriatric horses. *The Veterinary Journal* **155**: 19–25.

25 Frey LP, Kline KH, Foreman JH, Lyman JT and Butadom P (1999). Effects of alternate alkalinizing compounds on blood plasma acid–base balance in exercising horses. *Proceedings of the 16th Equine Nutrition and Physiology Society Symposium*, Raleigh, NC, pp. 161–162.

26 Greenhaff PL, Harris RC, Snow DH, Sewell DA and Dunnett M (1991). The influence of metabolic alkalosis upon exercise metabolism in the Thoroughbred horse. *European Journal of Applied Physiology and Occupational Physiology* 63129–63134.

27 Greenhaff PL, Snow DH, Harris RC and Roberts CA (1990). Bicarbonate loading in the thoroughbred: dose, method of administration and acid–base changes. *Equine Veterinary Journal Supplement* **9**: 83–85.

28 Lawrence L, Kline K, Miller-Graber P, Siegel A, Kurcz E, Fisher M and Bump K (1990). Effect of sodium bicarbonate on racing Standardbreds. *Journal of Animal Science* **68**: 673–677.

29 Hinchcliff KW and McKeever KH (1993). Effects of oral sodium loading on acid:base response to exercise in horses. *Proceedings of the 13th Equine Nutrition and Physiology Society Symposium,* Gainesville, FL, pp. 121–123.

30 Lloyd DR and Rose RJ (1995). Effects of sodium bicarbonate on acid–base status and exercise capacity. *Equine Veterinary Journal Supplement* **18**: 323–325.

31 Soma LR, Uboh CE, Nann L and Gerber AL (1996). Prerace venous blood acid:base values in Standardbred horses. *Equine Veterinary Journal* **28**: 390–396.

32 Freestone JF, Carlson GP, Harrold DR and Church G (1988). Influence of furosemide treatment on fluid and electrolyte balance in horses. *American Journal of Veterinary Research* **49**: 1899–1902.

33 Freestone JF, Carlson GP, Harrold DR and Church G (1989). Furosemide and sodium bicarbonate-induced alkalosis in the horse and response to oral KCl or NaCl therapy. *American Journal of Veterinary Research* **50**: 1334–1339.

34 McKeever KH, Hinchcliff KW, Reed SM and Robertson JT (1993). Role of decreased plasma volume in hematocrit alterations during incremental treadmill exercise in horses. *American Journal of Physiology* **265**: R404–R408.

35 McKeever KH, Hinchcliff KW, Reed SM and Robertson JT (1993). Plasma constituents during incremental treadmill exercise in intact and splenectomised horses. *Equine Veterinary Journal* **25**: 233–236.

36 Steel JD and Whitlock LE (1960). Observations on the haematology of Thoroughbred and Standardbred horses in training and racing. *Australian Veterinary Journal* **36**: 136–142.

37 Judson GJ, Frauenfelder HC and Mooney GJ (1983). Plasma biochemical changes in Thoroughbred racehorses following submaximal and maximal exercise. In: Snow DH, Persson SGB & Rose RJ (eds.), *Equine Exercise Physiology.* Cambridge: Granta Editions, pp. 405–408.