



STANDARD ARTICLE

Traditional and quantitative analysis of acid-base and electrolyte imbalances in horses competing in cross-country competitions at 2-star to 5-star level

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Abstract

Background: Early recognition and management of acid-base, fluid, and electrolyte disorders are crucial for the maintenance of health and performance in equine athletes.

Objectives: To analyze changes in acid-base and electrolyte status associated with exercise during cross-country competitions at different levels using traditional and quantitative approaches.

Animals: Thirty-eight eventing horses.

Methods: Prospective observational study. Jugular venous blood samples were collected before and after the cross-country test of 25 international eventing competitions ranging from 2-star (formerly 1-star) to 5-star (formerly 4-star) level. Blood gas analysis was performed to determine pH, $p\text{CO}_2$, Na^+ , Cl^- , and K^+ and calculate HCO_3^- , $t\text{CO}_2$ base excess (BE_{ECF}), anion gap (AG), strong ion difference calculated from Na^+ , K^+ , Cl^- , and lactate $^-$ (SID_4), strong ion difference calculated from Na^+ , K^+ , and Cl^- (SID_3), strong ion gap (SIG), and total nonvolatile weak buffer concentration (A_{tot}). Postexercise acid-base imbalances, diagnosed on the basis of the traditional approach, and the simplified strong ion model were compared.

Results: Significant decreases in pH, Cl^- , SID_4 , $p\text{CO}_2$, HCO_3^- , $t\text{CO}_2$, and BE_{ECF} as well as increases in K^+ , SID_3 , AG, TP, and A_{tot} were observed between pre- and post-exercise samples. The changes in acid-base parameters were significantly affected by the competition level. Using the strong ion approach, a higher proportion of horses was diagnosed with postexercise metabolic acidosis.

Conclusions and Clinical Importance: Regarding the complex acid-base changes in horses competing at cross-country competitions, the quantitative approach provided

Abbreviations: AG, anion gap; AL, advanced level; A_{tot} , total plasma concentration of nonvolatile weak acids; BE, base excess; BE_{ECF} , base excess of extracellular fluids; CCI, Concours Complet International; Cl^- , chloride; CV, coefficient of variation; EMmean, estimated marginal mean; FEI, Fédération Equestre Internationale; HCO_3^- , bicarbonate; IL, intermediate level; K^+ , potassium; LMM, linear mixed effects model; Na^+ , sodium; $p\text{CO}_2$, partial pressure of carbon dioxide; RH, relative humidity; SID, strong ion difference; SIG, strong ion gap; T0, pre-exercise sampling; T10, sampling 10 minutes postexercise; T30, sampling 30 minutes postexercise; $t\text{CO}_2$, total concentration of carbon dioxide; TP, total plasma protein; WBGT, wet bulb globe temperature.

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a more detailed analysis of the different factors contributing to acid-base balance than did the traditional approach.

KEYWORDS

blood gas, equine, eventing, exercise, point-of-care analyzer, strong ion approach

1 | INTRODUCTION

Disturbances of fluid, electrolyte, and acid-base balance as a result of strenuous exercise during competitions are encountered commonly in athletic horses. Previous investigations have shown that significant fluid, electrolyte, and biochemical changes occur in horses competing in the endurance test of 3-day events.¹⁻⁹ However, these studies were conducted before modification of the classical competition format to the short format without “roads and tracks” and “steeplechase” phases.

Horses that develop severe acid-base and electrolyte imbalances during competition may be more prone to metabolic disorders, exhaustion, and delayed recovery. Early recognition and appropriate management of acid-base and electrolyte imbalances therefore are crucial for the maintenance of health and performance in equine athletes, and on-site monitoring of relevant blood variables may be beneficial.

The availability of relatively inexpensive, portable blood gas and electrolyte analyzers enables immediate determination of relevant acid-base parameters under field conditions at the competition site. Appropriate clinical assessment and interpretation of acid-base imbalances, however, is a matter of controversial debate. The traditional Henderson-Hasselbalch approach for the interpretation of acid-base status focuses on the relationship between pH, partial pressure of carbon dioxide ($p\text{CO}_2$), and bicarbonate concentration (HCO_3^-).^{10,11} The major disadvantage of this approach is the qualitative nature of the information it provides with regard to exercise-related acid-base changes.¹² This limitation has led to increased application of the quantitative strong ion model which simultaneously takes into account pH and $p\text{CO}_2$ as well as the influence of the concentrations of strong ions such as sodium (Na^+), potassium (K^+), chloride (Cl^-), and lactate and the concentration of nonvolatile weak buffer ions (A_{tot}), primarily plasma protein, on acid-base balance.^{13,14} The quantitative approach has been shown to provide a more thorough interpretation of the complex acid-base and electrolyte changes that occur in response to competitions in show jumpers and endurance horses.^{15,16} However, the usefulness of the strong ion approach in assessing acid-base and electrolyte disturbances that are associated with cross-country competitions at different levels has not yet been evaluated.

The purposes of our study were: (1) to compare changes in acid-base and electrolyte status in eventing horses associated with cross-country competitions at different competition levels and (2) to assess whether the physicochemical strong ion approach provides a better understanding of the contribution of each variable to changes in acid-base homeostasis during exercise compared to the traditional Henderson-Hasselbalch approach.

2 | MATERIAL AND METHODS

2.1 | Study conditions

This prospective study was performed as part of the “performance monitoring program” of the German Olympic Committee for Equestrian Sports the aim of which is to promote long-term health and performance of elite eventing horses by offering performance diagnostic measures including routine measurements of blood lactate concentration in response to cross-country competitions. From the group of horses being regularly sampled within this program, 38 horses competing at various levels were selected for study.

Data collection took place between 2014 and 2016 during 25 competitions held under the rules of the Fédération Equestre Internationale (FEI) for international eventing competitions and ranging from 2-star (until 2018 designated as 1-star) to 5-star (until 2018 designated as 4-star) level. The competitions were held during 15 different events at 8 different venues in Central, Western, and Northern Europe between March and October.

2.2 | Sample collection and analysis

Venous blood samples were collected at rest the evening before the cross-country competition (T0) as well as 10 minutes (T10) and 30 minutes (T30) after finishing the cross-country course. Blood samples were collected anaerobically by venipuncture of the jugular vein into 1.2 mL lithium heparinized syringes (SARSTEDT AG & Co. KG, Nümbrecht, Germany). Any air bubbles were expelled immediately after sample collection and the syringes were sealed to ensure that the blood samples were not exposed to air.

Immediately after sampling, blood lactate concentration was determined enzymatically from whole blood using a handheld photometer (Lactate Photometer Plus DP 110, Diaglobal, Berlin, Germany).

The $p\text{CO}_2$, pH, and concentrations of Na^+ , Cl^- , and K^+ were measured using a portable blood gas analyzer (IDEXX VetStat Electrolyte and Blood Gas Analyzer, IDEXX Laboratories, Ludwigsburg, Germany) which previously has been validated for use in the horse by comparison with a stationary analyzer.¹⁷ The VetStat analyzer measures the optical fluorescence by optodes. It performs sample analysis using disposable cassettes and automatically calibrates during each measurement process using a precision gas mixture and the cassette's internal storage buffer. Additionally, the measuring accuracy of the device was checked once each day before the analyzer was used by means of standard reference cassettes for 2 different measurement ranges. The

TABLE 1 Interpretation of acid-base status

Diagnostic approach	Parameters	Type of disorder	Acidosis	Alkalosis
Traditional (Henderson-Hasselbalch)	Measured: pH, pCO ₂ , Calculated: BE _{ECF} , (AG)	Respiratory	pCO ₂ ↑	pCO ₂ ↓
		Metabolic	BE _{ECF} ↓	BE _{ECF} ↑
Quantitative (simplified strong ion model)	Measured: pH, pCO ₂ , Na ⁺ , K ⁺ , Cl ⁻ , Lactate, TP Calculated: SID, A _{tot} , (SIG)	Respiratory	pCO ₂ ↑	pCO ₂ ↓
		Metabolic	SID↓	SID↑
			A _{tot} ↑	A _{tot} ↓

Abbreviations: ↑, above the reference range; ↓, below the reference range; AG, anion gap; A_{tot}, total plasma concentration of nonvolatile buffers; BE_{ECF}, base excess in extracellular fluid; Cl⁻, chloride concentration; K⁺, potassium concentration; Na⁺, sodium concentration; pCO₂, partial pressure of carbon dioxide; SID, strong ion difference; SIG, strong ion gap; TP, total protein concentration.

TABLE 2 Competition requirements and environmental conditions during 25 cross-country competitions during 15 events held at 8 different venues (A-H)

Venue	Event	Class	Horses N	Samples			Temperature (°C)	RH (%)	WBGT	Distance (m)
				n	T0	T10				
A	May 14	CCI3*-S	1	-	-	1	18	42	18	3500
A	May 14	CCI3*-L	2	-	-	2	18	42	18	4600
B	June 14	CCI4*-S	1	-	-	1	18	59	19	3744
C	July 14	CCI2*-S	1	-	1	1	16	92	20	2650
C	July 14	CCI3*-S	2	-	2	1	17	88	20	3370
D	October 14	CCI4*-L	1	1	-	-	15	77	18	5985
A	April 15	CCI2*-S	3	2	2	-	10	69	13	3030
A	April 15	CCI3*-S	11	11	7	-	13	47	14	3300
E	May 15	CCI4*-S	3	3	2	-	15	69	17	3480
A	June 15	CCI3*-S	4	4	4	4	25	57	25	3300
B	June 15	CCI4*-S	3	3	3	3	15	78	18	3658
B	June 15	CCI5*-L	1	1	1	1	15	78	18	6365
B	March 16	CCI3*-S	9	-	9	7	10	93	14	3160
A	April 16	CCI2*-S	2	-	2	2	8	86	12	3000
A	April 16	CCI3*-S	5	-	5	4	10	86	14	3400
E	May 16	CCI4*-S	4	-	4	1	20	43	19	3580
F	May 16	CCI2*-S	2	-	2	2	18	78	20	3120
F	May 16	CCI3*-S	3	-	3	1	22	54	22	3465
B	June 16	CCI4*-S	7	-	7	5	20	68	22	3741
B	June 16	CCI5*-L	1	-	1	1	20	68	22	6365
G	September 16	CCI3*-S	3	-	3	-	27	68	29	3230
H	October 16	CCI2*-L	1	-	1	-	10	87	14	3640
H	October 16	CCI3*-S	3	-	3	3	10	94	14	3025
H	October 16	CCI3*-L	5	-	5	1	10	87	14	4400
H	October 16	CCI4*-L	2	-	2	2	15	72	17	5868

Notes: A total of 38 horses was sampled between 1 and 7 times at different occasions leading to a total of 80 different combinations of horses and competitions with a total of 137 analyzed samples. The number of horses (N) sampled per competition and the number of samples (n) collected at each time point (T0, pre-exercise; T10, 10 minutes postexercise; T30, 30 minutes postexercise) are reported. The designation of competition classes refers to the international event classification which applies since 2019 (CCI-S, short format; CCI-L, long format). The optimum speed during 2-star competitions was 520 m/min, during 3-star competitions 550 m/min, and during 4-star and 5-star competitions 570 m/min. The climate conditions during each event were quantified by estimation of the wet bulb globe temperature (WBGT) index from temperature and relative humidity (RH) using the approximation proposed by the FEI (FEI Eventing Memorandum, 8th edition, April 2015).

analyzer was protected from direct sunlight and heat before and during sample analysis. The range of proper operating temperature reported by the manufacturer is 8°C-32°C.

The remaining blood samples were centrifuged at 12 000g for 90 seconds to obtain plasma samples and determine total plasma protein (TP) concentration by direct refractometry (Dr. Mueller Refraktometer MHRC-200 ATC, Mueller-Optronic, Erfurt, Germany).

2.3 | Calculation of variables and interpretation of acid-base imbalance

Values of HCO_3^- and total concentration of carbon dioxide (tCO_2) were calculated automatically by the blood gas analyzer from pH and $p\text{CO}_2$ values using the Henderson-Hasselbalch equation¹⁸:

$$\text{HCO}_3^- = S \times p\text{CO}_2 \times 10^{(\text{pH} - \text{pK}_1)}$$

$$\text{tCO}_2 = \text{HCO}_3^- + (S \times p\text{CO}_2)$$

using values for the CO_2 solubility constant (S) of 0.0307 mmol/L/mm Hg and for the carbonic acid dissociation constant (pK_1) of 6.129.¹⁸

Anion gap (AG) was calculated by the blood gas analyzer from the following equation¹⁹:

$$\text{AG} = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)$$

Traditional analysis of acid-base balance was completed by calculating extracellular base excess (BE_{ECF}) from the Henderson-Hasselbalch equation¹⁸:

$$\text{BE}_{\text{ECF}} = 0.93 \times \{ \text{HCO}_3^- - 24.4 + 14.83 \times (\text{pH} - 7.40) \}$$

Quantitative analysis of acid-base balance was conducted using the strong ion model as previously described.¹⁸

Strong ion difference (SID_4) and total concentration of nonvolatile buffers (A_{tot}) were calculated from following equations¹⁸:

$$\text{SID}_4 = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{lactate})$$

$$A_{\text{tot}} = 2.24 \times (\text{TP})$$

where total protein is in g/dL.

TABLE 3 Venous acid-base variables determined in 137 blood samples from 38 horses collected before (T0) as well as 10 minutes (T10) and 30 minutes (T30) after 25 different cross-country competitions at 2-star to 5-star level

Variable	Reference Range	Intermediate Level			Advanced Level		
		T0 n = 17(*17)	T10 n = 49(*33)	T30 n = 29(*20)	T0 n = 8(*7)	T10 n = 20(*16)	T30 n = 14(*9)
pH	7.31-7.45 ¹⁶	7.43 ± 0.02 ^a	7.38 ± 0.01 ^b	7.42 ± 0.02 ^{a,‡}	7.44 ± 0.02 ^a	7.35 ± 0.02 ^b	7.45 ± 0.02 ^{a,‡}
Na ⁺ (mmol/L)	133-150	148 ± 0.8	148 ± 0.5	148 ± 0.6	148 ± 0.9	148 ± 0.7	147 ± 0.8
Cl ⁻ (mmol/L)	97-109	107 ± 0.6	107 ± 0.5 [†]	107 ± 0.5	108 ± 0.7 ^a	106 ± 0.6 ^{b,†}	107 ± 0.6
K ⁺ (mmol/L)	3.0-5.3	3.2 ± 0.13 ^a	3.8 ± 0.08 ^b	3.6 ± 0.10 ^b	2.9 ± 0.15 ^a	3.8 ± 0.12 ^b	3.7 ± 0.13 ^b
Lactate (mmol/L)	<2 ¹⁶	-	8.7 ± 1.1 ^{a,*}	4.6 ± 1.1 ^{b,‡}	-	13.9 ± 1.3 ^{a,*}	6.3 ± 1.3 ^{b,‡}
SID ₄ (mEq/L)	38-43 ²¹	43.3 ± 1.4 ^a	36.9 ± 1.0 ^{b,*}	40.3 ± 1.1 ^{c,‡}	40.4 ± 1.6 ^a	31.4 ± 1.3 ^{b,*}	38.0 ± 1.4 ^{a,‡}
SID ₃ (mEq/L)	38-44 ²³	43.7 ± 0.9	45.6 ± 0.7	44.9 ± 0.8	42.7 ± 1.1 ^a	45.5 ± 0.9 ^b	44.4 ± 0.9
$p\text{CO}_2$ (mmHg)	41-53 ¹⁶	48.6 ± 1.8 ^a	40.6 ± 1.1 ^{b,†}	46.2 ± 1.3 ^{a,*†}	49.1 ± 2.0 ^a	35.2 ± 1.6 ^{b,†}	39.7 ± 1.7 ^{c,*†}
HCO_3^- (mEq/L)	24-30 ¹⁶	30.4 ± 1.4 ^a	22.7 ± 1.0 ^{b,*†}	28.0 ± 1.1 ^a	30.0 ± 1.6 ^a	17.8 ± 1.3 ^{b,*†}	25.0 ± 1.4 ^c
tCO_2 (mEq/L)	28-35 ¹⁶	31.9 ± 1.4 ^a	23.9 ± 1.0 ^{b,*†}	29.4 ± 1.1 ^c	31.5 ± 1.6 ^a	18.9 ± 1.4 ^{b,*†}	26.2 ± 1.4 ^c
AG (mEq/L)	7-15 ²³	13.2 ± 1.8 ^a	22.7 ± 1.4 ^{b,†}	16.8 ± 1.5 ^a	12.1 ± 2.0 ^a	27.3 ± 1.8 ^{b,†}	19.1 ± 1.8 ^c
BE_{ECF} (mEq/L)	-6 to 6 ¹⁶	6.2 ± 1.5 ^a	-1.9 ± 1.1 ^{b,*†}	3.6 ± 1.2 ^a	5.6 ± 1.7 ^a	-6.9 ± 1.4 ^{b,*†}	1.2 ± 1.5 ^a
TP* (g/dL)	5.5-7.5 ²²	5.8 ± 0.2 ^a	6.5 ± 0.2 ^{b,*}	6.3 ± 0.2 ^b	6.3 ± 0.2 ^a	7.0 ± 0.2 ^{b,*}	6.7 ± 0.2
A_{tot} * (mEq/L)	12-15 ¹⁶	13.0 ± 0.4 ^a	14.6 ± 0.4 ^{b,*}	14.1 ± 0.4 ^b	14.1 ± 0.5 ^a	15.7 ± 0.4 ^{b,*}	15.0 ± 0.4
SIG* (mEq/L)	-2 to 2 ²¹	-1.6 ± 1.8 ^a	-9.9 ± 1.4 ^{b,†}	-4.6 ± 1.4 [†]	-0.02 ± 1.9 ^a	-13.4 ± 1.5 ^{b,†}	-6.2 ± 1.7 ^{c,†}

Note: Data are presented as estimated marginal means ± SE.

Abbreviations: AG, anion gap; A_{tot} , total plasma concentration of nonvolatile buffers; BE_{ECF} , base excess in extracellular fluid; Cl⁻, chloride concentration; HCO_3^- , bicarbonate concentration; K⁺, potassium concentration; n, number of samples (*number of samples in which TP, A_{tot} , and SIG were determined); Na⁺, sodium concentration; $p\text{CO}_2$, partial pressure of carbon dioxide; SID₃, strong ion difference calculated from the concentrations of 3 strong ions (Na⁺, K⁺, Cl⁻); SID₄, strong ion difference calculated from the concentrations of 4 strong ions (Na⁺, K⁺, Cl⁻, lactate⁻); SIG, strong ion gap; tCO_2 , total concentration of carbon dioxide; TP, total plasma protein concentration.

^{a,b,c}Indicates a significant difference between sampling times within groups ($P \leq .05$).

*Indicates a significant difference between groups within the same sampling time ($P \leq .05$).

[†]Indicates a significant effect of the competition level on the difference to T0 ($P \leq .05$).

[‡]Indicates a significant effect of the competition level on the difference to T10 ($P \leq .05$).

To be able to distinguish between the relative contribution of the blood lactate concentration and the inorganic ions (Na^+ , Cl^- , and K^+) to SID, the inorganic component of the SID (SID_3) also was calculated as²⁰:

$$\text{SID}_3 = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^-)$$

Additionally, the strong ion gap (SIG) was calculated from following equation¹⁹:

$$\text{SIG} = \frac{A_{\text{tot}}}{1 + 10^{(\text{pKa} - \text{pH})}} - \text{AG}$$

using a value for the effective dissociation constant of plasma weak acids (pKa) of 6.7.¹⁸

Based on the measured and calculated parameters, acid-base imbalances were categorized using both the traditional and the quantitative strong ion approach according to the specifications presented in Table 1.¹⁸ The reference ranges used for the acid-base parameters

are reported in Table 3.²¹⁻²³ Device-specific reference ranges were provided for Na^+ , Cl^- , and K^+ concentrations.

2.4 | Statistical analysis

All figures and statistical analyses were performed using Excel (Microsoft Excel 2013, Microsoft Corporation, Redmond, Washington) and R (R, version 3.4.3, The R Foundation, Vienna, Austria). To evaluate whether the cross-country test induced changes in the measured and calculated parameters and if these changes were affected by competition level, linear mixed effects models (LMM) with sampling time (T0, T10, T30), level and the interaction between sampling time and level as fixed effect were fitted to the data using the "lmer" function in R. To account for within-subject, within-venue, and within-event correlations, subject (horse) and event within venue were included as random effects. Because only few samples were obtained from horses competing at 2-star and 5-star level, 2 groups were formed. The "Intermediate level" (IL) group included samples collected

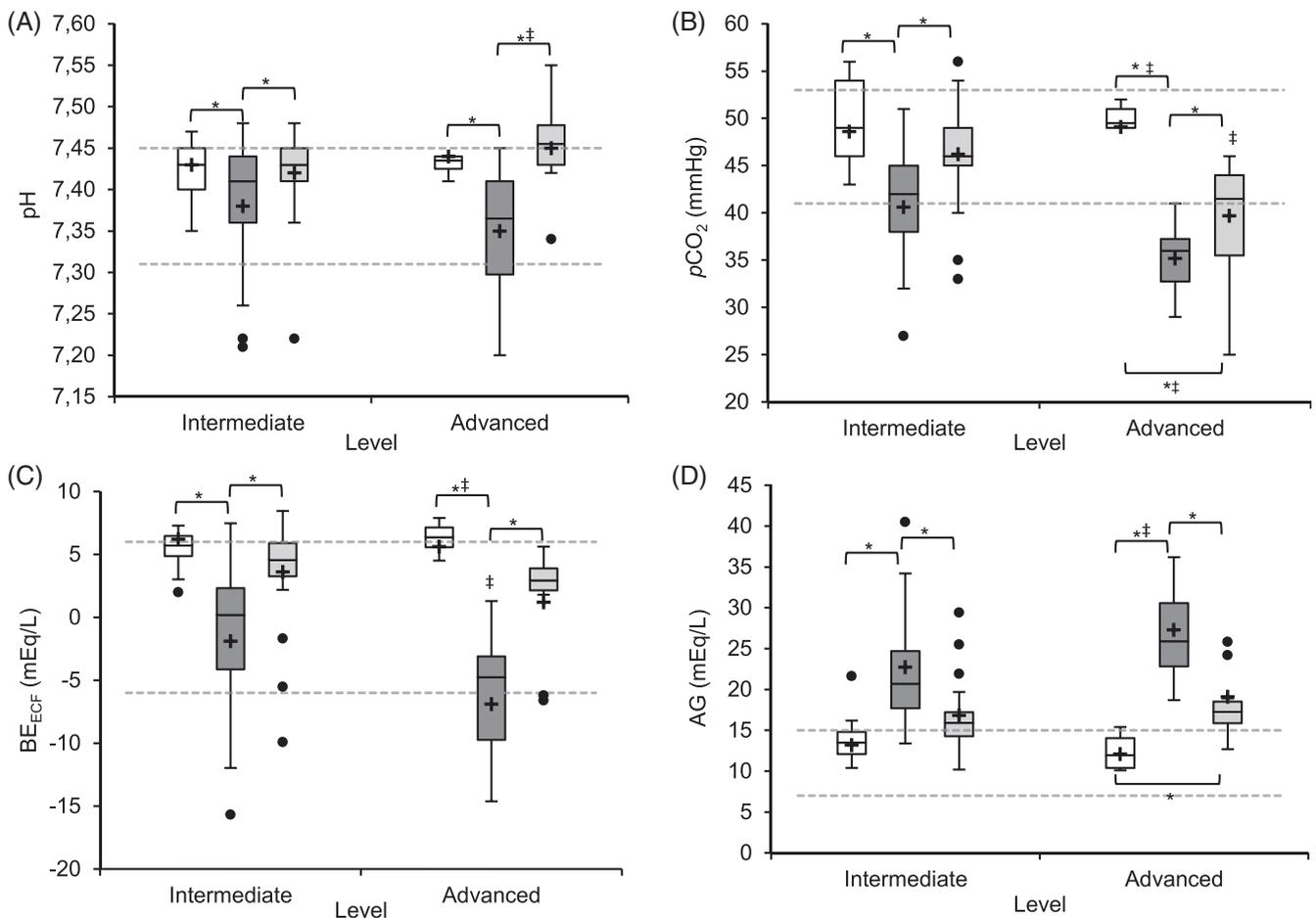


FIGURE 1 Venous (A) pH, (B) partial pressure of carbon dioxide (pCO_2), (C) base excess (BE_{ECF}), and (D) anion gap (AG) at rest (white boxplots), 10 minutes postexercise (dark grey boxplots) and 30 minutes postexercise (light grey boxplots) compared between cross-country competitions at Intermediate (2-star and 3-star) and Advanced (4-star and 5-star) level. The boxplots display the distribution of data by indicating the median and the interquartile range (IQR). Values that are larger than the upper quartile or smaller than the lower quartile by at least 1.5 times the IQR are displayed as dots. Marginal means, estimated by the linear mixed model are indicated by crosses. The upper and lower boundaries of the reference ranges for each parameter are indicated by dotted lines. *Indicates a significant difference between sampling times ($P \leq .05$). †Indicates a significant difference to Intermediate level ($P \leq .05$)

during competitions at 2-star and 3-star level and the “Advanced level” (AL) group included samples collected during competitions at 4-star and 5-star level. The significance of the fixed effects on the response variables was evaluated by comparing full models to models without the fixed effects by means of likelihood ratio tests using the “anova” function in R. Post hoc pairwise comparisons were performed by general linear hypothesis tests with Bonferroni-Holm correction using the “glht” function in R. Results are presented as estimated marginal means (EMmeans) \pm SE estimated by the LMM and in the case of pH, $p\text{CO}_2$, BE_{ECF} , AG, SID_4 , A_{tot} , and SIG additionally as median, 25 and 75% quartiles, minimum and maximum values. Normality of the error distributions of the linear regression models was evaluated by means of normal probability plots of the residuals. The level of statistical significance was set at $P \leq .05$ in all statistical analyses.

3 | RESULTS

3.1 | Study population

The study population included 30 geldings and 8 mares belonging to the following breeds: Warmblood ($n = 2$); Thoroughbred-cross

($n = 36$) with variable percentages of Thoroughbred blood: $<25\%$ Thoroughbred blood ($n = 14$), ≥ 25 and $<50\%$ Thoroughbred blood ($n = 15$), and $50\%-75\%$ Thoroughbred blood ($n = 7$). The horses were 6 to 16 years of age and constituted a heterogeneous group of horses at various levels of experience and training.

3.2 | Sampled competitions

The competition requirements and climate conditions as well as the number of sampled horses and analyzed blood samples are listed for each sampled competition in Table 2.

The mean speed during the course was 521 ± 17 m/min at 2-star level, 535 ± 22 m/min at 3-star level, 549 ± 21 m/min at 4-star level and 561 ± 18 m/min at 5-star level.

3.3 | Blood samples and missing values

Each of the 38 horses in the study population was sampled between 1 and 7 times at different occasions leading to a total of 80 different

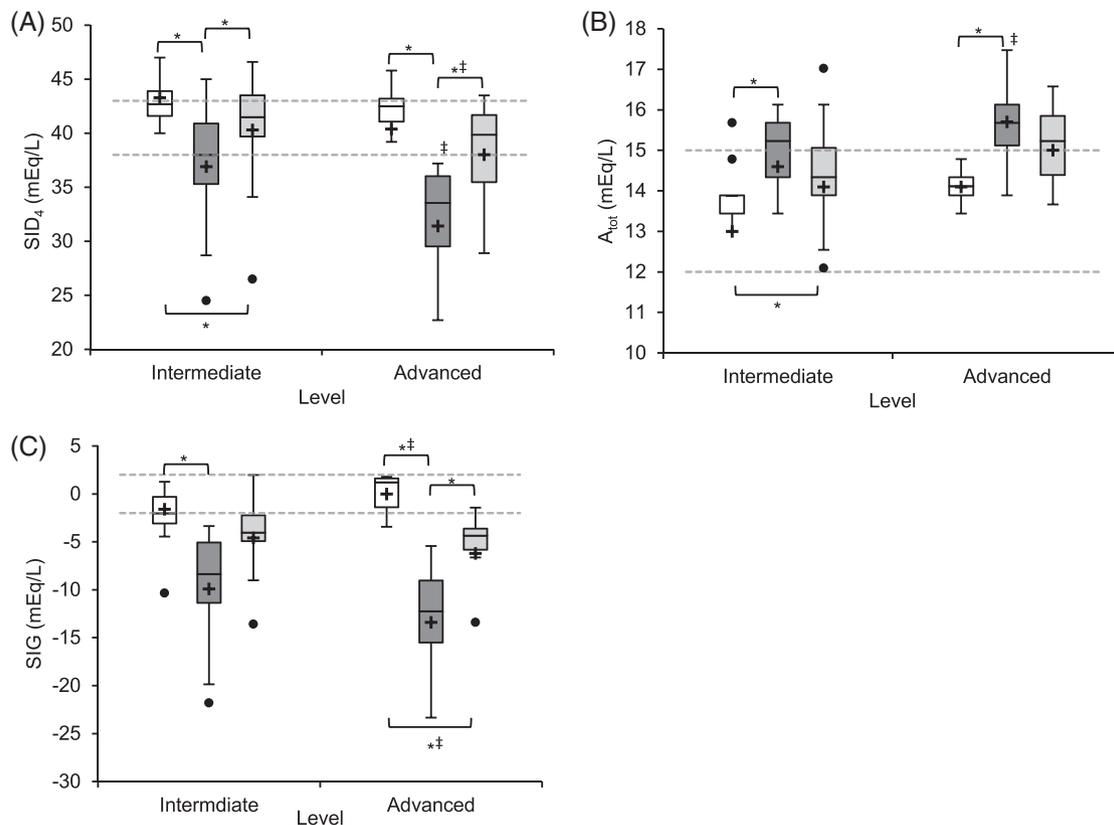


FIGURE 2 Venous (A) strong ion difference (SID_4), (B) concentration of weak acids (A_{tot}), and (C) strong ion gap (SIG) at rest (white boxplots), 10 minutes postexercise (dark grey boxplots) and 30 minutes postexercise (light grey boxplots) compared between cross-country competitions at Intermediate (2-star and 3-star) and Advanced (4-star and 5-star) level. The boxplots display the distribution of data by indicating the median and the interquartile range (IQR). Values that are larger than the upper quartile or smaller than the lower quartile by at least 1.5 times the IQR are displayed as dots. Marginal means, estimated by the linear mixed model are indicated by crosses. The upper and lower boundaries of the reference ranges for each parameter are indicated by dotted lines. *Indicates a significant difference between sampling times ($P \leq .05$). †Indicates a significant difference to Intermediate level ($P \leq .05$)

combinations of horses and competitions. In total, 137 blood samples were analyzed (25 at T0, 69 at T10, and 43 at T30). In 8 (10%) cases, blood samples were collected at all 3 time points. In 41 (51%) cases, there was 1 time point missing (in 31 cases [39%] the T0 sample was missing and in 10 cases [12%] the T30 sample was missing), and in 31 cases (39%) 2 time points were missing (in 4 cases [5%] the T0 and T10 samples were missing, in 20 cases [25%] the T0 and T30 samples were missing, and in 7 cases [9%] the T10 and T30 samples were missing). The relatively high proportion of missing results was mainly caused by logistic reasons because collection of samples at rest during the day before the cross-country test was not always feasible. Furthermore, at some events, T10 and T30 samples had to be collected at different locations to avoid interference of blood sample collection with the cool-down and grooming of the horses after competition. Because only 1 blood gas analyzer and 1 centrifuge were available, samples could not always be analyzed at both time points because transportation of blood

samples from the location of sample collection to the place where the analyzer was located would have caused too much delay between sample collection and analysis. Some samples were not collected because the riders failed to appear at the times scheduled for sampling. Because of technical problems, the TP concentration could not be determined in all samples (1 sample at T0, 20 samples at T10, and 5 samples at T30). Because the blood lactate concentration was not determined in pre-exercise samples, a resting blood lactate concentration of 1 mmol/L was assumed to calculate SID_4 for T0 samples.²⁴

3.4 | Acid-base analysis findings

The EMmeans \pm SE estimated by the LMMs for all measured and calculated variables compared between IL and AL are presented in Table 3. The changes of the traditional and quantitative acid-base variables in

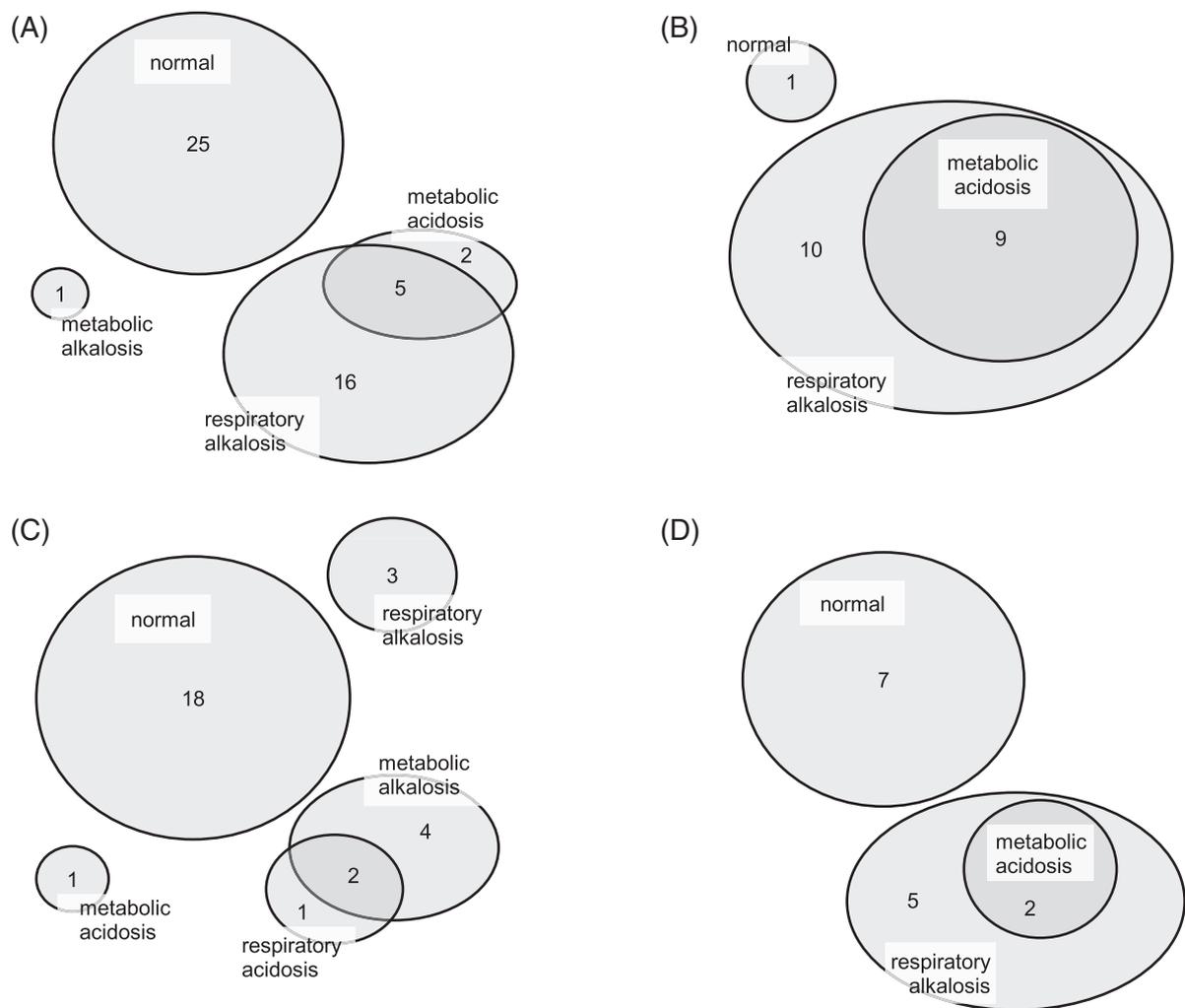


FIGURE 3 Distribution of postexercise acid-base imbalances based on the traditional approach. A, Analysis of blood samples collected 10 minutes postexercise in horses competing at 2-star ($n = 8$) or 3-star level ($n = 41$). B, Analysis of blood samples collected 10 minutes postexercise in horses competing at 4-star ($n = 18$) or 5-star level ($n = 2$). C, Analysis of blood samples collected 30 minutes postexercise in horses competing at 2-star ($n = 5$) or 3-star level ($n = 24$). D, Analysis of blood samples collected 30 minutes postexercise in horses competing at 4-star ($n = 12$) or 5-star level ($n = 2$). The proportion of circles and ovals corresponds to the percentage of samples assigned to the respective acid-base disorder. The figures indicate the numbers of blood samples

response to competitions at IL and AL are illustrated in Figures 1 and 2, and the distribution of individual acid-base imbalances detected at T10 and T30 in horses competing at IL and AL classified by the traditional and the quantitative strong ion approach are given in Figures 3 and 4.

Sampling time had a significant effect on pH ($\chi^2(2) = 37.74$; $P < .001$), Cl^- ($\chi^2(2) = 11.55$; $P = .003$), K^+ ($\chi^2(2) = 57.31$; $P < .001$), lactate ($\chi^2(1) = 49.31$; $P < .001$), SID_4 ($\chi^2(2) = 58.15$; $P < .001$), SID_3 ($\chi^2(2) = 17.18$; $P < .001$), pCO_2 ($\chi^2(2) = 60.61$; $P < .001$), HCO_3^- ($\chi^2(2) = 91.81$; $P < .001$), tCO_2 ($\chi^2(2) = 91.48$; $P < .001$), AG ($\chi^2(2) = 98.42$; $P < .001$), BE_{ECF} ($\chi^2(2) = 89.44$; $P < .001$), TP ($\chi^2(2) = 37.76$; $P < .001$), and SIG ($\chi^2(2) = 76.76$; $P < .001$). A significant interaction was found between sampling time and competition level for pH ($\chi^2(2) = 9.59$; $P = .008$), Cl^- ($\chi^2(2) = 8.56$; $P = .01$), lactate ($\chi^2(1) = 9.22$; $P = .002$), SID_4 ($\chi^2(2) = 7.21$; $P = .03$), pCO_2 ($\chi^2(2) = 6.58$; $P = .04$), HCO_3^- ($\chi^2(2) = 7.67$; $P = .02$), tCO_2 ($\chi^2(2) = 7.58$; $P = .02$), AG ($\chi^2(2) = 8.75$; $P = .01$), BE_{ECF} ($\chi^2(2) = 8.04$; $P = .02$), and SIG ($\chi^2(2) = 7.82$; $P = .02$).

The pH decreased from pre-exercise to T10 values by approximately 0.05 in the IL group and 0.09 in the AL group. However, the majority of T10 values remained within the reference range (16%

below the reference range in the IL group and 30% in the AL group). In both groups, pH returned to values that were not significantly different from pre-exercise values within 30 minutes postexercise. The magnitude of the increase from T10 to T30 values was higher in the AL group when compared to the IL group and 50% of the T30 values measured in the AL group were above the reference range.

In both groups, the T10 and T30 values of blood lactate concentration were considerably increased above the reference range with higher T10 values in the AL group when compared to the IL group. The lactate concentration decreased from T10 to T30 values by approximately 47% in the IL group and 55% in the AL group.

The HCO_3^- and BE_{ECF} decreased from pre-exercise to T10 values by approximately 8 mEq/L in the IL group and 12 mEq/L in the AL group. In the IL group, 14% of the T10 values of BE_{ECF} and 49% of the T10 values of HCO_3^- were below the reference range. In the AL group, 45% of the T10 values of BE_{ECF} and 90% of the T10 values of HCO_3^- were below the reference range. In both groups, the BE_{ECF} returned to values that were not significantly different from pre-exercise values within 30 minutes postexercise.

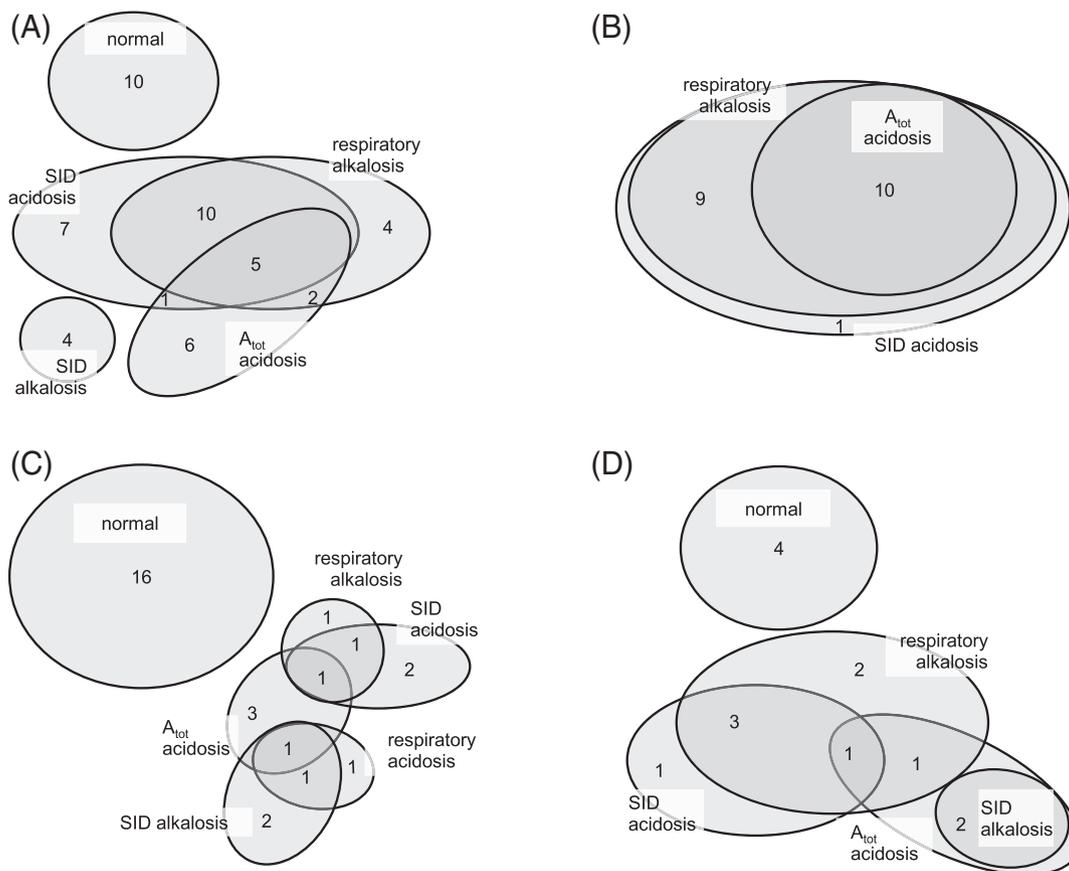


FIGURE 4 Distribution of postexercise acid-base imbalances based on the strong ion approach. A, Analysis of blood samples collected 10 minutes postexercise in horses competing at 2-star (n = 8) or 3-star level (n = 41). B, Analysis of blood samples collected 10 minutes postexercise in horses competing at 4-star (n = 18) or 5-star level (n = 2). C, Analysis of blood samples collected 30 minutes postexercise in horses competing at 2-star (n = 5) or 3-star level (n = 24). D, Analysis of blood samples collected 30 minutes postexercise in horses competing at 4-star (n = 12) or 5-star level (n = 2). The proportion of circles and ovals corresponds to the percentage of samples assigned to the respective acid-base disorder. The figures indicate the numbers of blood samples

The AG increased from pre-exercise to T10 values by approximately 10 mEq/L in the IL group and 15 mEq/L in the AL group. The AG values were not significantly different between groups at any measurement time, but the magnitude of the increase from pre-exercise to T10 values was higher in the AL group when compared to the IL group. In the IL group, 96% of the T10 values and 69% of the T30 values were above the reference range. In the AL group, 100% of the T10 values and 86% of the T30 values were above the reference range and the T30 values were still higher when compared to pre-exercise values.

The SID_4 decreased from pre-exercise to T10 values by approximately 6 mEq/L in the IL group and 9 mEq/L in the AL group. The T10 values were lower in the AL group when compared to the IL group, with 47% of the T10 values below the reference range in the IL group and 100% in the AL group. The magnitude of the increase between T10 and T30 values was higher in the AL group when compared to the IL group. In the AL group, however, 36% of the T30 values still were below the reference range compared to 14% in the IL group.

The A_{tot} increased from pre-exercise to T10 values by approximately 1.6 mEq/L in both groups with higher T10 values in the AL group when compared to the IL group. In the IL group, 42% of the T10 values and 25% of the T30 values and, in the AL group, 63% of the T10 values and 60% of the T30 values were above the reference range. The TP concentration increased from pre-exercise to T10 values by approximately 0.7 g/dL in both groups, but remained within the reference range in all sampled horses.

The SIG decreased from pre-exercise to T10 values by approximately 8 mEq/L in the IL group and 13 mEq/L in the AL group. The magnitude of the decrease in SIG from pre-exercise to T10 values was higher in the AL group when compared to the IL group, and the T30 values were still lower when compared to pre-exercise values. In both groups, 100% of the T10 values as well as 75% of the T30 values in the IL group and 80% of the T30 values in the AL group were below the reference range.

The pCO_2 decreased from pre-exercise to T10 values by approximately 8 mm Hg in the IL group and 14 mm Hg in the AL group. In the IL group, 43% of the T10 values and 11% of the T30 values were below the reference range. In the AL group, 95% of the T10 values and 50% of the T30 values were below the reference range, and the T30 values were still lower when compared to pre-exercise values.

Although Cl^- concentration decreased from pre-exercise to T10 values in the AL group and K^+ concentration increased from pre-exercise to T10 values in both groups, accompanied by an increase in the SID_3 in the AL group, these changes were very small.

On the basis of the quantitative approach, 74% of the horses sampled at T10 and 37% of the horses sampled at T30 were diagnosed with metabolic acidosis compared to 23% at T10 and 7% at T30 when using the traditional approach.

4 | DISCUSSION

Obtaining and analyzing blood samples from high-level performance horses in the field under competitive situations are affected by several uncontrollable factors and technical limitations that must be

addressed. One main limitation was the high proportion of missing observations with very few horses sampled at all 3 time points for logistical reasons. Because the exclusion of incomplete cases would have resulted in a large decrease in sample size and might have been biased, complete analysis of the collected data was performed by fitting the data to an LMM. Because missing data were random, this approach was believed to be an appropriate method to enable accurate analysis of the entire data set without the need to exclude cases with missing values. Because blood collection immediately after exercise was not feasible because of restrictions made at some events that blood samples should not be taken in public, the first postexercise blood sample was collected 10 minutes after finishing the course. As a result of this delay, the results do not accurately reflect the changes in acid-base balance during exercise because some changes that may have been present immediately after exercise already may have disappeared after 10 minutes of recovery.

The portable blood gas analyzer used in our study allowed determination of a wide range of blood variables at the competition site, enabling immediate analysis of acid-base and electrolyte status in response to cross-country competitions. However, point-of-care blood gas analyzers may not be as precise and accurate as benchtop analyzers. Inaccuracy of measurements therefore could have affected measured and calculated parameters, especially the physicochemical variables SID_4 , SID_3 , and SIG because these variables require accurate determination of several blood parameters. The portable analyzer used in our study previously has been determined to provide pH, pCO_2 , HCO_3^- , and K^+ results that were in agreement with a stationary analyzer, but overestimated Na^+ and Cl^- concentrations.¹⁷ The study indicated a constant positive bias for Na^+ and a constant positive as well as proportional bias for Cl^- concentrations. This deviation may explain the relatively high Na^+ and Cl^- concentrations observed in our study compared to previous studies in athletic horses.^{1-9,15,16} Because of the proportional bias, overestimation of Cl^- concentrations may have been especially relevant when the actual Cl^- concentrations were low. Deviations in measured acid-base and electrolyte variables also have been identified for other commercially available portable on-site analyzers.^{17,25,26} Comparisons between results obtained from different portable analyzers therefore generally should be interpreted with caution. However, because the precision of the analyzer used in our study was shown to be acceptable, and the bias was mainly systematic and constant, comparisons of results obtained at different occasions from the same analyzer should be valid. The lactate photometer used in our study was compared with another portable device that has already been validated for use in horse blood (Lactate Scout, SensLab GmbH, Leipzig, Germany).²⁷ The coefficient of variation (CV) calculated from 41 duplicate measurements with the photometer was 4.5%. The comparison between the photometer and Lactate Scout was made based on 183 measurements with lactate concentrations ranging from 0.9 to 24.0 mmol/L. A Bland-Altman analysis found a bias of -0.5 mmol/L with 95% limits of agreement of -2.1 and 1.0 mmol/L (unpublished data). The observed total error ($TE_{obs} = 2CV + Bias\%$) was 21%, which is lower than the allowable total error of 40% for measurement of blood lactate concentration

reported by the American Society for Veterinary Clinical Pathology guidelines.²⁸

Because albumin, globulin, and inorganic phosphate concentrations were not measured in our study, A_{tot} was calculated solely from the TP concentration. The formula used assigns the A_{tot} contribution of inorganic phosphate to total protein and assumes a normal albumin-to-globulin ratio and therefore may not be accurate for plasma with abnormal phosphate, albumin, and globulin concentrations. A more complete formula for estimating A_{tot} in horse plasma, accounting for inorganic phosphate, albumin, and globulin concentration would have required measurement of additional blood variables but might have been more accurate.¹⁴ A further limitation is that blood lactate concentration was not measured in pre-exercise samples and a resting blood lactate concentration of 1 mmol/L had to be assumed in order to calculate SID_4 .

The traditional approach for assessing acid-base status focuses on the relationship between pH, $p\text{CO}_2$, and HCO_3^- . The $p\text{CO}_2$ is considered as independent measure of the respiratory component of the acid-base balance. If the change in pH is due to changes in HCO_3^- , the mechanism for the alteration is considered metabolic in origin. However, the HCO_3^- , as it is calculated from pH and $p\text{CO}_2$, does not provide an independent measure of the metabolic component of an acid-base disturbance.¹⁸ This limitation has led to the BE_{ECF} concept.²⁹ The BE_{ECF} corrects for the fact that a shift in the HCO_3^- - CO_2 equilibrium reaction can change the HCO_3^- by adjusting the $p\text{CO}_2$ to 40 mm Hg and quantifying the amount of acid or base that must be added to restore the pH of 100% oxygenated human blood to 7.40. Therefore, the BE_{ECF} should be unaffected by acute changes in $p\text{CO}_2$ and provide an independent measure of the metabolic component of acid-base balance.¹⁸

The quantitative strong ion model developed by Stewart and simplified by Constable takes into account the influence of the concentration of strong ions such as Na^+ , K^+ , Cl^- , and lactate, as well as the concentration of nonvolatile weak buffer ions (primarily plasma protein) on acid-base balance. It provides a clear conceptual distinction between the dependent variables (pH and HCO_3^-) and the independent variables ($p\text{CO}_2$, SID , and A_{tot}).¹⁸ The major limitation in the practical clinical application of the strong ion model is the difficulty in obtaining accurate values for SID and A_{tot} because doing so would require identification and measurement of all strong and weak ions in plasma, which is impossible to do because unidentified ions may be present. Calculation of SID and A_{tot} therefore requires measurement of more plasma constituents, and derived values can only be estimates.

The assessment of acid-base status in response to cross-country competitions in our study identified considerable differences between the traditional and the quantitative approach regarding the classification of metabolic acidosis. Based on the BE_{ECF} , considerably fewer horses were diagnosed with metabolic acidosis 10 and 30 minutes postexercise when compared to the quantitative variables SID_4 and A_{tot} . Although the BE_{ECF} approach has been widely applied to acid-base disturbances in domestic animals, calculated BE_{ECF} values may not be equally valid in humans and horses. The concentration and buffer value of plasma protein and hemoglobin vary among species,

and normal blood pH and $p\text{CO}_2$ differ from those of humans.¹⁸ The original definition of the BE_{ECF} in humans assumes a linear relationship between $\log p\text{CO}_2$ and pH with the slope depending on the buffer capacity of the blood and relies on the fact that normal BE_{ECF} should be 0 mEq/L.¹⁸ The BE_{ECF} values determined at rest in our study suggest that normal values for the investigated horses were considerably higher (IL, 6.2 ± 1.5 mEq/L; AL, 5.6 ± 1.7 mEq/L) near the upper limit of the usually applied reference range for the BE_{ECF} value in horses (-6 to 6 mEq/L). The tendency to a slight metabolic alkalosis, as indicated by a relatively high proportion of pre-exercise samples with pH, HCO_3^- , BE_{ECF} , SID_4 , and $p\text{CO}_2$ values that were increased above the expected range, previously has been reported in athletic horses^{5,15} and may be the result of training-induced increased buffer capacity of the blood or the composition of the diet. Diets with high cation-anion difference induce a mild systemic strong ion alkalosis that may be characterized by increased plasma Na^+ , HCO_3^- , and tCO_2 concentrations and decreased plasma Cl^- concentrations.³⁰ Because no information about the diets of the investigated horses was obtained, an influence of the diet on resting acid-base values cannot be excluded. Because the high resting BE_{ECF} values observed in our study would violate its original definition, the validity of BE_{ECF} may have been limited in the investigated horses. Furthermore, the increases in red blood cell count and hemoglobin concentration associated with splenic contraction and intercompartmental fluid shifts during exercise considerably enhance the buffer capacity of the blood.³¹ This may have led to an underestimation of the metabolic acidosis by the determined BE_{ECF} values which may explain the discrepancies between the traditional and quantitative approaches.

The advantage of the traditional approach is that it enables assessment of acid-base status by measurement of only 2 blood variables ($p\text{CO}_2$ and pH). The major disadvantage is that it is merely qualitative and incapable of quantifying acid or base loads that lead to acid-base disorders.¹⁸ Furthermore, this approach does not take into account the effect of electrolyte and nonvolatile buffer concentrations on acid-base alterations. Therefore, the effect of variable plasma protein and electrolyte concentrations on BE_{ECF} may lead to erroneous conclusions as to the cause of acid-base disturbances, and the quantitative approach may be better suited to assess acid-base and electrolyte imbalances in exercising horses.

Our results are based on observations made on a heterogeneous group of horses with different physical ability and experience competing in various events under different environmental conditions. The physiological response to competitions at a certain level therefore was affected by external factors, such as the terrain, quality of the footing, and environmental conditions as well as internal factors, such as the inherent physical ability and training state of the horses. As one would therefore expect, the variability of the determined blood variables was relatively high. Furthermore, it is likely that horses competing at higher levels were preselected on the basis of greater fitness. Nevertheless, our results indicate that the increase in physical demands between cross-country competitions at IL and those at AL was high enough to induce more pronounced acid-base imbalances in horses competing at a higher level. These differences however likely were partly offset by different compensatory mechanisms.

Despite the higher blood lactate concentrations in response to competitions at AL indicating a higher contribution of anaerobic metabolism to energy supply, the decrease in pH between pre- and postexercise samples was not significantly different between groups. Higher postexercise blood lactate concentrations at AL were counteracted by stronger compensatory decreases in HCO_3^- and $p\text{CO}_2$. At AL, the increase in K^+ concentration and the decrease in Cl^- concentration at constant Na^+ concentration led to a significant but small increase in SID_3 , which partly compensated the decrease in SID_4 . The TP concentration significantly increased from pre- to postexercise values causing a significant increase in A_{tot} at both levels with higher TP concentrations at T10 in the AL group compared to the IL group. The increase in TP concentration that typically occurs in exercising horses primarily results from a rapid osmotic fluid shift from the vascular compartment into contracting skeletal muscle with the onset of exercise. The increase in extracellular K^+ concentration additionally results from the efflux of K^+ from contracting skeletal muscle to the extracellular compartment³⁰ and is associated with the occurrence of muscle fatigue.³² The decrease in Cl^- concentration may have been associated with Cl^- losses via sweating or an increased Cl^- shift from plasma into red blood cells in exchange for HCO_3^- .¹² Prolonged exercise that is associated with increased losses of water and electrolytes via sweat eventually may lead to dehydration and hypochloremic alkalosis associated with markedly decreased Cl^- concentrations and increased TP concentrations.¹² The increases from pre-exercise to T10 TP concentrations observed in our study corresponded to an approximately 12% (IL) and 11% (AL) decrease in plasma volume and were slightly less pronounced than those previously observed in horses competing in the classical format speed and endurance test of 3-day event competitions.^{2-4,6,7,9} In contrast to endurance horses, which may exhibit severe fluid and electrolyte losses, the changes in fluid and electrolyte balance associated with cross-country competitions observed in our study were rather small and therefore seem to be of little clinical relevance.

In horses competing at AL, $p\text{CO}_2$ was still significantly decreased compared to resting values at 30 minutes recovery and significantly lower than in horses competing at IL although blood lactate concentrations at 30 minutes recovery were not significantly different between competition levels. Consequently, the increase in pH from T10 to T30 values was higher in the AL group when compared to the IL group. Furthermore, a higher proportion of horses competing at AL exhibited respiratory alkalosis that was not necessarily associated with metabolic acidosis indicating a primary respiratory alkalosis. These findings suggest that the major cause for hyperventilation in these horses was thermoregulation rather than metabolic acidosis and that the heat load appeared to be higher in horses competing at AL. The climate conditions during the sampled competitions were predominantly moderate with wet bulb globe temperature (WBGT) indices between 12 and 29. Following the recommendations made by the FEI, increased heat stress requiring additional precautions to prevent the horses from overheating is expected to occur when the WBGT index increases above 27 (www.fei.org). It is therefore to be expected that the investigated horses mainly were not subjected to exceptionally high levels of heat stress during the cross-country test. Nevertheless, our results indicate that the heat accumulated during the cross-country test

induced a respiratory response that still was present after 30 minutes of recovery and more pronounced after competitions at a higher level.

The magnitude of the decrease in the blood lactate concentration from T10 to T30 values was significantly higher in the AL group when compared to the IL group. The decrease in blood lactate concentration after exercise normally occurs at a linear rate and is independent of the concentration.³³ Lactate removal after exercise can be accelerated by low-intensity postexercise activity.³⁴ The horses investigated in our study usually were walked during the first 30 minutes of recovery. The ability to remove lactate is directly associated with the oxidative capacity of muscle fibers and it has been shown that the rate of lactate disappearance increases with training.³⁵ Horses competing at higher level therefore may have been able to remove lactate at a higher rate compared to those competing at lower level as a result of higher oxidative capacity of their muscles.

5 | CONCLUSIONS AND CLINICAL IMPLICATIONS

Although a high proportion of horses competing at high-level cross-country competitions exhibited mild to moderate metabolic acidosis that persisted for >30 minutes postexercise, this metabolic acidosis could be partly compensated by respiratory alkalosis so that the pH remained within the reference range in the majority of horses. The changes in electrolyte and protein concentrations observed in response to cross-country competitions were small and therefore may be less important contributors to postexercise acid-base balance than previously observed in endurance horses.¹⁶ However, their contribution increased with higher demands at AL and they should be taken into account when assessing acid-base status in eventing horses, especially at higher performance levels and in hot and humid conditions. Regarding the complex mechanisms of acid-base changes in horses competing at cross-country competitions, the physicochemical approach provided more detailed insight into the different contributors to acid-base balance in response to cross-country competitions than did the traditional approach. Particularly, separate evaluation of alterations in SID_4 , SID_3 , and A_{tot} in terms of their contribution to acid-base homeostasis enabled a more precise differentiation between acid-base imbalances at different competition levels. On-site monitoring of acid-base and electrolyte variables in eventing horses during competitions may help veterinarians make more educated decisions on how to manage individual horses during strenuous competitions so as to prevent severe disturbances in acid-base, fluid, and electrolyte balance associated with cross-country competitions. Further research is needed to evaluate if the severity of acid-base disorders is related to performance in the cross-country test as well as in the subsequent show-jumping test and how acid-base homeostasis during exercise may be affected by training.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The commission for the ethical use of animals of the University of Liège, Belgium approved the study and all riders gave their informed consent that the data of their horses may be used for scientific purposes. The research was conducted within the scope of the "performance monitoring program" of the German Olympic Committee for Equestrian Sports.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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