EQUINE VETERINARY JOURNAL



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*Equine vet. J.* (2010) **42** (Suppl. 38) 76-82
doi: 10.1111/j.2042-3306.2010.00213.x

# Acid-base imbalances during a 120 km endurance race compared by traditional and simplified strong ion difference methods

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**Keywords:** horse; endurance competition; acid-base; electrolyte alterations; strong ion approach

## Summary

***Reasons for performing study:*** **Acid-base disturbances are traditionally assessed using the Henderson-Hasselbach equation. The simplified strong ion approach describes more accurately the complex acid-base and electrolyte abnormalities present in endurance horses.**

***Objective:*** **To describe acid-base and electrolytes changes in fit horses competing in a FEI*** 120 km endurance race and to compare the traditional vs. strong ion approaches.**

***Methods:*** **Thirty horses were initially enrolled in the study. Venous blood samples were obtained before the race (n = 25), at the second (n = 29; 65.4 km) and third vet-gates (n = 23, 97.4 km) and upon race completion (n = 17). Blood gas analysis was performed to determine pH, $PCO_2$, $PO_2$, $Na^+$, $K^+$ and $iCa^{++}$, and calculate $HCO_3^-$, base excess and $tCO_2$. Packed cell volume and total protein, globulin, albumin, lactate, phosphate, glucose and creatinine concentrations, as well as muscle enzymes activities, were also determined. Calculated variables included strong ion difference (SIDm), strong ion gap (SIG) and nonvolatile buffer concentration ($A_{tot}$). A longitudinal linear model using the general estimating equation methodology was used for statistical analysis.**

***Results:*** **Mild but significant increases in $PCO_2$, SIDm, lactate, plasma protein, globulins and $A_{tot}$, as well as a decrease in potassium concentrations were observed from the second vet-gate to race finish when compared to prerace values ($P<0.05$). Using the strong ion approach, 67% samples showed acid-base disturbances vs. 70% when using the traditional method, but their interpretations only matched in 24% of measurements.**

***Conclusions:*** **A complex acid-base imbalance characterised by a mild strong ion alkalosis (hypochloraemia attenuated by hyperlactataemia), nonvolatile buffer acidosis and compensatory mild respiratory acidosis were present in most horses, although pH did not significantly change during a 120 km endurance race. The strong ion approach to interpretation of acid-base balance should be favoured over the traditional approach in endurance horses, given the frequent and complex alterations in $PCO_2$, SIDm and $A_{tot}$ during a race.**

## Introduction

Acid-base and electrolyte disorders have been described in horses associated with prolonged exercise and competitive endurance races (Rose *et al*. 1980; Rose and Sampson 1982; Snow *et al*. 1982; Schott *et al*. 2006). Two different methods have been used during recent decades to describe these alterations in horse plasma: the traditional approach uses the Henderson-Hasselbach equation and is clinically adequate to describe acid-base imbalances. However, it is more descriptive than mechanistic and is only accurately applied to plasma with approximately normal protein, albumin and haemoglobin concentrations (Constable 1997). On the other hand, the simplified strong ion model offers a quantitative indepth insight into the pathophysiology of acid-base disorders. This quantitative approach explains how pH can be affected by alterations in plasma protein and phosphate concentrations, as well as by changes in the concentration of strong ions such as sodium and chloride (Constable 1997), which can be very useful in deciding, specific treatments if horses develop metabolic disorders.

The interpretation of changes in acid-base balance occurring during athletic events is complicated because the main variables change simultaneously, often in opposite directions. These complex alterations require a method that evaluates acid-base equilibrium not focused on dependent variables (pH, bicarbonate) like the traditional approach, but an alternative method that evaluates alterations using independent variables (such as partial venous pressure of $CO_2$, strong ion difference and nonvolatile weak buffer concentrations).

Recent studies of acid-base balance in endurance horses have determined the alterations of some variables using a quantitative approach (Hoffman *et al*. 2002; Hess *et al*. 2005) or electrolyte disturbances (Schott *et al*. 2006), but no interpretation of acid-base balance was made according to this method. To the authors' knowledge, no comparison between the traditional and quantitative methods has been previously described in endurance competitions or a comprehensive characterisation of acid-base disorders by the quantitative approach in an endurance race setting. The purpose of this study was to compare changes detected in acid-base balance and electrolyte status using traditional and quantitative approaches

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*[Paper received for publication 05.01.10; Accepted 11.06.10]*

and to describe the observed alterations during a 120 km FEI*** endurance race.

## Materials and methods

### *Horses and study protocol*

The study was performed in a 120 km regional championship race equivalent to a FEI*** endurance race with mean environmental temperature of 10°C (9–14.5°C) and relative humidity of 96% (73–100%). After being informed about the purpose and requirements of the study, 30 competitors consented to participate. Horses were Arabians, or Arabian-crossbreds, had been transported varying distances and arrived at a common stable 12–24 h before the race. Sex distribution was 19 geldings, 5 mares and in 6 cases the information was not registered. Mean ± s.d. age was 9 ± 1.3 years. Horses enrolled in the study received various types of electrolyte supplement and nutrition before and during the race. A standardised FEI veterinary examination (vet-gate) was performed before the race, at 30, 65.4, 97.4 km and at the end of the race (120 km). The mean ± s.d. velocity of horses was 15.2 ± 1.6 km/h.

### *Sample collection and analysis*

Blood samples were taken within 1–2 h of starting the race from 25 of 30 horses, at the second vet-gate (65.4 km) from 29 horses, at the third vet-gate (97.4 km) from 23 horses and within 10 min of finishing the race (120 km) from 17 horses. Blood samples were obtained anaerobically by jugular venipuncture into 10 ml evacuated heparinised tubes (lithium heparin, Venoject)[1]. Blood gas determination was performed immediately after sample collection. Samples were kept in ice for up to 20 min, after which packed cell volume (PCV), blood glucose and lactate were determined. The remaining heparinised blood samples were centrifuged at 1500 ***g*** for 15 min to obtain plasma samples, which were divided into 3 aliquots of 1–1.5 ml and frozen at –20°C until further analysis. Plasma samples were maintained in dry ice for shipping. Rectal temperature was recorded for each horse at the time of venous sampling. Blood gas analysis was performed using a portable analyser (I-Stat with EG7+ cartridges)[2] to determine pH, partial venous pressure of $CO_2$ ($PCO_2$) and partial venous pressure of $O_2$ ($PO_2$) corrected by temperature and concentrations of sodium [$Na^+$], potassium [$K^+$], ionised calcium [$iCa^{++}$] and haemoglobin (Hb). The handheld device calculated bicarbonate [$HCO_3^-$] and total $CO_2$ ($tCO_2$) concentrations using pH and $PCO_2$ values and the Henderson-Hasselbach equation (West 1995). Blood glucose and lactate concentrations were determined using portable hand-held devices (Accucheck Glucose, Accucheck Lactate)[3]. PCV was determined using a microhaematocrit centrifuge and total plasma protein (TP) by direct refractometry. Plasma concentrations of chloride [$Cl^-$], creatinine, creatine kinase (CK), aspartate aminotransferase (AST), albumin, globulins and phosphates ($P_i$) were determined by standard colorimetric biochemical procedures.

### *Calculated parameters*

Traditional analysis was completed calculating anion gap (AG) from the equation described by Emmett and Narins (1977) and base excess (BE) obtained from the Henderson-Hasselbach formula in conjunction with Siggaard-Anderson equation:

$$AG = \left(Na^+ + K^+\right) - \left(Cl^- + HCO_3^-\right)$$

$$BE = 0.02786 \times PCO_2 \times 10^{(pH-6.1)} + 13.77 \times (pH - 124.58)$$

Values of $HCO_3^-$ and $tCO_2$ were calculated by the handheld device using the following equations:

$$HCO_3^- = S_{CO2} \times pCO_2 \times 10^{(pH-pK'1)}$$

$$tCO_2 = HCO_3^- + 0.03 \times pCO_2$$

where $pK'_1$ is the apparent dissociation constant, which has an estimated value of 6.1 and is obtained from the sum of pKs (6.038 at 37°) and the negative logarithm of the activity coefficient of the hydrogen ion (0.091) generating a value of 6.129 and the value used for $CO_2$ solubility ($S_{CO2}$) in plasma at 37°C was 0.03 mEq/l (Constable 1997).

Quantitative analysis of acid base balance was assessed using the method described by Stewart (1983) and simplified by Constable (1997). Measured strong ion difference (SIDm), strong ion gap (SIG) and total nonvolatile buffers ($A_{tot}$) were calculated using the following formulas:

$$SIDm = \left(Na^+ + K^+\right) - \left(Cl^- + lactate\right)$$

$$A_{tot} = 2.25 \times albumin + 1.4 \times globulin + 0.59 \times P_i$$

$$SIG = \frac{Atot}{1 + 10^{(pKa - pH)}} - AG \quad \text{(Constable } \textit{et al.} \text{ 1998)}$$

The value used for $K_a$ of plasma ($2.22 \times 10^{-7}$ Eq/l; $pK_a = 6.65$) is that experimentally determined for horses by Constable (1997).

Finally, plasma osmolarity was calculated as:

$$\text{Osmolarity} = 2(Na + K) + Glucose + \frac{Urea + 0.47}{2.8}$$

Interpretation of acid-base status was performed using a bicarbonate based traditional approach and the quantitative strong ion difference based analysis, and the agreement between the two methods of interpretation was assessed. In order to categorise acid-base status by the traditional and quantitative methods the measured values of pH, $PCO_2$, $HCO_3^-$, blood lactate concentration, SIDm and $A_{tot}$ were compared to the reference range detailed on Table 1. For interpretation of SIDm and $A_{tot}$, the mean ± 2 s.d. of prerace values were used as reference range due to the lack of reported values in endurance horses.

### *Statistical analyses*

Data are shown as mean ± s.e. for all dependent variables, except for CK and AST, which are reported as median (25th–75th percentiles). The study variables (i.e. pH, [$K^+$], [$Na^+$], [$Cl^-$], [$iCa^{++}$], [$HCO_3^-$], $tCO_2$, PCV, SIDm, SIG, $A_{tot}$, lactate, CK, AST, creatinine, glucose, BE, albumin, globulins, TP and calculated osmolarity) were analysed by means of a longitudinal linear model using the general estimating equation (GEE) methodology to account for intrasubject correlations for phases completed with the assumption of unstructured correlation matrix and, in the case of nonconvergence, first degree dependence of data was assumed. Of the 30 horses enrolled in the study, prerace blood samples were not obtained from 5 horses (*Horses 1*, *15*, *23*, *25* and *27*) and we assumed the average prerace values of the other 25 horses was an

adequate approximation when performing the statistical analysis. The last observation carried forward (LOCF) method was applied to impute the missing values on the dependent variables of eliminated horses at the second or third vet-gates. Bonferroni correction was used when performing multiple comparisons. All data were analysed with statistical analysis software (SPSS version 15) and values of $P \leq 0.05$ were considered statistically significant.

## Results

Seventeen horses completed the race; 10 horses were eliminated for lameness (4 in the second vet-gate and 6 in the third vet-gate) and 3 for metabolic problems (2 at the second control and one at the end of the race, all due to mild dehydration). One of the 30 horses was eliminated for the purpose of this study (*Horse 22*) because it was retired for lameness before the race mid-point and a second blood sample was not obtained. Mean speed of finishers was 15.6 km/h with a range of 9.5–21 km/h.

Parameters used in the quantitative acid base balance evaluation are shown in Figure 1 and the available data plus imputed missing values for all dependent variables are presented in Table 1. A mild but significant ($P = 0.047$; Table 1) decrease in blood pH was detected with increasing distance (Fig 1), but no statistically significant differences were found between phases, whilst $PCO_2$, $HCO_3^-$, $tCO_2$ and calculated BE showed an initial increase (Table 1, $P<0.001$) with a tendency to decrease from the race mid-point to the end. Endurance exercise resulted in a mild but significant increase in blood lactate that reached a plateau from mid-race onwards (Table 1, $P = 0.04$). Distance completed had an effect on plasma or blood electrolyte concentrations ($P<0.001$; Table 1), characterised by a modest and sustained decrease in sodium, chloride and ionised calcium and a slight decrease in potassium concentrations that recovered by the end of the race. Strong ion difference (SIDm) showed a significant (Fig 1 and Table 1, $P<0.001$) increase due to the combined effects of a moderate decrease in $Cl^-$ and mild increase of blood lactate concentrations. PCV, TP and creatinine concentrations increased significantly (Table 1, $P<0.001$) with increasing distance. Similarly, albumin, globulin, phosphate and $A_{tot}$ concentrations initially increased significantly (Table 1, $P<0.001$) but these stabilised during the second half of the race. Strong ion gap increased significantly ($P<0.001$; Table 1) with distance until third phase, returning to resting values by the end of the race. Blood glucose concentration remained stable except for a ~20% decrease detected at the third vet-gate ($P<0.001$; Table 1). Finally, no significant changes in calculated plasma osmolarity along the race were detected.

The interpretation of acid-base balance of each horse at the end of the race (or at the time of elimination) showed a poor agreement between the traditional and quantitative approaches given that it only matched in 3 cases (Table 2 and Fig 2). All 94 blood gas determinations performed in this study were evaluated using a quantitative and traditional approach and the interpretation only matched in 33 of 94 of these. Using a traditional approach, 66/94 (70%) determinations of acid-base status presented detectable alterations (25 lactic metabolic acidosis, 2 respiratory acidosis and 5 metabolic alkalosis) and the remaining 34 of these 66 had complex imbalances (18 had a combination of the 3 above mentioned derangements and the other 16 had combinations of 2 of these conditions). Using a quantitative evaluation of acid-base balance, 63/94 (67%) blood gas analyses presented alterations of which 12 were nonvolatile buffers ($A_{tot}$) metabolic acidosis, one metabolic acidosis due to decrease in SIDm, 4 metabolic alkalosis due to SIDm and 7 respiratory acidosis and the remaining 39 of these 63 analyses showed mixed acid base disturbances (9 respiratory acidosis with

**TABLE 1: Mean ± s.e. values (except for CK and AST reported as median and 25th–75th percentiles) before, during and at the end of a 120 km FEI ride for all horses**

| | Reference range† | Prerace n = 25 | Second vet-gate n = 29‡ | Third vet-gate n = 23‡ | Finish n = 17‡ |
|---|---|---|---|---|---|
| pH | 7.31–7.45 | 7.40 ± 0.01 | 7.40 ± 0.01 | 7.39 ± 0.01 | 7.39 ± 0.01 |
| SIDm (mmol/l) | 35–38.5 | 37 ± 0.2 | 39 ± 0.5* | 39 ± 0.8* | 38 ± 0.5* |
| $PCO_2$ (mmHg) | 41–53 | 47 ± 0.5 | 52 ± 0.9* | 52 ± 0.9* | 50 ± 0.9* |
| $HCO_3^-$ (mEq/l) | 24–30 | 29 ± 0.3 | 32 ± 0.7* | 31 ± 0.6* | 30 ± 0.6 |
| $Na^+$ (mEq/l) | 134–144 | 137 ± 0.33 | 137 ± 0.6 | 135 ± 0.8 | 134 ± 1.0 |
| $K^+$ (mEq/l) | 3.5–4.5 | 3.5 ± 0.1 | 3.1 ± 0.1* | 3.1 ± 0.1* | 3.2 ± 0.1* |
| $Cl^-$ (mEq/l) | 90–100 | 102 ± 0.4 | 99 ± 0.1* | 97 ± 1.3* | 96 ± 1.3* |
| Lactate (mmol/l) | <2 | 1.2 ± 0.1 | 2.6 ± 0.1* | 2.7 ± 0.1* | 2.6 ± 0.2* |
| $P_i$ (mg/l) | 15–45 | 27 ± 1.0 | 33 ± 2.0* | 37 ± 2.0* | 35 ± 2.0* |
| BE (mEq/l) | −6 to +6 | 4.4 ± 0.3 | 7.2 ± 0.8* | 6.2 ± 0.7* | 5.3 ± 0.7 |
| $tCO_2$ (mmHg) | 28–35 | 30.4 ± 0.3 | 33.3 ± 0.7* | 32.5 ± 0.7* | 31.1 ± 0.7 |
| PCV (%) | 36–44 | 38.2 ± 0.8 | 45.2 ± 0.9* | 46.5 ± 1* | 45.8 ± 1.1* |
| TP (g/l) | 65–75 | 66 ± 1.0 | 74 ± 1.0* | 75 ± 1.0* | 75 ± 1.0* |
| Albumin (g/l) | 34–47 | 35 ± 0.4 | 4.0 ± 0.6* | 4.0 ± 0.7* | 4.0 ± 0.6* |
| Globulin (g/l) | 26–36 | 30 ± 0.7 | 34 ± 0.9* | 33 ± 0.9* | 33 ± 0.9* |
| $A_{tot}$ (mEq/l) | 12–15 | 12.9 ± 0.14 | 15.6 ± 0.3* | 15.8 ± 0.3* | 15.7 ± 0.3* |
| SIG | −2 to +6 | 1.8 ± 0.2 | 3.4 ± 0.5* | 4.4 ± 0.7* | 2.6 ± 0.5 |
| Glucose (mmol/l) | 5.45–5.98 | 6.2 ± 0.1 | 6.4 ± 0.21 | 5.4 ± 0.3* | 6.3 ± 0.3 |
| $iCa^{++}$ (mmol/l) | 1.4–1.6 | 1.6 ± 0.01 | 1.5 ± 0.03* | 1.4 ± 0.03* | 1.5 ± 0.03* |
| Creatinine (mg/l) | <20 | 12 ± 0.3 | 16 ± 0.6* | 17 ± 0.8* | 17 ± 0.7* |
| CK (iu/l) | 100–300 | 250 (181.3–299.5) | 594 (460–1705) | 1,035 (767–2943) | 1,649 (1254–3037)* |
| AST (iu/l) | 150–400 | 325 (276–379) | 399 (334–542) | 450 (370–630) | 450 (416–603)* |
| Osmolarity (mOsm/kg) | 270–300 | 292.5 ± 0.6 | 293.9 ± 1.15 | 290.9 ± 1.5 | 290 ± 1.7 |

Summarised data are all available observations plus missing values imputed by LOCF to perform statistical analysis by GEE models. *Significant difference compared to prerace values; †(Soma *et al.* 1996; Constable 1997; Kingston 2004; Navarro *et al.* 2005). ‡Number of horses at the end of each phase.

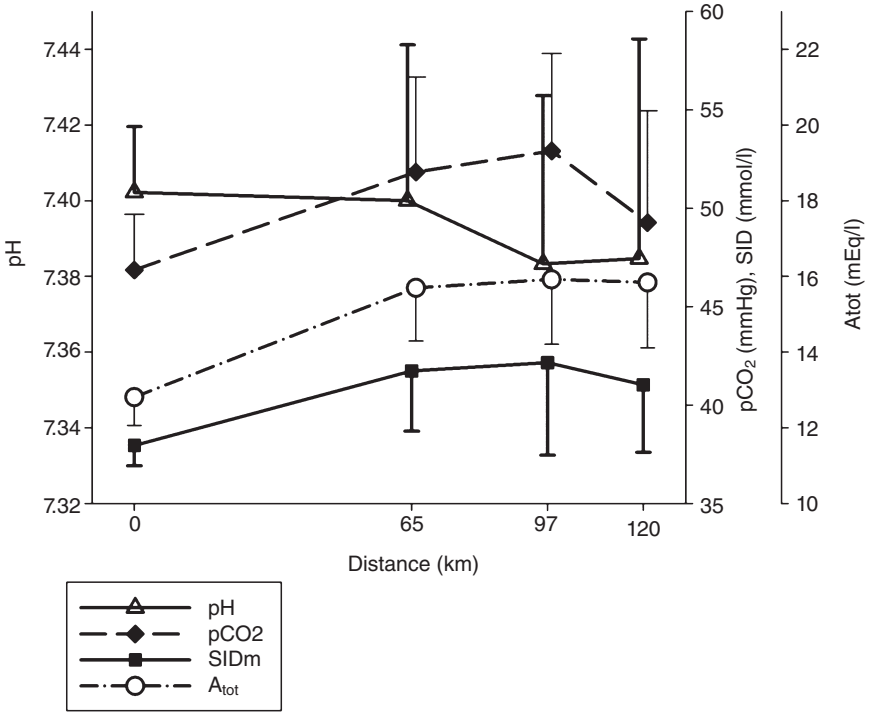


*Fig 1: Mean ± s.e. temperature corrected pH, strong ion difference (SIDm), $PCO_2$ and nonvolatile weak buffer concentration ($A_{tot}$) in horses during a 120 km FEI*** endurance race. All available data are presented without imputation of missing values by LOCF.*

**TABLE 2: Interpretation of acid-base balance of all horses at their last time point (at the time of elimination or the race finish, n = 29) and of all finishing horses (n = 17) using the traditional and the quantitative approaches**

| | Competitors (n = 29) | Finishers (n = 17) | Interpretation |
|---|---|---|---|
| Traditional approach | 11 | 6 | Lactic metabolic acidosis |
| | 6 | 2 | Metabolic alkalosis + lactic metabolic acidosis |
| | 1 | 1 | Respiratory acidosis |
| | 5 | 2 | Respiratory acidosis + metabolic alkalosis + lactic metabolic acidosis |
| | 3 | 3 | Respiratory acidosis + lactic metabolic acidosis |
| | 3 | 3 | Normal |
| Quantitative approach | 3 | 0 | Lactic metabolic acidosis |
| | 2 | 2 | SIDm metabolic acidosis |
| | 1 | 1 | SIDm metabolic alkalosis |
| | 2 | 1 | SIDm metabolic alkalosis + lactic metabolic acidosis |
| | 4 | 3 | $A_{tot}$ metabolic acidosis + lactic metabolic acidosis |
| | 8 | 4 | $A_{tot}$ metabolic acidosis + SIDm metabolic alkalosis + lactic metabolic acidosis |
| | 3 | 2 | Respiratory acidosis + lactic metabolic acidosis |
| | 1 | 1 | Respiratory acidosis + $A_{tot}$ metabolic acidosis + Lactic metabolic acidosis |
| | 1 | 1 | Respiratory acidosis + $A_{tot}$ metabolic alkalosis + Lactic metabolic acidosis |
| | 1 | 1 | Respiratory acidosis + $A_{tot}$ metabolic acidosis + SIDm metabolic alkalosis |
| | 3 | 1 | Respiratory acidosis + $A_{tot}$ metabolic acidosis + SIDm metabolic alkalosis + lactic metabolic acidosis |

metabolic alkalosis due to SIDm and acidosis due to $A_{tot}$, 14 metabolic alkalosis due to SIDm with metabolic acidosis due to $A_{tot}$ and other mixed disorders).

## Discussion

Recent studies in endurance horses evaluated changes in acid-base balance and electrolyte concentrations using a quantitative approach (Hoffman *et al*. 2002; Hess *et al*. 2005), but a detailed description of quantitative acid-base alterations was not provided. The main findings of the present study of acid-base balance in fit endurance horses during a 120 km FEI*** endurance race are: 1) the presence of a complex acid-base imbalance in most horses characterised by a mild strong ion alkalosis (hypochloraemia) attenuated by mild lactic acidosis, nonvolatile buffer ion acidosis and compensatory mild respiratory acidosis and 2) poor agreement between the interpretation of acid-base balance using the traditional and quantitative approaches.

The traditional or Henderson-Hasselbach approach has been used applied to horse plasma since 1964 (Gillespie *et al*. 1964). This is a simple method of interpretation that does not require determination of a large number of parameters, but has 4 important limitations: 1) inexact results when protein or electrolytes alterations are present; 2) the approach is based on 2 dependent

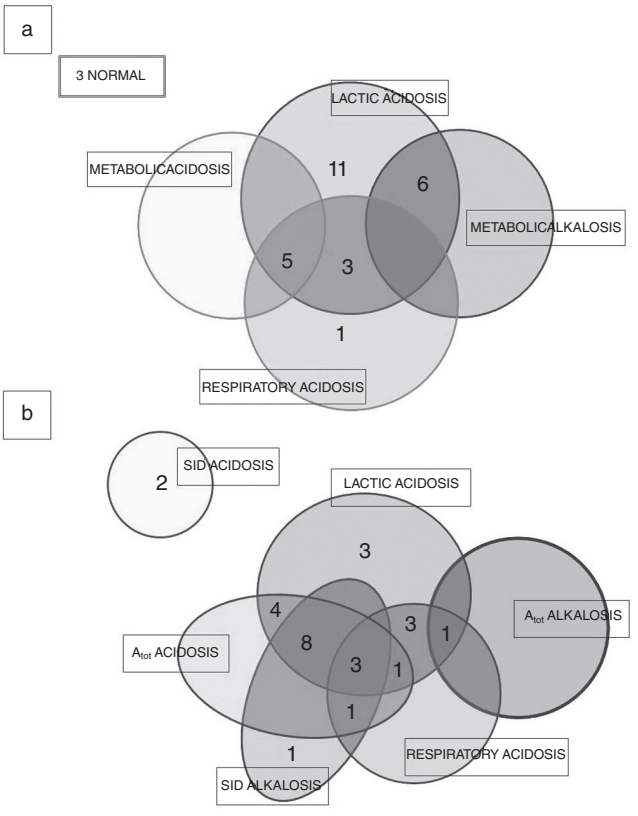


*Fig 2: Interpretation of acid-base balance at the race finish (or at the vet-gate where eliminated) of each horse using the traditional (a) or the quantitative approaches (b).*

variables (pH and $HCO_3^-$) and an independent variable ($PCO_2$); 3) disorders are classified into a limited number of categories compared with the quantitative method and 4) it does not help guide a fluid therapy regime, should it be necessary. For these reasons the Henderson-Hasselbach approach to acid-base disturbances is not the most appropriate method when there are alterations in protein, $Na^+$ or $Cl^-$ concentrations (Constable 1997). These derangements are common in endurance horses where large changes in electrolyte and protein concentrations can occur due to sweat losses and haemoconcentration.

Using the traditional approach to acid-base assessment the most consistent alterations found in this study were mild metabolic lactic acidosis and complex changes characterised by mild metabolic alkalosis plus mild compensatory respiratory acidosis.

Previous studies in endurance horses have shown changes in pH, $PCO_2$, $Na^+$, $K^+$, $iCa^{2+}$, $Cl^-$, lactate, glucose, CK, AST, TP, albumin and creatinine concentrations as a function of speed (Hoffman *et al*. 2002; Schott *et al*. 2006), reporting more severe disturbances in horses running at higher speed (15–20 km/h) when compared to lower speed (8–12 km/h). In the present study, performed with high level competition horses and high mean velocity (15.6 km/h), alterations of the different variables were evaluated for disturbances related to distance rather than speed. Changes observed in this study were similar to those found in studies that correlate the evaluated variables with high velocity. However, our study was performed in favourable environmental conditions at fast speed and only mild changes were observed in the studied variables.

The first variable used for traditional assessment is pH. In the present study statistically but not clinically significant changes in pH were detected with increasing distance, as was expected from the previously reported positive association with speed (Hoffman *et al*. 2002; Hess *et al*. 2005).

The resting $PCO_2$ values in this study were slightly high compared with reference values for horses in some (Navarro *et al*. 2005) but not all studies (Soma *et al*. 1996). The initial decrease of $PCO_2$ described in the literature (Lindinger and Waller 2008) due to increased alveolar ventilation was not found in our study probably because the first blood sample during exercise was delayed until the second vet-gate at 65.4 km and at this point of the race the initial hyperventilatory response was exceeded and $PCO_2$ had began to accumulate.

The third variable used in traditional approach to acid-base balance is $HCO_3^-$. Plasma concentration of $HCO_3^-$ is determined by $PCO_2$ and should decrease to compensate for a lactate increase (Aguilera-Tejero *et al*. 2000). In the present study, an increase of $PCO_2$ was detected, which would lead to an increase in $HCO_3^-$, but the simultaneous increase in lactate blunted this elevation in $HCO_3^-$. Base excess is used to quantify the nonrespiratory aspect of acid-base balance determinations. In this study it had a tendency to increase indicating a slight increase of $HCO_3^-$ not clearly detected with direct determinations.

Finally, in the traditional approach an increase in anion gap is used as indirect evidence of increased blood lactate concentration when direct measurement is not possible. In the present study blood lactate concentration was directly measured with a portable analyser, which has been previously validated in horses (Tennent-Brown *et al*. 2007) and used in studies of exercise-induced changes in blood lactate (Baldari *et al*. 2009).

Evaluation of acid-base balance using traditional approach only takes into account pH, $PCO_2$, BE, $HCO_3^-$ and AG. This provides limited information and only allows classification of alterations in respiratory alkalosis or acidosis, and metabolic alkalosis or acidosis. In endurance horses it is necessary to evaluate the magnitude of electrolyte changes and effect of haemoconcentration (increase in protein concentration). Most of the endurance horses in the present study showed mixed alterations that did not result in significant changes in pH, but important alterations can be present and not detected using the traditional approach. In contrast, quantitative analyses allows differentiating metabolic acidosis or alkalosis due to changes in electrolytes (SIDm), lactate or due to changes in nonvolatile buffer concentration ($A_{tot}$) and respiratory alkalosis or acidosis.

Stewart's approximation of acid-base balance has been previously used in man before and after exercise using venous blood samples (Weinstein *et al*. 1991) and has also been evaluated for showjumper horses (Aguilera-Tejero *et al*. 2000). To the authors' knowledge, there are no studies comparing the quantitative and traditional assessment of acid-base analyses in endurance horses. The quantitative approach uses 3 independent ($PCO_2$, SIDm and $A_{tot}$) and one dependent variable (pH) for the assessment of acid-base equilibrium. This approach has important advantages for the evaluation of endurance horses: 1) it takes into account the contribution of nonvolatile weak buffers such as proteins (albumin and globulins) and of strong ions on pH and 2) complex alterations of acid-base balance are easier to detect. The quantitative approach allows improved interpretation of acid-base equilibrium when electrolytes or protein concentrations are altered, but requires measurement of multiple parameters and use of complex equations.

In the quantitative method, pH and $PCO_2$ are interpreted as in the traditional approach. The difference between the methods is focused on assessment of metabolic disturbances. In the present study, the most frequent alteration found using the quantitative method was metabolic alkalosis due to increased SIDm, as well as complex alterations characterised by metabolic alkalosis due to SIDm plus metabolic acidosis due to $A_{tot}$. Hoffman *et al*. (2002) reported that the strongest determinant of plasma pH in an 80 km race was SIDm. Changes in SIDm values in exercising horses are due to electrolyte losses in sweat and increases of blood lactate concentration. Horse sweat is iso- or slightly hypertonic with the same concentration of sodium as plasma, but higher chloride concentration. Due to electrolyte concentrations of sweat, chloride losses are more severe than of other ions (Jose-Cunilleras 2004; Lindinger and Waller 2008). Plasma chloride concentration also decreased in the initial minutes of jumping exercise due to an influx into muscle and red cells (Aguilera-Tejero *et al*. 2000). The combination of these conditions produces a fast decrease in plasma $Cl^-$. In the present study, a greater decrease of $Cl^-$ concentration was detected (6 mmol/l) compared with values reported in previous studies (Barton *et al*. 2003), but similar to that reported in horses competing in a 160 km race (Schott *et al*. 2006). The decrease of $Cl^-$ can lead to metabolic alkalosis, but usually an increased lactate concentration will attenuate this.

Decreased sodium concentration was probably associated with a combination of prolonged sweating and addition of water to extra cellular fluid (ECF) space by voluntary drinking of water or hypotonic electrolyte solutions (more important in the second half of a race), or a shift of intracellular fluid (ICF) poor in $Na^+$ and $Cl^-$ to the ECF space, in particular from tissues that are less metabolically active during prolonged exercise (McKeever 2008). Hypotonic fluid shifts or water consumption also contributes to dilution of other electrolytes and plasma components (McKeever 2008).

Plasma $K^+$ concentration showed a mild tendency to decrease during the race, but recovered by the finish line. Previous studies report an increase of plasma $K^+$ concentration beginning at 4 m/s (14 km/h) and explain the decrease in plasma $K^+$ concentration detected after the race due to rapid redistribution (3 min) of this electrolyte during recovery inside red blood cells and muscle cells (Hess *et al*. 2005).

A slight increase in SIDm values was detected due to a decrease in $Cl^-$, despite a mild decrease in $Na^+$ and mild increase in lactate concentrations. Strong ion difference was higher during exercise suggesting that the main determinant of it was $Cl^-$ loss leading to metabolic alkalosis (increase in SIDm). Usually when SIDm increases pH does increase as well (i.e. tendency towards alkalosis) but in the present study no changes in pH were observed. During the race a complex derangement developed in which pH did not clearly change due to the combined effects of: 1) mild increase in SIDm (tendency towards alkalosis); 2) an increase in $PCO_2$ (leading to acidosis), and 3) an increase in $A_{tot}$ (it also causes a tendency toward acidosis).

Plasma proteins (mainly albumin) and phosphates are weak acids that are not fully dissociated at physiological pH and, as such, are capable of buffering hydrogen ions. In the simplified strong ion approach, $[A_{tot}]$ represents the total plasma concentration of nonvolatile weak buffers. Another important contribution to pH imbalances in endurance horses is the increase of $A_{tot}$ due to increased plasma proteins as a consequence of dehydration. An increase of TP has been documented during long distance endurance rides (120–160 km) but values at the end of the ride generally returned to the resting values (Barton *et al*. 2003). This finding suggests that haemoconcentration is greater during the first 60–80 km of exercise and the return to preride results could be explained by a decrease in exercise intensity, due to losses of proteins from vascular space or addition of water to extra cellular fluid. A marked increase in TP (12%) was observed during the initial 65 km in the present study and a tendency to stabilisation from midpoint to race finish. This tendency was more evident for plasma creatinine, which had an initial increase of 42% during the first half of the race and no statistical changes were detected from midpoint to the end. Similar changes of ~14% were detected in albumin and globulin concentrations. All these alterations were indicative of more marked dehydration tendency during the first half of race and a posterior plateau maintained until race end, probably due to lack of drinking during the first half of the race. This is consistent with previous studies reporting that thirst is not stimulated during the first stages of an endurance race, leading to sustained dehydration during and after the event (Nyman *et al*. 1996; Butudom *et al*. 2002). Nonvolatile buffer concentration also followed a tendency to increase because two-thirds of $A_{tot}$ is albumin, but the former increased more than expected due to higher increase of $P_i$ concentration. Prerace $A_{tot}$ was slightly lower than previously reported values (Navarro *et al*. 2005), probably associated with a mild hypoglobulinaemia as seen in dog athletes during periods of endurance training (McKenzie *et al*. 2007).

The interpretation of 33 out of 94 analyses matched using the 2 methods. The agreement between the traditional and quantitative methods of interpretation of acid-base balance was not as poor as expected, given that large alterations in $PCO_2$, SIDm or $A_{tot}$ were not found in this study. However, the concordance was poor when the interpretation was compared for each horse at the race finish (or at the time of elimination) and the authors suggest use of the quantitative approach whenever possible. The most common acid base disorder observed was a mild SIDm alkalosis due to mild hypochloraemia combined with mild nonvolatile weak buffer acidosis due to mild increase in plasma protein concentrations. These derangements are detected using the quantitative approach but would be missed using the traditional approach to acid base status.

In addition to the limitations inherent in field investigations, there were additional limitations in this study that warrant mention. First, no complete data collection and detailed list of management factors were recorded. No information about supplement or electrolyte administration was obtained during the race. This fact made impossible to determine whether the mild electrolyte derangements seen in these horses were related to administration of supplements by riders or exercise induced. The use of vacutainers for analysis of blood gases is currently not considered as the best sampling method according to Clinical and Laboratory Standards Institute (D'Orazio 2009) given that an anaerobic sample cannot be maintained. However, the authors believe minimal changes are expected due to our sampling method (samples for blood gas analysis were immediately performed) and the same sampling method has previously been used in other equine studies (Soma *et al*. 1996; Aguilera-Tejero *et al*. 2000; Navarro *et al*. 2005).

Finally, point-of-care blood analysers may not be as precise or accurate as bench top analysers. However, the hand-held analysers used in this study have been validated for use in field studies of exercising horses (Foreman *et al*. 1999; Silverman and Birks 2002) and also are currently used by team and treating veterinarians. It was demonstrated, however, that these analysers underestimate

blood potassium concentration (by ~0.4 mEq/l), and overestimates plasma $Cl^-$ (by ~3 mEq/l) (Grosenbaugh *et al*. 1998) and blood lactate concentrations (by 0.6 mmol/l for values <5 mmol/l) (Tennent-Brown *et al*. 2007). This deviation may explain the observed lower prerace SIDm values reported in this study.

Recently, a new quantitative method was suggested for acid-base interpretation in man. In contrast to traditional and Stewart's methods, this new approach is not based on the principle of electroneutrality, but on the principles of mass conservation and pre-equilibrium proton concentration. The predictive value of pH using this new formula has a very good correlation with measured values (Nguyen *et al*. 2009), but these equations have only been applied in protein-free multiple-buffered aqueous solutions and not yet in plasma.

In conclusion, traditional analysis should not be used for evaluation of endurance horses because mild alterations in pH are mainly caused by electrolyte and protein ($A_{tot}$) changes that are only taken into account using Stewart's method of assessment of acid-base balance. Although pH did not significantly change in fit endurance horses during a 120 km race, a complex acid-base imbalance characterised by mild strong ion alkalosis (hypochloraemia) attenuated by mild lactic acidosis, nonvolatile buffer acidosis and compensatory mild respiratory acidosis was present in most horses. Using the quantitative approach these disturbances were detected and a more rational treatment could be elaborated. Complex acid-base alterations are present frequently in endurance horses and the quantitative method is more suitable to detect these kinds of disturbances.

## Manufacturers' addresses

[1]Terumo Europe, Leuven, Belgium.
[2]Abbott Laboratories, Abbott Park, Illinois, USA.
[3]Roche Diagnostics, Basel, Switzerland.

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