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Hydroelectrolytic and Energetic Replenisher in Horses Undergoing Marcha Training

José D. Ribeiro Filho[1*], Bruna M. Ribeiro[1], Micheline O. Silva[1], Samuel R. Alves[1], Anaïs C. Benitez[1], Waleska M. F. Dantas[1], Marcel F. B. Avanza[1], Raffaella B. C. Teixeira[1], Pedro Ancelmo N. Ermita[2], Laice A. Silva[3], Rinaldo B. Viana[4], Hélio C. Manso Filho[5]

[1]Department of Veterinary, Federal University of Viçosa, Viçosa, Brazil
[2]Institute of Studies of the Wet Tropic, Federal University of Southern and Southeastern Pará, Xinguara, Brazil
[3]Department of Veterinary Clinic and Surgery, Federal University of Minas Gerais, Belo Horizonte, Brazil
[4]Institute of Animal Health and Production, Federal Rural University of Amazonia, Belém, Brazil
[5]Zootecnics Department, Federal Rural University of Pernambuco, Recife, Brazil
Email: *jdribeirofilho@gmail.com

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Abstract

The study aimed to assess the clinical, laboratory, and blood gas analysis of horses undergoing Marcha training and the effects of voluntary ingestion of hydroelectrolytic and energy replenishers after exercise. Eight horses of both genders aged between 5 and 10 years, were included in the study. The exercise consisted of a 10-min warm-up followed by 45 min uninterrupted Marcha on a flat dirt track in the morning. After exercise, the horses received one of the following treatments: Drinking water (control group); Hydroelectrolytic and energy replenisher containing sodium chloride, potassium chloride, calcium acetate, magnesium chloride, sodium citrate, dextrose, maltodextrin, and sucrose in three different concentrations (Replenishers A, B, and C). The horses were distributed across the four treatments in a 4 × 4 Latin Squares design using a Split-plot system with 48-hr intervals. Clinical and laboratory evaluations were conducted at four time points: T0 - 5 min before exercise; T1 - up to 5 min after exercise; T2 - 2 hr after starting treatment; and T4 - 4 hr after beginning treatment. Concentrations of urea, creatinine, lactate, phosphorus, and ionized calcium significantly changed after exercise. An increase in blood pH and a decrease in chloride concentrations were observed when replenishers B and C were offered after exercise. The replacements were ingested spontaneously by the animals in a volume greater than that of the control group (water). Replacement B was the most ingested by the animals, demonstrating its greatest potential.

Keywords

Dehydration, Electrolyte Replacement, Exercise, Rehydration, Voluntary Intake

1. Introduction

The growth of equestrian sports has resulted in heightened competitiveness, leading to increased intensity in training and exercise. This escalation in physical activity can induce hydroelectrolytic, acid-base, and energetic imbalances in horses. It's important to recognize that all forms of physical activity instigate changes in homeostasis, eliciting an acute physiological response [1]. Physical exercise serves as a biological stressful stimulus, inducing reversible alterations in various homeostatic variables, which can be identified through laboratory analysis.

Solutions employed for hydroelectrolytic, and energy replacement ought to include sufficient quantities of major electrolytes lost through sweating, including Na^+ (Sodium), K^+ (Potassium), Cl^- (Chloride), Ca^{++} (Calcium), Mg^{++} (Magnesium), and an energy source such as glucose, dextrose, or maltodextrin [2]. The incorporation of carbohydrates into hydroelectrolytic replenishers provides several advantages, as it helps prevent a drop in blood glucose levels, stimulates the absorption of fluid and electrolytes, and enhances palatability, particularly in cases where solutions are intended for spontaneous ingestion by horses.

A diverse range of solutions and electrolyte pastes are administered to horses to enhance animal performance. While some rehydration and supplementation practices are well-founded, others lack consistent scientific support. When selecting a rehydration method, it is advisable to choose the one that best aligns with the handling and training conditions of the athletic animal. Preference should be given to solutions that are safe, and practical and prioritize the welfare of the horse. Although there is no universally ideal replacement solution, a beneficial option for enhancing electrolyte and energy absorption involves the voluntary or *ad libitum* intake of replenisher solutions. The physiological form of rehydration is represented by hydroelectrolytic and energy replacement through spontaneous ingestion [3].

The conditioning of horses and the replenishment of fluids, electrolytes, and energy after exercise are fundamental for improving performance. Ingesting only water after periods of body fluid and electrolyte loss is not as effective in promoting hydration compared to electrolyte replacement solutions containing carbohydrates [4]. Therefore, this study was conducted to evaluate the use of hydroelectrolytic and energetic replenishers in horses undergoing Marcha training. The aim was to analyze the effects of these replenishers through a laboratory approach.

2. Materials and Methods

The experimental procedures were approved by the ethics committee in animal

use of Universidade Federal de Viçosa (CEUA/UFV process number 14/2016) following the guidelines of Brazilian legislation edited by the National Council for the Control of Animal Experimentation (CONCEA).

Study design: The study was conducted during the first half of 2016 at a farm located in the state of Minas Gerais, Brazil, at an altitude of approximately 660 meters, latitude - 20˚, 45', 16.3", and longitude 42˚, 52', 57.02", with an average temperature of 24˚C. Eight Mangalarga Marchador horses, comprising 6 males and 2 females, with a mean age of 7.5 years and an average weight of 414 kg, were included in the study. All horses were actively engaged in training, participating regularly in Marcha competitions, and were well-conditioned for the type and level of exercise they undertook.

The horses were kept in stalls and were fed twice daily with 10 - 12 kg/animal of chopped elephant grass (*Pennisetum purpureum*) and twice daily with 2 kg/animal of a commercially balanced concentrate. They had access to clean water and mineral supplements *ad libitum*. During the days of the experiment, the horses received the same feed, excluding the mineral supplement that was withheld. Water or the replenishers were provided *ad libitum* in graduated buckets throughout the experiment.

All animals were ridden in the morning on a dirt track, where they underwent the following training regimen: Initially, a 10-min warm-up session was conducted, followed by 45 min of uninterrupted Marcha exercise. Following the training session, the saddle and bridle were removed, and the horses received a bath, followed by a 10-min exposure to the sun for drying. Subsequently, the animals were placed in individual stalls where they remained for four hr (observational time). During this period, the animals were provided with feed and either hydroelectrolytic and energy replenishers or potable water (control).

Three hydroelectrolytic and energy replenisher solutions were employed, each comprising sodium chloride, potassium chloride, calcium acetate, magnesium chloride, sodium citrate, dextrose, maltodextrin, and sucrose. These components were diluted in water at varying concentrations, as detailed in Table 1 (hydroelectrolytic and energy replenisher A (176 mOsm/l), hydroelectrolytic and energy replenisher B (150 mOsm/l), and hydroelectrolytic and energy replenisher C (131 mOsm/l).

The four treatments (replenisher solutions A, B, C, and water) were supplied *ad libitum* over a four-hr period, using 18 *l* graduated plastic buckets positioned within the stalls. The contents of the buckets were replenished as the solutions were consumed.

Eight animals were allocated to four treatments in a 4 × 4 Latin Squares design using a Split-plot system, with a 48-hr interval between treatment cycles. The experiment consisted of two phases, with a four-day gap between the first and second phases. In each stage, all animals underwent testing, and the same set of animals were subjected to all treatments throughout the entire duration of the experiment.

Table 1. Composition in grams and osmolarity of hydroelectrolytic and energy replenisher A, hydroelectrolytic and energy replenisher B, and hydroelectrolytic and energy replenisher C supplied to horses after Marcha exercise training, diluted in 1000 ml of water (H_2O).

Composition	Treatments		
	A	B	C
Sodium Chloride (NaCl)	1.4 g	1.0 g	0.6 g
Potassium Chloride (KCl)	0.7 g	0.5 g	0.3 g
Calcium Acetate ($C4H_6CaO_4$)	0.7 g	0.5 g	0.3 g
Magnesium Chloride ($MgCl_2$)	0.35 g	0,25 g	0,15 g
Sodium Citrate ($C_6H_5Na_3O_7$)	0.7 g	0,5 g	0,5 g
Dextrose ($C_6H_{12}O_6$)	5 g	5 g	5 g
Maltodextrin ($C_6H_{10}O_5$)	5 g	5 g	5 g
Sucrose ($C_{12}H_{22}O_{11}$)	20 g	20 g	20 g
Osmolarity (mOsm/L)	**176**	**150**	**131**

Collection of biological samples and laboratory evaluations: Clinical evaluations and sample collections for laboratory tests were conducted at the following time points: T0 - five min before the beginning of exercise (without fasting); T1 - within five min after the conclusion of the exercise (following removal of the saddle and bridle); T2 - two hr after the initiation of the treatment phase (horses were in the stall, receiving one of the four treatments); and T4 - four hr after the initiation of the treatment phase (horses were in the stall, receiving one of the four treatments).

The voluntary intake of the solutions was assessed using 18 *l* graduated buckets every two hr (T2 and T4) for each horse to calculate the total volume ingested in liters (*l*) during the four hr of treatments. For biochemical evaluation, blood was collected using needles and vacuum tubes without anticoagulant to obtain serum and with sodium fluoride to obtain plasma. An automatic multi-biochemical analyzer was utilized for all biochemical analyses. In the serum, the following variables were determined: Magnesium (Mg^{++}), Phosphorus (P), Urea (UR), and Creatinine (CR). In the plasma, Lactate (LAC) and Glucose (GLU) were measured using commercial kits (Human® commercial reagent).

Two m*l* of blood was collected anaerobically via jugular venipuncture using lithium heparin syringes. The samples were immediately placed in ice water (0˚C - 4˚C) and stored until blood gas analysis, which occurred within 2 hr of collection. The following variables were measured: Sodium (Na^+), Potassium (K^+), Ionized Calcium (iCa^{++}), Chloride (Cl^-), venous blood pH (pH), Partial carbon dioxide pressure of the venous blood (pCO_2), Venous blood bicarbonate concentration (HCO_3^-), Base excess concentration (BE). Additionally, the Anion

GAP (AG) was calculated using the formula: AG = ([Na^+] + [K^+]) − ([Cl^-] + [HCO_3^-]) and the Strong Ion Difference (SID) was determined using the formula quoted by Constable [5]: SID = ([Na^+] + [K^+]) − ([Cl^-] + [LAC]).

Statistical Analyses: The Statistical Analysis System program was utilized, and the data were presented as mean and standard deviation. The experiment was conducted using two Latin Squares (4 × 4) in a Split-plot design. Mathematical models were applied for the ingested volume of treatments: $Yijkl = \mu + Ai + Pj + Trk + e(a)ijk$, and clinical laboratory variables: $Yijkl = \mu + Ai + Pj + Trk + e(a)ijk + Tel + (Tr * Te)kl + e(b)l$, where $Yijkl$ = observed response; μ = general constant; Ai = effect of the animal; Pj = period effect; Trk = treatment effect; $e\ (a)\ ijk$ = parcel error (residue a); Tel = time effect; $(Tr * Te)\ kl$ = treatment and time interaction; $e\ (b)\ l$ = error concerning the subplots (residue b). Data were subjected to analysis of variance, and the LS-means were compared using the Tukey-Kramer method at a 5% probability [6].

3. Results

All horses remained well throughout the experimental period. After exercise, they showed intense sweating, but without signs of fatigue. The volume ingested by horses showed a difference between treatments ($P < 0.05$). Animals that ingested replenishing solution B showed higher consumption in T4 (9 liters) when compared to animals in the Control group (4, 7 liters) (Table 2).

Table 2. Mean values and standard deviations of ingested volume (*l*) over time in horses undergoing Marcha training and subsequently treated with water (control) or hydroelectrolytic replenisher.

	T2	T4 (Total Volume)
Control (water)	2.70 ± 2.80^A	4.7 ± 2.66^B
Replenisher A	5.00 ± 3.46^A	7.5 ± 4.63^{AB}
Replenisher B	6.20 ± 5.76^A	10.8±7.43^A
Replenisher C	5.25 ± 2.88^A	9.0 ± 4.46^{AB}

Mean values and standard deviations followed by capital letters are different in the same column and differ statistically ($P < 0.05$).

Urea (UR) concentration did not change significantly between treatments and over time ($P > 0.05$), except treatment C which showed a significant increase ($P < 0.05$) after Marcha training (T1) (Table 3). Concentrations of Creatinine (CR) did not display significant variation between treatments ($P > 0.05$), although the interaction of treatments with time revealed an increase ($P < 0.05$) after training (T1) in treatment Control, B, and C (Table 3). Lactate (LAC) levels increased after Marcha training (T1) only in treatment B ($P < 0.05$). No significant differences in LAC were observed between treatments ($P > 0.05$). Glucose (GLU) levels did not vary ($P > 0.05$) over time or between treatments (Table 3).

Table 3. Mean values and standard deviations of Urea (UR), Creatinine (CR), Lactate (LAC), and Glucose (GLU) in horses undergoing Marcha training and subsequently treated with water (control) or with hydroelectrolytic repositories.

Treatment	Time			
	T0	T1	T2	T4
		UR (mg/dl)		
Control (water)	30 ± 3.96^{Aa}	31 ± 2.28^{Aa}	31 ± 5.25^{Aa}	31 ± 7.40^{Aa}
Repository A	31 ± 4.21^{Aa}	30 ± 4.79^{Aa}	32 ± 5.79^{Aa}	31 ± 4.05^{Aa}
Repository B	30 ± 4.03^{Aa}	33 ± 5.68^{Aa}	31 ± 5.88^{Aa}	31 ± 6.78^{Aa}
Repository C	27 ± 9.70^{Ab}	32 ± 2.48^{Aa}	30 ± 4.41^{Aab}	30 ± 3.62^{Aab}
		CR (mg/dl)		
Control (water)	1.55 ± 0.21^{Ab}	1.74 ± 0.17^{Aa}	1.64 ± 0.19^{Aab}	1.54 ± 0.19^{Ab}
Repository A	1.58 ± 0.23^{Aa}	1.74 ± 0.27^{Aa}	1.68 ± 0.28^{Aa}	1.60 ± 0.26^{Aa}
Repository B	1.53 ± 0.23^{Ab}	1.71 ± 0.20^{Aa}	1.65 ± 0.34^{Aab}	1.53 ± 0.18^{Ab}
Repository C	1.50 ± 0.23^{Ab}	1.71 ± 0.19^{Aa}	1.56 ± 0.28^{Aab}	1.53 ± 0.29^{Ab}
		LAC (mmol/l)		
Control (water)	0.70 ± 1.00^{Aa}	2.21 ± 24.29^{Aa}	0.80 ± 1.77^{Aa}	0.76 ± 1.19^{Aa}
Repository A	0.66 ± 0.92^{Aa}	1.46 ± 6.30^{Aa}	1.09 ± 2.92^{Aa}	0.88 ± 1.83^{Aa}
Repository B	0.63 ± 1.41^{Ab}	2.69 ± 29.00^{Aa}	1.20 ± 4.79^{Aab}	1.00 ± 2.95^{Aab}
Repository C	0.68 ± 1.31^{Aa}	1.64 ± 6.90^{Aa}	1.05 ± 1.51^{Aa}	0.95 ± 2.07^{Aa}
		GLU (mg/dl)		
Control (water)	107 ± 11.26^{Aa}	99 ± 16.08^{Aa}	95 ± 14.97^{Aa}	94 ± 7.68^{Aa}
Repository A	107 ± 17.25^{Aa}	100 ± 11.69^{Aa}	112 ± 27.45^{Aa}	92 ± 19.06^{Aa}
Repository B	104 ± 12.67^{Aa}	100 ± 8.70^{Aa}	113 ± 30.70^{Aa}	98 ± 13.06^{Aa}
Repository C	103 ± 8.97^{Aa}	103 ± 8.18^{Aa}	123 ± 32.70^{Aa}	100 ± 19.54^{Aa}

Mean values and standard deviations, followed by different lowercase and uppercase letters in the same row and column, differ statistically ($P < 0.05$).

There were no significant differences in sodium (Na^+), potassium (K^+), and magnesium ion (Mg^{++}) concentrations between treatments and across the evaluated time points ($P > 0.05$) (Table 4). Phosphorus (P) concentrations decreased significantly in horses after Marcha training (T1) only in treatment A ($P < 0.05$). In turn, ionized calcium (iCa^{++}) decreased significantly in horses after Marcha training (T1) across all treatments ($P < 0.05$) but did not differ between treatment groups ($P > 0.05$) (Table 4).

As can be seen in Table 4, chloride (Cl^-) concentrations exhibited significant variation over time ($P < 0.05$), except treatment A. Chloride decreased after ex-

ercise (T1), but a significant decrease was observed at T2 and T4 in the Control group and treatment C, while in treatment B this only occurred in T4. Significant differences in Cl^- were noted between treatments A and C at T2 and T4, with treatment C reaching the lowest values ($P < 0.05$).

Table 4. Mean values and standard deviations of Sodium (Na^+), Potassium (K^+), Magnesium (Mg^{++}), Phosphorus (P), Ionized Calcium (iCa^{++}) and Chloride (Cl^-) in horses undergoing Marcha training and subsequently treated with water (control) or with hydroelectrolytic replenisher.

Treatment	Time			
	T0	T1	T2	T4
			Na^+ (mmol/l)	
Control (water)	137 ± 1.89^{Aa}	137 ± 1.98^{Aa}	138 ± 1.77^{Aa}	137 ± 1.41^{Aa}
Repository A	137 ± 0.93^{Aa}	137 ± 1.13^{Aa}	137 ± 1.28^{Aa}	137 ± 0.64^{Aa}
Repository B	137 ± 0.64^{Aa}	137 ± 1.13^{Aa}	137 ± 1.91^{Aa}	137 ± 0.89^{Aa}
Repository C	137 ± 1.46^{Aa}	137 ± 1.81^{Aa}	136 ± 1.07^{Aa}	136 ± 1.36^{Aa}
			K^+ (mmol/l)	
Control (water)	4.07 ± 0.30^{Aa}	3.90 ± 0.50^{Aa}	4.08 ± 0.48^{Aa}	4.23 ± 0.52^{Aa}
Repository A	4.22 ± 0.30^{Aa}	3.86 ± 0.36^{Aa}	3.95 ± 0.30^{Aa}	4.20 ± 0.33^{Aa}
Repository B	4.21 ± 0.25^{Aa}	3.98 ± 0.42^{Aa}	3.77 ± 0.37^{Aa}	4.01 ± 0.21^{Aa}
Repository C	4.25 ± 0.41^{Aa}	3.89 ± 0.46^{Aa}	3.93 ± 0.36^{Aa}	4.13 ± 0.25^{Aa}
			Mg^{++} (mmol/l)	
Control (water)	2.01 ± 0.48^{Aa}	1.90 ± 0.55^{Aa}	2.11 ± 0.54^{Aa}	2.06 ± 0.63^{Aa}
Repository A	2.00 ± 0.69^{Aa}	1.94 ± 0.63^{Aa}	2.03 ± 0.59^{Aa}	1.67 ± 0.72^{Aa}
Repository B	1.86 ± 0.65^{Aa}	1.95 ± 0.69^{Aa}	1.93 ± 0.49^{Aa}	1.99 ± 0.53^{Aa}
Repository C	1.76 ± 0.47^{Aa}	1.83 ± 0.42^{Aa}	1.92 ± 0.44^{Aa}	1.96 ± 0.50^{Aa}
			P (mmol/l)	
Control (water)	3.19 ± 0.43^{ABa}	2.98 ± 0.45^{Aa}	3.03 ± 0.48^{Aa}	3.39 ± 0.44^{Aa}
Repository A	4.01 ± 1.62^{Aa}	2.70 ± 0.73^{Ab}	3.11 ± 0.56^{Aab}	3.44 ± 1.07^{Aab}
Repository B	3.03 ± 0.33^{ABa}	2.89 ± 0.48^{Aa}	2.39 ± 0.34^{Aa}	2.75 ± 0.50^{Aa}
Repository C	2.80 ± 1.03^{Ba}	2.84 ± 0.59^{Aa}	2.63 ± 0.55^{Aa}	2.96 ± 0.70^{Aa}
			iCa^{++} (mmol/l)	
Control (water)	1.68 ± 0.08^{Aa}	1.50 ± 0.06^{Ab}	1.62 ± 0.10^{Aa}	1.61 ± 0.11^{Aab}
Repository A	1.68 ± 0.05^{Aa}	1.48 ± 0.09^{Ab}	1.65 ± 0.08^{Aa}	1.66 ± 0.11^{Aa}
Repository B	1.66 ± 0.08^{Aa}	1.47 ± 0.12^{Ab}	1.65 ± 0.07^{Aa}	1.66 ± 0.08^{Aa}
Repository C	1.67 ± 0.09^{Aa}	1.49 ± 0.09^{Ab}	1.66 ± 0.10^{Aa}	1.67 ± 0.09^{Aa}

Continued

			Cl^- (mmol/l)	
Control (water)	100 ± 3.52^{Aa}	99 ± 4.11^{Aab}	97 ± 4.00^{ABb}	97 ± 3.94^{Ab}
Repository A	100 ± 4.29^{Aa}	99 ± 3.87^{Aa}	99 ± 5.10^{Aa}	98 ± 4.96^{Aa}
Repository B	100 ± 4.45^{Aa}	99 ± 3.77^{Aab}	98 ± 4.47^{ABab}	97 ± 3.44^{Ab}
Repository C	100 ± 1.91^{Aa}	98 ± 2.97^{Aab}	95 ± 2.50^{Bbc}	95 ± 2.39^{Bc}

Mean values and standard deviations, followed by different lowercase and uppercase letters in the same row and column, differ statistically ($P < 0.05$).

As can be seen in Table 5, following Marcha training (T1), venous pH increased significantly in the control group and treatment C ($P < 0.05$). Treatment B showed a significant increase in pH only at T4. No significant changes in pH were observed between groups. Partial carbon dioxide pressure (pCO_2), bicarbonate concentration (HCO_3^-) base excess (BE), anion gap (AG), and strong ion difference (SID) did not show significant changes between treatments and within treatments over time ($P > 0.05$).

Table 5. Mean values and standard deviations of venous blood pH (pH), carbon dioxide partial pressure (pCO_2), blood bicarbonate (HCO_3^-), base excess (BE), anion gap (AG), and strong ion difference (SID) in horses submitted to Marcha training and subsequently treated with water (control) or with hydroelectrolytic replenisher.

Treatments	Times			
	T0	T1	T2	T4
			pH	
Control (water)	7.41 ± 0.02^{Ab}	7.44 ± 0.03^{Aa}	7.43 ± 0.02^{Aab}	7.43 ± 0.03^{Aab}
Replenisher A	7.42 ± 0.02^{Aa}	7.44 ± 0.02^{Aa}	7.43 ± 0.02^{Aa}	7.42 ± 0.03^{Aa}
Replenisher B	7.41 ± 0.02^{Ab}	7.43 ± 0.03^{Aab}	7.42 ± 0.02^{Aab}	7.44 ± 0.02^{Aa}
Replenisher C	7.40 ± 0.03^{Ab}	7.44 ± 0.03^{Aa}	7.41 ± 0.02^{Aab}	7.43 ± 0.02^{Aab}
			pCO_2 (mmHg)	
Control (water)	47.1 ± 3.13^{Aa}	43.0 ± 3.26^{Aa}	44.8 ± 3.52^{Aa}	45.7 ± 3.61^{Aa}
Replenisher A	46.2 ± 3.16^{Aa}	43.7 ± 3.23^{Aa}	43.7 ± 1.22^{Aa}	45.0 ± 3.45^{Aa}
Replenisher B	45.4 ± 3.28^{Aa}	43.4 ± 4.04^{Aa}	44.0 ± 3.14^{Aa}	42.8 ± 2.96^{Aa}
Replenisher C	46.4 ± 4.83^{Aa}	44.1 ± 4.21^{Aa}	45.5 ± 3.08^{Aa}	45.6 ± 3.52^{Aa}
			HCO_3^- (mmol/l)	
Control (water)	28.8 ± 1.56^{Aa}	28.1 ± 2.34^{Aa}	28.7 ± 2.28^{Aa}	29.4 ± 1.79^{Aa}
Replenisher A	28.8 ± 1.02^{Aa}	28.4 ± 1.22^{Aa}	27.8 ± 1.20^{Aa}	28.4 ± 0.96^{Aa}
Replenisher B	28.0 ± 1.17^{Aa}	27.5 ± 2.51^{Aa}	27.9 ± 2.19^{Aa}	28.6 ± 1.31^{Aa}
Replenisher C	28.2 ± 1.17^{Aa}	28.6 ± 1.94^{Aa}	28.2 ± 2.23^{Aa}	29.4 ± 1.75^{Aa}

Continued

		Base Excess (mmol/l)		
Control (water)	4.1 ± 1.35^{Aa}	4.8 ± 2.33^{Aa}	4.5 ± 2.07^{Aa}	5.1 ± 1.66^{Aa}
Replenisher A	4.3 ± 0.74^{Aa}	5.1 ± 0.89^{Aa}	3.7 ± 1.33^{Aa}	4.1 ± 0.65^{Aa}
Replenisher B	3.6 ± 1.04^{Aa}	4.2 ± 2.18^{Aa}	3.7 ± 1.91^{Aa}	4.6 ± 1.30^{Aa}
Replenisher C	3.7 ± 0.84^{Aa}	5.19 ± 1.51^{Aa}	3.8 ± 2.09^{Aa}	5.1 ± 1.50^{Aa}
		AG (mmol/l)		
Control (water)	12.06 ± 3.61^{Aa}	14.07 ± 3.95^{Aa}	15.59 ± 3.10^{Aa}	15.30 ± 2.35^{Aa}
Replenisher A	12.04 ± 4.57^{Aa}	13.42 ± 4.28^{Aa}	14.38 ± 5.16^{Aa}	14.66 ± 4.43^{Aa}
Replenisher B	13.18 ± 4.40^{Aa}	14.84 ± 4.88^{Aa}	15.12 ± 3.77^{Aa}	15.09 ± 3.73^{Aa}
Replenisher C	13.41 ± 2.51^{Aa}	14.55 ± 1.71^{Aa}	16.37 ± 1.94^{Aa}	15.84 ± 1.55^{Aa}
		DIF (mmol/l)		
Control (water)	40.14 ± 3.83^{Aa}	39.95 ± 2.99^{Aa}	43.55 ± 2.76^{Aa}	44.00 ± 2.69^{Aa}
Replenisher A	40.22 ± 4.98^{Aa}	40.39 ± 3.56^{Aa}	41.12 ± 4.92^{Aa}	42.21 ± 4.54^{Aa}
Replenisher B	40.58 ± 8.83^{Aa}	39.66 ± 2.95^{Aa}	41.81 ± 3.33^{Aa}	42.65 ± 2.78^{Aa}
Replenisher C	40.96 ± 1.77^{Aa}	41.50 ± 1.52^{Aa}	43.50 ± 1.92^{Aa}	44.31 ± 1.92^{Aa}

Mean values and standard deviations, followed by different lowercase and uppercase letters in the same row and column, differ statistically ($P < 0.05$).

4. Discussion

Hydroelectrolytes and energy replenishers for horses are solutions that contain carbohydrates such as glucose, sucrose, and maltodextrin, along with electrolytes. By combining water, electrolytes, and energy replenishment, these solutions contribute to hydration in addition to nutritional support [7]. In this test, hydroelectrolyte and energy replenishers were consumed in greater quantities than water, demonstrating that all of them had good palatability, especially replenisher B. The average volume ingested by animals that ingested replenisher B during the four hours of supply (T2 to T4) was 10.8 L, while treatments A and C had averages of 7.5 and 9 L, respectively. Meanwhile, the animals in the control group consumed 4.7 liters (Table 2).

In pilot studies that preceded this trial, it was observed that horses demonstrated a preference for solutions with sweet flavors, mild aromas, and no color. Therefore, no aromas, artificial flavors, or palatalizing agents were used, as some of them, when tested in pilot studies, always caused a decrease in the voluntary intake of the solutions. These results are in line with other studies that found animals less receptive to the inclusion of these substances. The flavor and aroma of the solutions developed in this test resulted from the combination of the composition of electrolytes (salts) and carbohydrates and their respective quantities. Certainly, one of the factors that most contribute to the consumption of replacement solutions is

their palatability [8], [9]. It is mainly responsible for the increase in voluntary intake of sports drinks compared to water. In this respect, replenisher B was the highlight, demonstrating its great potential among the solutions tested.

The results of the present test are similar to those obtained by Avanza *et al.* [3], who reported higher ingested volumes of experimental replenishers compared to water after the Marcha exercise, and by Monteiro *et al.* [10] in polo horses. On the other hand, Puoli Filho *et al.* [11], in a study comparing the intake of water and isotonic solution containing carbohydrates and electrolytes, observed greater water consumption compared to isotonic solution in horses undergoing resistance exercise. Meanwhile, Butudom *et al.* [12] and Nyman *et al.* [13] observed significant voluntary consumption of saline solutions (0.45% and 0.9%) instead of water, concluding that saline treatment is a more effective rehydration strategy than water alone.

Activities that induce fluid loss through sweat cause a reduction in renal blood flow and glomerular filtration rate that reflects the transient elevation of post-exercise urea (UR) and creatinine (CR) concentrations. These changes are dependent on the intensity of the exercise and their increase is typically associated with muscular activity and the workload to which animals are subjected [14]. In this study, all animals showed increased post-exercise CR values (T1), which decreased at T4. Replenishers B and C likely caused a more pronounced decrease in CR values at T4 due to their higher ingestion rate, as can be seen in **Table 3**. The control treatment also caused the greatest decrease in CR values, despite its lower rate of ingestion, demonstrating its high rate of intestinal absorption. Unfortunately, urinary volume was not measured; perhaps if it had been done, we would have had a better explanation for this result.

Depending on the intensity of the exercise, there is an increase in plasma lactate (LAC) values. However, in the present study, the animals demonstrated a mean plasma LAC level of 2.0 mmol/L at the end of Marcha training, indicating aerobic exercise with a tendency to transition from aerobic to anaerobic muscle activity, confirming that Marcha is predominantly a low-intensity and medium-duration aerobic exercise [15], [16].

There was an increase in lactate values in T1 in animals from all treatments, although a significant difference occurred only in animals from treatment B. In T2 and T4, there was a gradual decrease in LAC in all treatments. Although the replenishers contain sucrose, dextrose, and maltodextrin, there was no excessive production of acidic substances resulting from fermentation by the intestinal microbiota, demonstrating their safety.

Changes in blood glucose concentration depend on exercise type, duration, intensity, and the horse's training and feeding status. According to Votion [17], the plasma concentration of GLU usually increases during short and high-intensity exercises, despite varied results in the literature. In long-duration and endurance exercises (>3 hr), GLU tends to decrease. In the present trial, the combination of dextrose, maltodextrin, and sucrose present in the replacements, although not significant, caused a small increase in the glycemic rate of treat-

ments A, B, and C in T2. If the amount of these carbohydrates in the replacements had been greater, the glycemic rate could have increased. However, larger quantities might have reached the large intestine, causing more fermentation and production of acidic substances, which could lead to dysbiosis in animals. Although replenishers are also important as precursors [7], it is necessary to be careful with the amount of carbohydrates these solutions contain.

There were no differences in serum concentrations of Na^+, K^+, and Mg^{++} throughout the experimental phase (T0 to T4) in animals from all treatments. During exercise, depending on its intensity and duration, there is a decrease in K^+ due to its loss through sweating and increased renal excretion of Na^+. Renal reabsorption of Na^+ occurs at the expense of the excretion of K^+ and H^+, and consequently, the retained ions increase the expansion of extracellular fluid in response to dehydration [18]. However, as this event was not detected by the serum values of these electrolytes, it is believed that Marcha, being a low-intensity and medium-duration exercise, did not cause sufficient electrolyte losses to trigger this type of mechanism. It should be noted that if the concentration of these electrolytes had been measured in urine, we might have observed a difference between animals that received replacements and those in the control group.

In the present study, a decrease in serum P concentrations was observed after exercise, but it was significant only in animals in treatment A, which may indicate an attempt to restore intracellular P reserves. Furthermore, the lower serum P concentration may be due to increased carbohydrate metabolism, which depends on the duration and intensity of exercise [19]. During the treatment period (T2 and T4), P values showed changes. None of the three replenishers tested contained a source of phosphorus; perhaps for this reason, variations in serum phosphorus values were recorded in animals from all treatments. These results point to the possibility of incorporating a phosphate source into the composition of these replenishers.

There was a decrease in iCa values in animals from all treatments at T1. García *et al.* [20] found a significant decrease in iCa after exercise, as in the present study. In T2 and T4, values recovered in animals from all treatments. Despite the lack of statistical significance, it can be seen that in animals that received replenishers, iCa values were slightly higher at those times, demonstrating the positive effect of the calcium contained in treatments A, B, and C. Therefore, in animals subjected to exercises that may generate greater decrements in calcium, the use of electrolyte and energy replenishers containing a source of calcium in specified amounts may be more appropriate to treat exercise deficit in athletic animals compared to water alone.

The lack of a significant decrease in plasma Cl^- concentration after gait training (T1) can be explained by the exercise's inability to cause major changes in the dynamics of this ion. When comparing treatments, the animals that received treatment C recorded the lowest chloride values. When evaluating treatments over time, the lowest chloride values during the treatment period (T2 and T4) were also detected in the animals that received solution C. This event occurred

because treatment C contains the lowest concentrations of chloride in its composition, indicating that in animals that have decreased serum chloride due to the action of exercise, solution C is not the best choice.

Marcha training in the animals in the present study caused a slight increase in pH values in animals from all treatments. Despite this increase, the values remained within the reference range [18], [21]. During the treatment phase (T2 and T4), the values showed similar results, demonstrating the absence of significant clinical changes. In low-intensity exercise, such as walking, there are generally no changes in acid-base status, anion gap, and strong ion difference. When changes do occur, they are discreet. This can be seen in the small changes detected in pH values and the absence of changes in pCO_2, HCO_3^-, BE, anion gap, and strong ion difference in the animals in the present test. The absence of these changes was due to the lack of significant electrolyte imbalances. These results demonstrate that the tested solutions do not cause significant changes in the acid-base balance, confirming their safety.

The limitations of the present research are the small number of animals used, the intensity of exercise imposed on the animals, and the lack of measurement of urinary volume and electrolytes in urine.

It is concluded that the hydroelectrolytes and energy replenishers were ingested spontaneously by the animals in a greater volume than that of the control group (water). However, replenisher B, by restoring and maintaining electrolyte concentrations after exercise and being the most palatable, as indicated by the greater volume ingested by the animals, demonstrated its greatest potential. Despite these results, new trials subjecting animals to greater intensity exercises are needed to prove the effectiveness of the tested replacements.

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Conflicts of Interest

The authors have no competing or conflict of interests in submitting this article. The datasets generated for this study are available on request to the corresponding author.

References

[1] Bartolomé, E. and Cockram, M.S. (2016) Potential Effects of Stress on the Performance of Sport Horses. *Journal of Equine Veterinary Science*, **40**, 84-93. https://doi.org/10.1016/j.jevs.2016.01.016

[2] Filho, J.D.R., Pessin, A.E., Atoji, K., Souza, M.V., Gomes, C.L.N. and Silva, A.R. (2014) Enteral Fluid Therapy: Biochemical Profile of Horses Treated with Hypotonic Enteral Electrolyte Solutions Associated with Energy Sources. *Journal of Equine Veterinary Science*, **34**, 759-764. https://doi.org/10.1016/j.jevs.2014.01.004

[3] Avanza, M.F.B., Ribeiro Filho, J.D., Ribeiro, B.M., Alves, S.R., Silva, M.O., Dantas,

W.M.F., Manso Filho, H.C. and Viana, R.B. (2018) Ingestão ad libitum de repositores hidroeletrolíticos e energéticos em cavalos submetidos ao treinamento de marcha. *Revista Brasileira de Medicina Veterinaria*, **40**, e033318.

[4] Marlin, D.J., Scott, C.M., Mills, P.C., Louwes, H. and Vaarten, J. (1998) Rehydration Following Exercise: Effects of Administration of Water versus an Isotonic Oral Rehydration Solution (ORS). *The Veterinary Journal*, **156**, 41-49. https://doi.org/10.1016/s1090-0233(98)80060-9

[5] Constable, P.D. (1999) The Physicochemical Approach for Evaluating Acid-Base Balance in Exercising Horses. *Equine Veterinary Journal*, **31**, 636-638. https://doi.org/10.1111/j.2042-3306.1999.tb05301.x

[6] Statistical Analysis System (2002) SAS/STAT® 9.0. Cary: SAS Institute, Inc.

[7] Shirreffs, S.M., Aragon-Vargas, L.F., Chamorro, M., Maughan, R.J., Serratosa, L. and Zachwieja, J.J. (2005) The Sweating Response of Elite Professional Soccer Players to Training in the Heat. *International Journal of Sports Medicine*, **26**, 90-95. https://doi.org/10.1055/s-2004-821112

[8] Guerra, I. (2004) A importância da hidratação e da alimentação do atleta. *Revista Mineira de Educação Física*, **12**, 159-73.

[9] Aparecida Edvim Cruz, M., Costa Cabral, C.A. and Bouzas Marins, J.C. (2009) Nível de conhecimento e hábitos de hidratação dos atletas de mountain bike. *Fitness & Performance Journal*, **8**, 79-89. https://doi.org/10.3900/fpj.8.2.79.e

[10] Monteiro, L.C., Ribeiro Filho, J.D., Dantas, W.D.M.F., Ribeiro, B.M., Silva, M.O.D., Costa, C.M., *et al.* (2018) Effect of Ad Libitum Intake of an Electrolyte Repository in Horses That Underwent a Polo Game. *Semina*: *Ciências Agrárias*, **39**, 187-198. https://doi.org/10.5433/1679-0359.2018v39n1p187

[11] Puoli Filho, J.N.P., Barros Neto, T.L.d., Rodrigues, P.H.M. and Garcia, H.P.L. (2007) Parâmetros fisiológicos do desempenho de cavalos de alta performance hidratados voluntariamente com água ou solução isotônica contendo carboidrato. *Brazilian Journal of Veterinary Research and Animal Science*, **44**, 122-131. https://doi.org/10.11606/issn.1678-4456.bjvras.2007.26650

[12] Butudom, P., Schott, H.C., Davis, M.W., Kobe, C.A., Nielsen, B.D. and Eberhart, S.W. (2002) Drinking Salt Water Enhances Rehydration in Horses Dehydrated by Frusemide Administration and Endurance Exercise. *Equine Veterinary Journal*, **34**, 513-518. https://doi.org/10.1111/j.2042-3306.2002.tb05475.x

[13] Nyman, S., Jansson, A., Dahlborn, K. and Lindholm, A. (1996) Strategies for Voluntary Rehydration in Horses during Endurance Exercise. *Equine Veterinary Journal*, **28**, 99-106. https://doi.org/10.1111/j.2042-3306.1996.tb05037.x

[14] Piccione, G., Vazzana, I., Giannetto, C., Gianesella, M. and Errantelli, V. (2008) Modification of Some Haematological and Haematochemical Parameters in Horse during Long Distance Rides. *Research Journal of Veterinary Sciences*, **1**, 37-43. https://doi.org/10.3923/rjvs.2008.37.43

[15] Silva, F.S., Melo, S.K.M., Manso, H.E.C.C.C., Abreu, J.M.G. and Manso Filho, H.C. (2014) Heart Rate and Blood Biomarkers in Brazilian Gaited Horses during a Standardised Field Gaited Test. *Comparative Exercise Physiology*, **10**, 105-111. https://doi.org/10.3920/cep140003

[16] Avanza, M.F.B. (2016) Effects of Ad Libitum Intake of Hypotonic and Energy Hydroelectrolyte Replacers in Horses Submitted to Marcha Training. Master's Thesis, Federal University of Viçosa.

[17] Votion, D. (2014) Metabolic Responses to Exercise and Training. In: Hinchcliff,

K.W., Kaneps, A.J. and Geor, R.J., Eds., *Equine Sports Medicine and Surgery*, Elsevier, 747-767. https://doi.org/10.1016/b978-0-7020-4771-8.00033-8

[18] Carlson, G.P. and Bruss, M. (2008) Fluid, Electrolyte, and Acid-Base Balance. In: Kaneko, J.J., Harvey, J.W. and Bruss, M.L., Eds., *Clinical Biochemistry of Domestic Animals*, Elsevier, 529-559. https://doi.org/10.1016/b978-0-12-370491-7.00017-9

[19] Andrews, F.M., Geiser, D.R., White, S.L., Williamson, L.H., Maykuth, P.L. and Green, E.M. (1995) Haematological and Biochemical Changes in Horses Competing in a 3 Star Horse Trial and 3-Day-Event. *Equine Veterinary Journal*, **27**, 57-63. https://doi.org/10.1111/j.2042-3306.1995.tb05009.x

[20] Garcia, T.R., Rezende, A.S.C.d., Santiago, J.M., Almeida, F.Q.d., Fonseca, M.G. and Munõz, A. (2013) Venous Hemogasometry and Blood Electrolytes in Mangalarga Marchador Mares Submitted to Aerobic Training. *Ciência e Agrotecnologia*, **37**, 559-565. https://doi.org/10.1590/s1413-70542013000600009

[21] Weiser, G. (2012) Laboratory Evaluation of Acid-Base Disorders. In: Thrall, M.A., Weiser, G., Allison, R.W. and Campbell, T.W., Eds., *Veterinary Hematology and Clinical Chemistry*, 2nd Edition, Wiley-Blackwell, 393-400.