University Library

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Bailey, SR;Forbes, G;Selvadurai, N;McLarney, K;Jones, S;Steel, CM

Title:
The effect of serial administration of bicarbonate on plasma total CO2 concentrations in horses

Date:
2021-02

Citation:
Bailey, S. R., Forbes, G., Selvadurai, N., McLarney, K., Jones, S. & Steel, C. M. (2021). The effect of serial administration of bicarbonate on plasma total CO2 concentrations in horses. Drug Testing and Analysis, 13 (2), pp.397-403. https://doi.org/10.1002/dta.2937.

Persistent Link:
https://hdl.handle.net/11343/276587

Check for updates

Author Manuscript

RESEARCH ARTICLE

The effect of serial administration of bicarbonate on plasma total CO_2 concentrations in horses

Short title: Effect of serial bicarbonate administration on plasma CO_2 in horses

Simon R. Bailey[a], Grace Forbes[b], Naomi Selvadurai[c], Katelin McLarney[a], Susan Jones[a], Catherine M. Steel[a]

Correspondence:
Catherine M. Steel, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia
csteel@unimelb.edu.au

[a]Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia
[b]Racing Victoria Ltd, 400 Epsom Road, Flemington, Victoria, Australia 3031
[c]Racing Analytical Services Limited, 400 Epsom Road, Flemington, Victoria, Australia 3031

ABSTRACT

The administration of alkalinizing agents including bicarbonate is of concern to racing authorities because resultant alkalosis may enhance performance and interfere with the detection of drugs in post-race urine. A threshold for total carbon dioxide (TCO_2) of 36.0 mmol/L in plasma (with action limit of 37.0 mmol/L) has been set. Serial dosing of sodium bicarbonate has gained popularity in human athletes but has not been studied in horses previously. Sodium bicarbonate (200 g per horse) and 60 g of an electrolyte-vitamin complex was administered in 2 L water *via* nasogastric intubation to five Standardbred horses for three consecutive days (total dose bicarbonate 0.42 ±0.02 g/kg). Serial blood samples were taken over Days 1-5, with the final day (5) intended to simulate a 'clear day', and TCO_2 was analysed. Following the first bicarbonate administration, plasma TCO_2 peaked at 6 hours (34.8 ±1.3 mmol/L), returning to baseline by 23 hours. On Day 2, four out of the five horses showed a peak greater than 36.0 mmol/L (mean 37.0 ±2.1 mmol/L). With daily repeated dosing, plasma TCO_2 peaked progressively earlier, and by Day 3 the peak occurred at 2 hours and concentrations declined more rapidly. On Days 4 and 5, TCO_2 levels remained low (<32.1 mmol/L on Day 4 and between 27.0 – 31.2 mmol/L on Day 5). These studies demonstrate that serial dosing of a 'split dose' of sodium bicarbonate on three consecutive days does not result in the accumulation or carry-over of plasma TCO_2 levels beyond the levels observed following a single dose.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/dta.2937

Keywords: equine, alkalinising agent, alkalosis

This article is protected by copyright. All rights reserved.

1. INTRODUCTION

During intense exercise, working muscles have a greater reliance on non-oxidative energy pathways and there is intracellular accumulation of hydrogen ions (H+) with resultant acidification of muscle that is associated with fatigue.[1] Alkalinising agents such as sodium bicarbonate ($NaHCO_3$) which minimise H^+ accumulation may be given to horses[2-8] and human athletes[9,10] in attempts to maintain exercise performance when acidosis limits anaerobic capacity. Sodium bicarbonate aids intracellular pH regulation by acting as an extracellular (blood) buffer, raising the extracellular pH and HCO_3 concentrations and enhancing efflux of H^+ from muscle cells during high-intensity exercise, potentially delaying the onset of fatigue and enhancing performance.[11-13] This 'buffering' effect can be achieved in human athletes using both acute and chronic $NaHCO_3$ supplementation.[14]

The practice of administering mixtures containing sodium bicarbonate (commonly known in the horse racing industry as a "milk shake") by nasogastric intubation 3 to 5 hours before racing was considered widespread, particularly in the harness racing industry, before pre-race testing was introduced in Australia over 25 years ago.[4] The administration of alkalinizing agents including bicarbonate is of concern to horse racing authorities not only because it may improve performance,[2,5] but also because it can interfere with the detection of other drugs in post-race urine and cause gastrointestinal disturbances.[15] Sodium bicarbonate causes an increase in urine flow and increase in urine pH, in order to conserve plasma volume in response to the fluid shift from the extravascular space to the vascular compartment, and to re-establish acid-base balance, respectively[16]. This may mask the administration of other performance-enhancing drugs by reducing their concentration in urine, and is therefore of importance from an integrity perspective, as well as being a welfare concern.

Measurement of plasma total carbon dioxide concentration (TCO_2) is used as an indicator of alkalinizing agent use. TCO_2 refers to the sum of the concentrations of carbon dioxide liberated from bicarbonate, carbonate, carbonic acid and carbon dioxide bound to plasma proteins during the analytical process, together with the dissolved carbon dioxide. The mean TCO_2 concentration in Australian Thoroughbred horses, known not to have been treated with alkalinizing agents, has been determined to be 30.8 (SD 1.4 mmol/L).[17] A threshold level of plasma TCO_2 of 36.0 mmol/L (with an action limit of 37.0 mmol/L, allowing for uncertainty of measurement) for horses has been set. [18] Furthermore, as outlined in the Australian Rules of Racing (for Thoroughbred horses), a horse must not be administered an alkalinizing agent in any manner within 1 clear day (a period of 24h from 12.01am to 12 midnight) of an official trial or race; and in the Australian Harness Racing rules (for Standardbred horses) must not be stomach tubed within 48 hours of a trial or race or given any alkalinizing agent on the day of competition.

Previous studies of the effects of alkalinizing agents, including bicarbonate, in horses have used the administration of a single dose.[2,5,6] Following a single dose of 0.3 to 1.0 g/kg body weight, the TCO_2 concentration increases until it peaks at around 4-7 hours, then slowly declines to return to pre-administration levels after 20-30 hours. The effect varies between horses, depends on the dose, the route of administration, and the alkalinizing agent administered.[5,6] The effect of serial administration of lower doses of bicarbonate over several days has not been investigated although this practice is currently of concern for racing authorities. Serial dosing (or 'serial loading') of bicarbonate for 3-5 days to build up blood buffer levels, that may remain elevated for longer after the last ingestion compared to after a single dose, is considered by some to have ergogenic effects for at least 24 hours after the last

This article is protected by copyright. All rights reserved.

Author Manuscript

dose.[9, 19-24] The additional benefit of reducing gastrointestinal problems associated with administration on the day of competition has been reported in human athletes,[9] potentially prompting racehorse trainers to try a similar technique.

The overall aim of this study was to investigate the effect of serial dosing of sodium bicarbonate on three consecutive days on the plasma total CO_2 (TCO_2) concentration in horses. The specific objective was to determine whether serial dosing of sodium bicarbonate (200 g) on three consecutive days can result in a TCO_2 level that exceeds the permitted threshold of 36.0 mmol/L 47 h later (i.e. on the morning of competition, even with one clear day since the last dose).

2. MATERIALS AND METHODS

2.1 Subjects

Five adult Standardbred horses were used for this study. All procedures were approved by the University of Melbourne Animal Ethics Committee prior to starting the trial (Permit number 1814425.2).

For the duration of the study the horses were kept in adjacent dirt yards (10 m x 15 m) with open shelter, in pairs. A veterinary health check was conducted on arrival and no abnormalities were detected. An acclimatization period of 3 days was provided after arrival. Body weight was accurately assessed using calibrated weigh scales (Horseweigh, UK) and weight range was 487-543 kg. The horses had unrestricted access to water. They were fed hay *ad libitum* and were also given a small feed of soaked soybean hulls and lucerne chaff each afternoon.

2.2 Materials

Sodium bicarbonate was obtained from Sigma-Aldrich Pty Ltd, Castle Hill NSW, and had a purity of >99.5%. Supervites® product (15 kg tub) was obtained from Randlab, Chipping Norton, NSW.

2.3 Procedures

Sodium bicarbonate (200 g per horse) was dissolved in 2 L of tap water, along with 60 g of Supervites® (Randlab; 1 scoop), which is an electrolyte and vitamin complex commonly given with bicarbonate to horses in race training. These products were weighed on calibrated laboratory scales and added to 2 L of clean tap water shortly before each administration with adequate mixing to ensure that the full dose was received on each occasion. As 1 scoop of Supervites® may provide quite a variable amount of product depending on the packing density of the powder (1 scoop giving between approximately 44 – 62 g), in this study a weighed amount of product was used, rather than relying on the scoop measure. This suspension was administered *via* a standard nasogastric tube (16 mm outer diameter; siliconized PVC tubing) to each of the horses at 9 am (± 25 mins) for three consecutive days.

Author Manuscript

This article is protected by copyright. All rights reserved.

Horses were placed in stocks for stomach tubing, and afterwards were returned to their yards, where further blood samples were collected. Horses were catheterised (14G intravenous jugular catheter; BD Angiocath, Becton Dickinson, Macquarie Park, NSW, Australia) at the start of the study and baseline blood samples collected. Following delivery of the suspension containing bicarbonate, blood samples were taken at 2, 4, 6, 8, 10 and 23 hours (8 am the following morning); 10 mL of blood was collected into lithium heparin tubes. On day 4, further blood samples were collected (23, 26, 28, 30 and 32 h after the final bicarbonate dose), with a final sample taken at 47 hours (8 am on Day 5). The timeline of dosing and sample collection is shown in Figure 1.

2.4 Health monitoring of the horses

During and after stomach tubing, the nostrils and tube were assessed for any traces of blood, and the nostril used was alternated each day. Horses were monitored in their yards every 2 hours for 10 hours following each administration, observing for any signs of discomfort, abdominal pain (colic) or diarrhea. They also underwent a daily general monitoring check.

2.5 Sample handling

After each 10 mL of blood was collected into a lithium heparin tube (BD Vacutainer; Becton Dickinson, Macquarie Park, NSW, Australia), the sample was promptly chilled at 4 °C. Samples were then transported chilled in a cool box to the laboratory (Racing Analytical Services Limited (RASL), Flemington), delivered each day before 12 noon. This ensured that samples could be analysed that same day. TCO_2 was measured in plasma using the standard analytical method used for all race day blood samples.

2.6 Sample analysis

Samples were analysed using a Beckman Coulter Unicel DxC 600 (Beckman Coulter Australia, Mount Waverley). Briefly, this instrument employs an ion selective electrode to measure the rate of pH change caused by the liberation of CO_2 when acid is added to plasma. The rate of change of pH is directly proportional to the total amount of CO_2 carbon dioxide in the sample.

The instrument was calibrated using calibrators at 0 mmol/L and 30 mmol/L supplied by Beckman Coulter. A calibration graph covering the range of interest was then constructed by analysing the ASE Linearity Set (Australian Scientific Enterprises Pty Ltd, Hornsby, Australia) containing bicarbonate at 27, 30, 33, 36 and 39 mmol/L. The trueness of the calibration was then verified using a certified reference material (E22c), containing TCO_2 at 35.26 ± 0.2(k = 2) mmol/L, supplied by ASE and independently certified by the National Measurement Institute.

Blood samples were centrifuged in their vacutainers at 3,500 rpm for 10 minutes. An aliquot of plasma was removed and transferred to a sample cup for analysis. Samples were analysed in duplicate, the mean of the duplicates calculated and their TCO_2 concentrations then interpolated from the calibration graph.

Calibration graphs showed linear response over the range 27-30 mmol/L with r^2 >0.998. Aliquots of the CRM were analysed between every 10 samples and measured concentrations

This article is protected by copyright. All rights reserved.

were within the accepted range of ± 0.5 mM throughout. The uncertainty of measurement at 36.0 mmol/L was less than 1 mmol/L at > 99% confidence.[25]

3. RESULTS

All horses tolerated the stomach tubing well and none required additional restraint with a nose twitch or sedation. All horses received the full dose of bicarbonate on each occasion. There was trace blood on the stomach tube on one occasion, but otherwise there were no adverse effects of the bicarbonate administration, and all horses remained bright and healthy throughout the study and no signs of gastrointestinal problems were observed. Blood samples were taken at the timepoints described in the methods. This was well tolerated and there were no missing data points.

The concentrations of plasma TCO_2 are represented graphically in Figures 2 and 3, and data for the individual horses is provided as supplementary information.

Following the first bicarbonate administration, plasma TCO_2 peaked at 6 hours, at a mean peak concentration of 34.8 ±1.3 mmol/L (mean ± standard deviation), with only one of the horses showing a peak greater than 36 mmol/L. These moderately elevated levels were still increased at 10 hrs post administration, but the TCO_2 had returned to baseline by 23 hours.

Following the second bicarbonate administration, plasma TCO_2 peaked at 4 hours, at a mean peak concentration of 37.0 ±2.1 mmol/L (mean ±SD), with 4 out of the 5 horses showing a peak greater than 36.0 mmol/L and 2 exceeding the action limit of 37.2 mmol/L. After 4 hours the plasma levels rapidly decreased, below the 36 mmol/L threshold by 6 hours and continued to steadily decline down to 31.1 ±0.8 mmol/L by 23 hours.

Following the third bicarbonate administration, plasma TCO_2 peaked even earlier, at 2 hours (mean peak concentration of 35.6 ±0.7 mmol/L (mean ±SD), with 2 out of the 5 horses showing a peak greater than 36 mmol/L. After 2 hours the plasma levels rapidly decreased, below the 36 mmol/L threshold by 4 hours and were back down below baseline by 10 hours (to 30.4 ±1.2 mmol/L).

On Day 4, the 'clear day' with no bicarbonate administration, plasma TCO_2 levels remained low, not exceeding 32.1 mmol/L in any of the horses. One horse exhibited levels varying between 27.4 and 32.1 mmol/L, however the other horses remained in the range between 27.0 mmol/L (the lower limit of reporting in the analysis by the laboratory) and 30.2 mmol/L, which was below the starting baseline on Day 1 (30.9 ±0.8 mmol/L).

On Day 5, the final sample indicated that plasma TCO_2 levels were remaining in the normal baseline range, with a mean value of 28.6 ±1.7 mmol/L (range 27.0 – 31.2 mmol/L).

Author Manuscript

This article is protected by copyright. All rights reserved.

4. Discussion and conclusions

These studies demonstrate that serial dosing of a 'split dose' of sodium bicarbonate on three consecutive days does not result in the accumulation or carry-over of plasma TCO_2 levels beyond the levels observed following a single dose. Nor does this dosing regimen result in sustained values of TCO_2 above the permitted threshold of 36.0 mmol/L beyond the day of the last treatment. After each administration of bicarbonate, the levels of plasma TCO_2 had returned to baseline by 23 hours later and following the third administration the levels were back at their baseline values within 10 hours. The lack of accumulation is largely to be expected, since sodium bicarbonate is a salt and its component ions in solution, sodium and bicarbonate, are electrolytes. Therefore, excess amounts of these ions are cleared rapidly by the kidneys. In the case of bicarbonate, it is readily filtered through the renal glomerulus and then reabsorbed from the proximal tubule and collecting ducts, as required to maintain acid-base balance in the body. [16] Accumulation would normally only be seen with drug compounds where clearance is slower relative to dose and dosing interval.

It was observed in the present study that the peaks in TCO_2 did not consistently exceed the threshold level set by racing authorities of 36 mmol/L, and only two horses exceeded the action limit of 37.0 mmol/L. However, the dose used in the present study was considerably lower than that used in some previous experimental studies in horses showing a performance-enhancing effect and was specifically chosen to represent a divided dose split over 3 days. In one of the original studies in this area in horses,[5] a single dose of 1g/kg bicarbonate was used, and this produced peak plasma bicarbonate values (after 5 hours) of 39.6 mmol/L (although it should be noted that bicarbonate values will be marginally lower than TCO_2 because TCO_2 also includes dissolved carbon dioxide, carbonic acid and carbon dioxide bound to plasma proteins) and significantly increased exercise capacity. Calculating the dose of bicarbonate used in the present study on a g/kg basis, including the bicarbonate present in the Randlab Supervites® product (21.2 g per 60 g dose) equates to 0.42 ±0.02 g/kg. Doses ranging from 0.1-0.5 g/kg body mass have been investigated in humans to improve athletic performances. However, when using a single administration 0.3- 0.4 g/kg bicarbonate is generally used, although an individual approach to dosage and timing is recommended and not all studies support an ergogenic effect (for a recent review see Lopes-Silva *et al* 2019)[14]. However, Greenhaff *et al* (1990) suggested that in horses a higher dose of 0.6 g/kg may be necessary to produce an increase in blood bicarbonate similar to that found after 0.3 g/kg in man.[26]

In the present study, following the first bicarbonate administration the peak in the total plasma CO_2 was not as high as the subsequent administrations, and occurred later (at 6 hours). These observations suggest that the rate of absorption from the intestine was relatively slower to begin with and became more efficient with each dose. With each administration, the peak occurred within a shorter time interval: 6 hours for the first dose, 4 hours for the second dose and only 2 hours after the third dose. Absorption of bicarbonate ions occurs in the duodenum and jejunum (bicarbonate is secreted into the duodenum in both pancreatic secretions and bile, in order to neutralise the acidic stomach contents entering the duodenum). In an active but indirect mechanism, bicarbonate ions are transported out of the intestinal enterocyte cells towards the bloodstream and hydrogen ions secreted into the gut lumen, combining with bicarbonate to form CO_2, which then diffuses into the blood and is exhaled by the respiratory system. The net effect is the absorption of bicarbonate ions. The increased rate of absorption observed with each consecutive dose in the current study may have been due to increased active ion transport in the intestinal epithelium or upregulation of the enzyme carbonic anhydrase.

Author Manuscript

This article is protected by copyright. All rights reserved.

The clearance of bicarbonate from the blood also appeared to become progressively more rapid with each dose. At 10 hours after the first administration, plasma TCO_2 was still elevated around the same levels as the peak concentration (34.8 ±1.3 mmol/L after 6 hours and 34.7 ±1.5 mmol/L after 10 hours). After the second administration, levels had sharply declined from the peak concentration by 10 hours but had not yet returned to baseline. Following the third administration however, there was an even more rapid reduction in plasma concentrations, decreasing to below the pre-administration levels by 10 hours (to 30.4 ±1.2 mmol/L). This suggests that the mechanisms by which excess bicarbonate is cleared from the bloodstream (principally into the urine by the kidneys) became more efficient with each dose. Since the reabsorption of bicarbonate from the renal filtrate is the major mechanism for controlling how much bicarbonate is returned to the blood, presumably this mechanism was able to be suppressed to a greater extent with each repeated dose, increasing bicarbonate excretion in the urine. Alternatively, the exhalation of CO_2, which has entered the blood stream from the intestinal lumen as part of the bicarbonate absorption process, may have been increased. In comparison, in human athletes, McNaughton and others,[19,20] reported an increase in blood sodium, pH, bicarbonate and base excess (measured only once daily) over baseline for the duration of the trials when gelatin capsules containing sodium bicarbonate were ingested four times daily for a total daily dose of 0.5 mg/kg for 5-6 days and an ergogenic effect that lasted several days after bicarbonate ingestion ceased. [20] The authors proposed that the body stores some of the extra bicarbonate after chronic administration. However, the blood bicarbonate concentration and base excess, although remaining above baseline level, declined over the 6-day bicarbonate administration period, [20] also suggesting enhanced clearance in that study.

One other study has examined repeat doses of sodium bicarbonate in equids, giving 2 doses of 0.4 g/kg either 4 days or 7 days apart, to 3 pony geldings.[26] Blood samples were taken up to five and a half hours post administration, and no carry-over effect was observed (i.e. there were no significant differences between the first and second responses with either time interval). Given the findings of the present study, it is not surprising that no carry-over effect was observed. A preliminary study mentioned in the paper by Kowalski *et al* (1991),[27] giving two different doses (0.4 and 0.5 g/kg for the first and second doses, respectively), two days apart, produced rather inconsistent responses for the second dose in the 3 ponies, causing them to suspect the possibility of a carry-over effect. However, it seems very unlikely that a carry-over effect could have been responsible for the inconsistent responses.

On Day 4 of the current study, the 'clear day' with no bicarbonate administration, plasma TCO_2 concentrations remained low, not exceeding 32.1 mmol/L in any of the horses. Four out of the five horses remained in the range between 27.0 mmol/L (the lower limit of reporting in the analysis by the laboratory) and 30.2 mmol/L, which was below the starting baseline on Day 1 (30.9 ±0.8 mmol/L). On Day 5, the final sample indicated that plasma TCO_2 concentrations were remaining in the normal baseline range (28.6 ±1.7 mmol/L). This confirmed that after the TCO_2 concentrations rapidly returned to baseline after the third bicarbonate administration, they remained low and did not fluctuate to any significant extent.

Unlike human athletes in which beneficial efforts on performance during high intensity exercise may occur for up to 2 days after chronic sodium bicarbonate administration has ceased,[14] the potential ergogenic effect of serial administration of bicarbonate in horses has not been studied and was beyond the scope of this current study. Driller *et al*. (2012)[28] suggested that any ergogenic effect in humans may be due to increased sodium levels rather

This article is protected by copyright. All rights reserved.

than improved buffering capacity which cannot always be demonstrated after serial dosing, and that this may result in improved perfusion of muscles. [29] We did not find an alkalinising effect that persisted the day after the third administration in the current study and did not measure sodium levels. Whether an ergogenic effect may still occur in horses following chronic administration remains to be determined.

The variability in TCO_2 response to bicarbonate administration between horses is worthy of mention. The timing of when the peak TCO_2 concentrations occurred showed considerable variability after the first dose (ranging between 2 and 10 hours; Supplementary Figure 1). However the time of peak concentration became very consistent between all of the horses following the second and third doses (4 and 2 h respectively in all cases). In terms of peak response and AUC, the horses gave reasonably consistent responses throughout and there were no outliers. One horse consistently showed a slightly lower peak and AUC compared with the others, and another achieved a peak of greater than 36.0 mmol/L on each occasion (which the others did not quite achieve); but particularly by the third administration the peak responses were very similar in all horses (mean peak concentration of 35.6 mmol/L with standard deviation of only 0.7).

Regarding the time points at which samples were obtained in the present study, the timing of the peak TCO_2 concentrations were adequately captured following the first and second bicarbonate administrations, but the peak as early as 2 h following the third dose was unexpected. Therefore future studies of this type would include a 1 h sample at the very least. Also, due to the relatively flat peak and slow decline in TCO_2 after the first administration, further samples could have been included between 12 and 16 h, although after the third administration the levels were back down to baseline by 10 h.

Therefore, the conclusions from the present study are that, following the administrations of 200 g sodium bicarbonate plus 60g of Randlab Supervites® on three consecutive days, there was no apparent carry-over or accumulation of TCO_2 in the blood of horses. Following the administration of sodium bicarbonate the increase in plasma TCO_2 was short-lived and did not extend beyond 23 hours, even shortening to 10 hours by the final dose (Day 3). There was no significant increase above normal baseline levels evident during Day 4 or at the start of Day 5, and furthermore on these days the plasma TCO_2 concentration did not approach the threshold level set by racing authorities of 36.0 mmol/L.

FUNDING

Funding for this study was provided by Racing Victoria Ltd.

CONFLICT OF INTEREST

The authors have no competing interests to declare.

This article is protected by copyright. All rights reserved.

Author Manuscript

REFERENCES

1. Keyser RE. Peripheral Fatigue: High-Energy Phosphates and Hydrogen Ions. *PM R.* 2010;2(5):347-358. DOI:10.1016/j.pmrj.2010.04.009.
2. Lawrence L, Kline K, Miller-Graber P, *et al*. Effect of sodium bicarbonate on racing Standardbreds. *J Anim Sci.* 1990;68(3):673-677. DOI:10.2527/1990.683673x
3. Auer D, Skeleton K, Tay S, Baldcock F. Detection of bicarbonate administration (milkshake) in Standardbred horses. *Aust Vet J.* 1993;70(9):336-340. DOI:10.1111/j.1751-0813.1993.tb00879.x
4. Rose RJ, Lloyd DR. Sodium bicarbonate: more than just a 'milkshake'? *Equine Vet J.* 1992;24(2):75-76. DOI:10.1111/j.2042-3306.1992.tb02785.x
5. Lloyd DR, Rose RJ. Effects of sodium bicarbonate on fluid,electrolyte and acid-base balance in racehorses. *Br Vet J.* 1995;151(5):523-545. DOI:10.1016/s0007-1935(05)80024-7
6. Scarth J, Davis M, Houghton R, Houghton E, Williams R, Webbon P. The effect of sodium bicarbonate administration on equine plasma total carbon dioxide concentrations. Paper presented at: Proc 13th Int Conf Racing Anal Vet. R&W Publications, Newmarket 2001.
7. Heffron B, Benoit M, Bishop J, *et al*. Equine total carbon dioxide testing in Illinois in 2012. *J AnalToxicol.* 2014;38(8):536-540. DOI:10.1093/jat/bku095
8. Kallings P. Bicarbonate administration and its control in the horse. Paper presented at: 13th International Conference of Racing Analysts and Veterinarians 2000; Cambridge, UK.
9. Burke LM, Pyne DB. Bicarbonate loading to enhance training and competitive performance. *Int J Sports Physiol Perform.* 2007;2(1):93-97. DOI:10.1123/ijspp.2.1.93
10. Carr AJ, Hopkins WG, Gore CJ. Effects of acute alkalosis and acidosis on performance A meta-analysis. *Sports Med.* 2011;41(10):801-814. DOI:10.2165/11591440-000000000-00000
11. Siegler JC, Marshall PW, Bishop D, Shaw G, Green S. Mechanistic insights into the efficacy of sodium bicarbonate supplementation to improve athletic performance. *Sports Med Open.* 2016;2(1):41. DOI:10.1186/s40798-016-0065-9
12. Hadzic M, Eckstein ML, Schugardt M. The Impact of Sodium Bicarbonate on Performance in Response to Exercise Duration in Athletes: A Systematic Review. *J Sports Sci Med.* 2019;18(2):271-281.
13. Grgic J, Garofolini A, Pickering C, Duncan MJ, Tinsley GM, Del Coso J. Isolated effects of caffeine and sodium bicarbonate ingestion on performance in the Yo-Yo test: A systematic review and meta-analysis. *J Sci Med Sport.* 2019. DOI:10.1016/j.jsams.2019.08.016
14. Lopes-Silva JP, Reale R, Franchini E. Acute and chronic effect of sodium bicarbonate ingestion on Wingate test performance: a systematic review and meta-analysis. *J Sports Sci.* 2019;37(7):762-771. DOI:10.1080/02640414.2018.1524739
15. Frey L, Kline KH, Foreman JH, Brady A, Cooper S. Effects of warming-up, racing and sodium bicarbonate in Standardbred horses. *Equine Vet J.* 1995;27(S18):310-313.
16. Rivas L, Hinchcliff K, Kohn C, Sams R, Chew D. Effect of sodium bicarbonate administration on renal function of horses. *Am J Vet Res.* 1997;58(6):664-671.
17. Auer D, Hughes K, Vine J, *et al*. Measurement of total plasma carbon dioxide concentrations in thoroughbreds using ASE linearity set. Paper presented at: Proceedings of the 13th International Conference of Racing Analysts and Veterinarians 2001; Newmarket, UK.
18. Hibbert DB, Armstrong N and Vine JH. Total CO_2 measurements in horses: where to draw the line. *Accreditation and Quality Assurance.* 2011;16(7):339-345.
19. McNaughton L, Backx K, Palmer G, Strange N. Effects of chronic bicarbonate ingestion on the performance of high-intensity work. *Eur J Appl Physiol Occup Physiol.* 1999;80(4):333-336. DOI:10.1007/s004210050600
20. McNaughton L, Thompson D. Acute versus chronic sodium bicarbonate ingestion and anaerobic work and power output. *J Sports Med Phys Fitness.* 2001;41(4):456.
21. Tobias G, Benatti FB, de Salles Painelli V, *et al*. Additive effects of beta-alanine and sodium bicarbonate on upper-body intermittent performance. *Amino Acids.* 2013;45(2):309-317. DOI:10.1007/s00726-013-1495-z

This article is protected by copyright. All rights reserved.

Author Manuscript

22. Douroudos II, Fatouros IG, Gourgoulis V, *et al*. Dose-related effects of prolonged NaHCO3 ingestion during high-intensity exercise. *Med Sci Sports Exerc.* 2006;38(10):1746-1753. DOI:10.1249/01.mss.0000230210.60957.67
23. Oliveira L, de Salles Painelli V, Nemezio K, *et al*. Chronic lactate supplementation does not improve blood buffering capacity and repeated high-intensity exercise. *Scand J Med Sci Sports.* 2017;27(11):1231-1239. DOI:10.1111/sms.12792
24. Delextrat A, Mackessy S, Arceo-Rendon L, Scanlan A, Ramsbottom R, Calleja-Gonzalez J. Effects of Three-Day Serial Sodium Bicarbonate Loading on Performance and Physiological Parameters During a Simulated Basketball Test in Female University Players. *Int J Sport Nutr Exerc Metab.* 2018;28(5):547-552. DOI:10.1123/ijsnem.2017-0353
25. Hibbert DB, Stenhouse A, Murphy C, *et al*. A GUM-compliant uncertainty budget for the analysis of total carbon dioxide (TCO_2) in equine plasma. *Accred Qual Assur.* 2008;13:523-530.
26. Greenhaff P, Snow D, Harris R, Roberts C. Bicarbonate loading in the Thoroughbred: dose, method of administration and acid-base changes. *Equine Vet J Suppl.* 1990;22(S9):83-85. DOI:10.1111/j.2042-3306.1990.tb04741.x
27. Kowalski J, Roberts A, Williams J, Hintz H, Daniluk P, Schryver H. Effect of interval between doses on response of the pony to sodium bicarbonate. *Cornell Vet.* 1991;81(1):59-66.
28. Driller MW, Gregory JR, Williams AD, Fell JW. The effects of serial and acute $NaHCO_3$ loading in well-trained cyclists. *J Strength Cond Res.* 2012;26(10):2791-2797. DOI:10.1519/JSC.0b013e318241e18a
29. Mitchell TH, Abraham G, Wing S, *et al*. Intravenous bicarbonate and sodium chloride both prolong endurance during intense cycle ergometer exercise. *Am J Med Sci.* 1990;300(2):88-97. DOI:10.1097/00000441-199008000-00004

Figure Legends

Figure 1. Timeline of sodium bicarbonate administrations and sample collections.

Figure 2. Plasma Total CO_2 in blood samples from 5 horses following serial administration of 200 g sodium bicarbonate via nasogastric tube. Each point represents the mean value ± standard deviation of the mean.

Figure 3. Plasma Total CO_2 in blood samples from 5 horses each day following 3 daily administrations of 200 g sodium bicarbonate via nasogastric tube. Each point represents the mean value ± standard deviation of the mean. The dotted line represents the threshold level of 36.0 mmol/L.

Supplementary Table 1. Bicarbonate administration protocol.

Supplementary Figure 1. Plasma Total CO_2 in blood samples from each individual horse following 3 daily administrations of 200 g sodium bicarbonate via nasogastric tube.

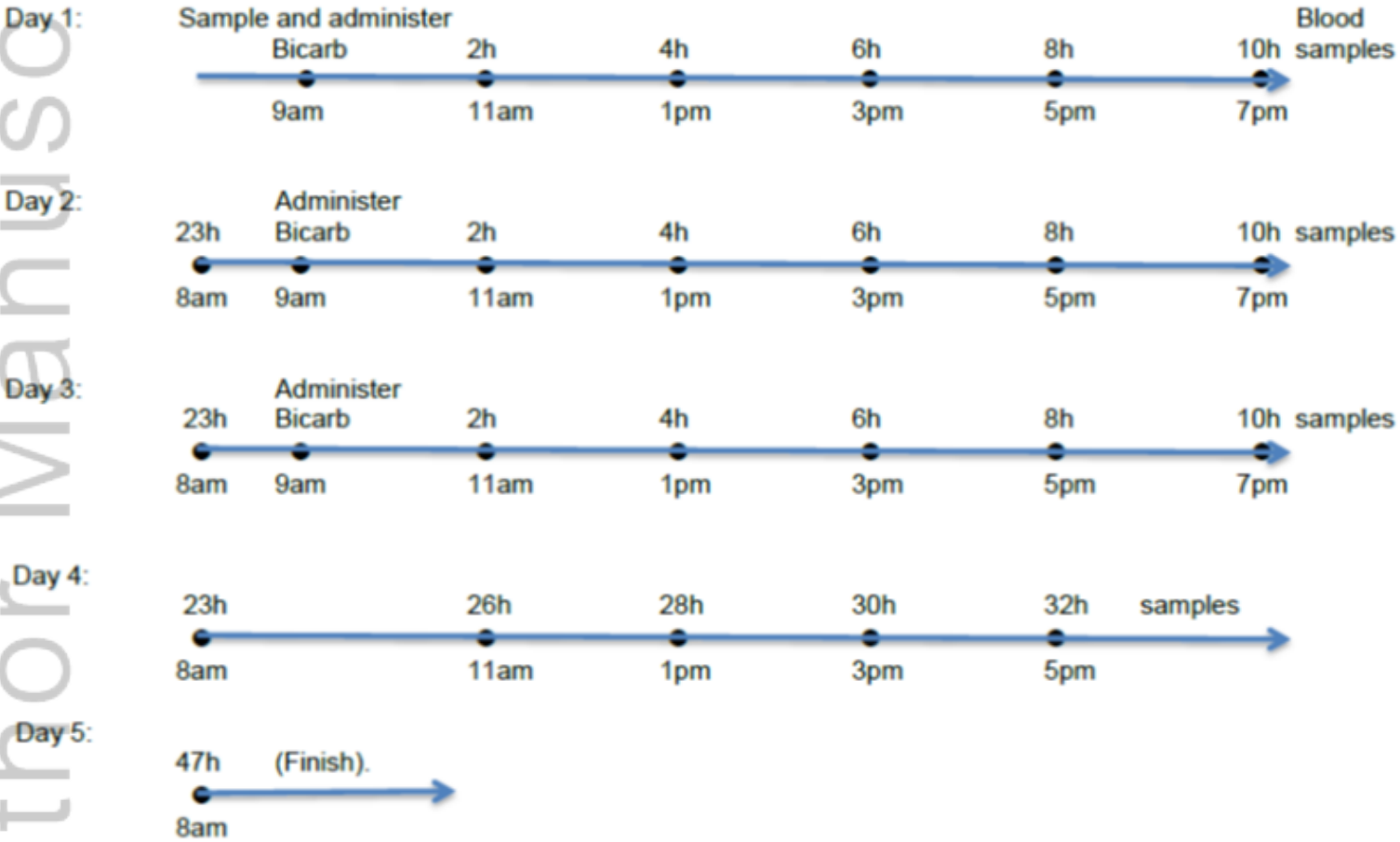
Supplementary Figure 2. Plasma Total CO_2 in blood samples from each individual horse following serial administration of 200 g sodium bicarbonate via nasogastric tube.

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

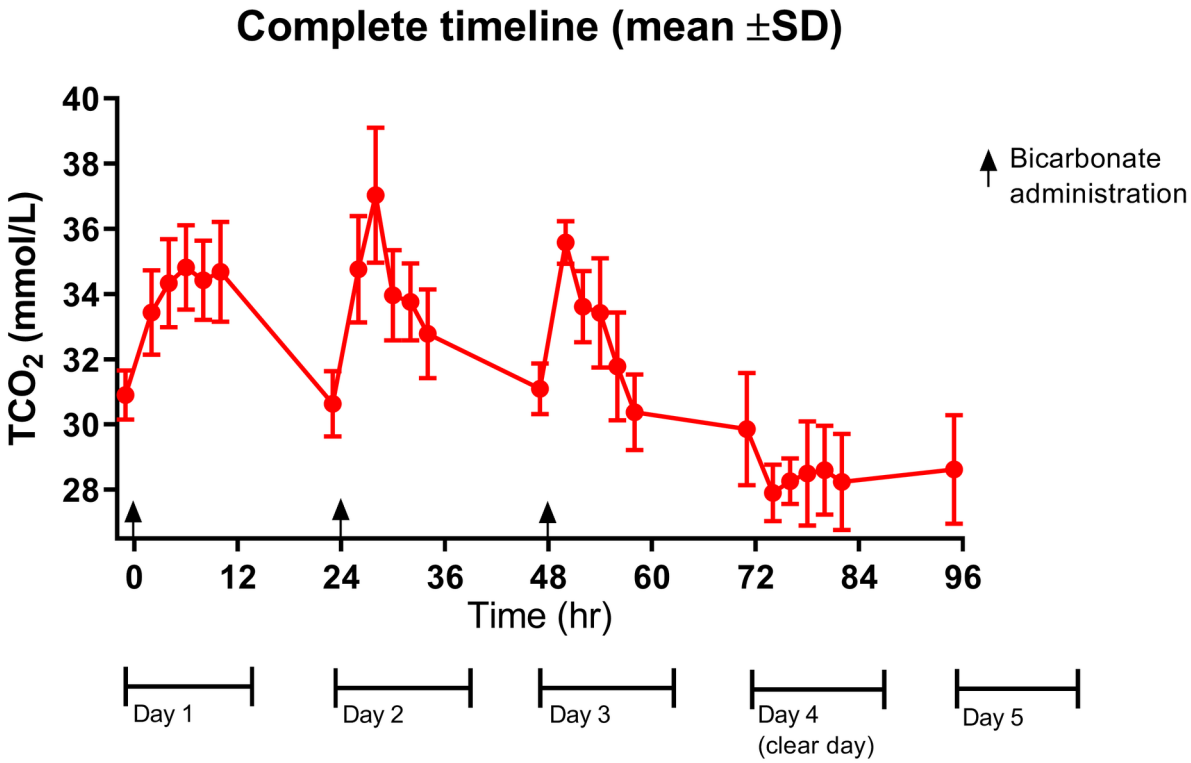
Figure 1: Study timeline.



DTA_2937_Figure1.tif

This article is protected by copyright. All rights reserved.

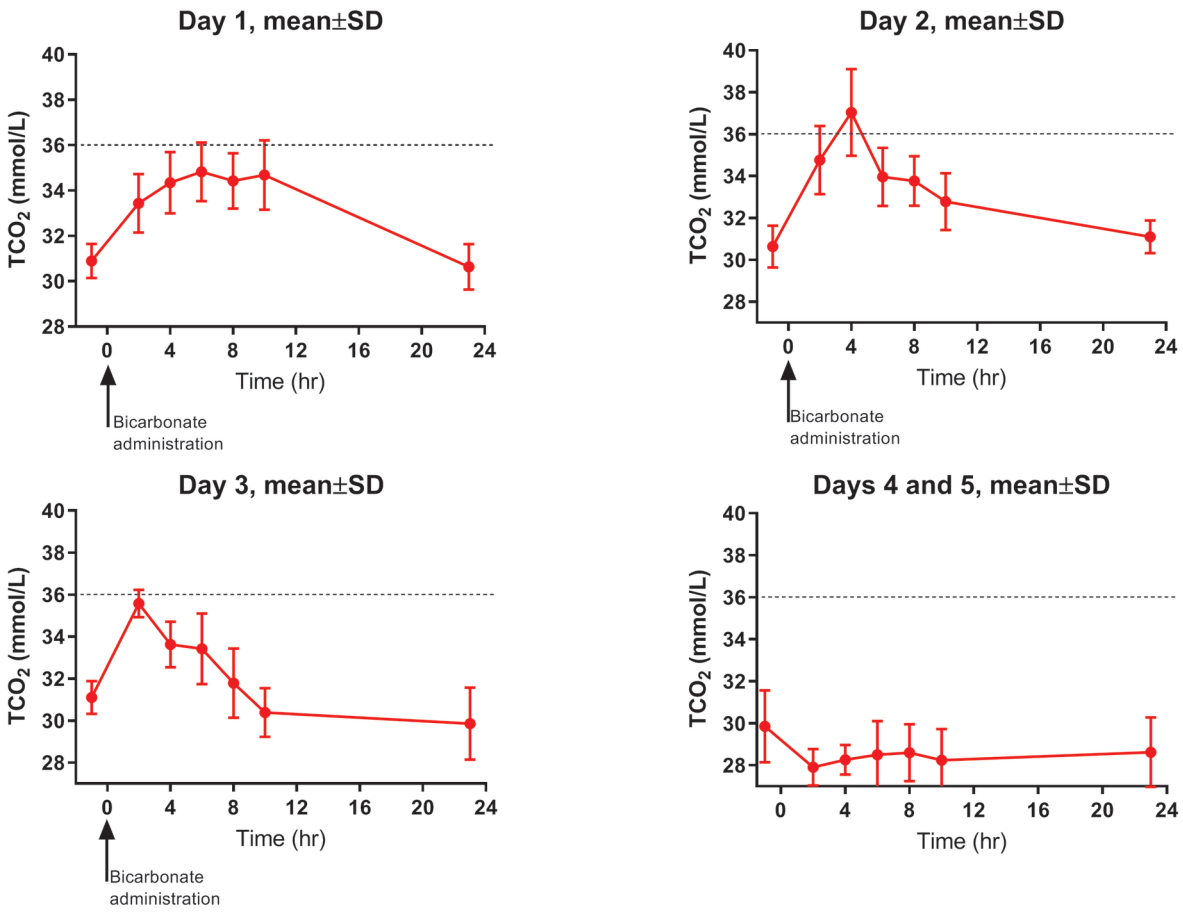
Author Manuscript



DTA_2937_Figure 2 Complete timeline means.tif

This article is protected by copyright. All rights reserved.

Author Manuscript



DTA_2937_Figure 3 Means by day.tif

This article is protected by copyright. All rights reserved.