FULL ARTICLE

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Evaluation of hydroelectrolytic, energetic supplementation, and clinical laboratory parameters in search and rescue dogs

Avaliação da suplementação hidroeletrolítica, energética e parâmetros clínico laboratoriais em cães de busca, resgate e salvamento

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ABSTRACT

Establishing methods and actions to improve performance and reduce changes during physical activity is essential in search and rescue dogs. Studying the changes in search and rescue dogs during working activities and how to correct them can help improve their performance and avoid complications. Six healthy adult dogs, which had participated in search and rescue operations for at least one year, were used. Animals had no evidence of clinical disease nor received any supplement or medication prior to the assessment. Dogs were evaluated before, during, and after exercise and submitted to volume replacement with mineral water (T1) and hydroelectrolytic supplementation (T2). Body temperature (BT), parameters of hydration (body weight, erythrogram, and total protein (TP)), energy indicators (glucose, lactate), electrolytes (K^+, Na^+, Cl^-, P^+, Ca^{2+}, $Mg2^+$), and hormone levels (cortisol, aldosterone, insulin) were determined. After exercise, isotonic dehydration was detected in both treatments, accompanied by erythrocytosis and weight loss. During the recovery phase, in both treatments, dogs presented a significant increase in BT and lactate and a significant decrease in insulin, TP, and P+. BT and lactate increased after exercise and returned to basal upon recovery. Insulin decreased after exercise without changes in glucose. The maintenance of cortisol indicated the adjustability of dogs to environmental stimuli and stress resistance, and aldosterone did not change during exercise. Both volume replacement with water or hydroelectrolytic and energetic supplementation can correct the isotonic dehydration exhibited by dogs.

Keywords: Dehydration. Electrolytes. Exercise. Hormone. Military dogs.

RESUMO

Estabelecer métodos e ações para melhorar o desempenho e reduzir alterações durante a atividade física em cães de busca, resgate e salvamento é importante. Estudar as alterações que ocorrem nesses cães durante a atividade de trabalho e as formas de corrigi-las pode ajudar a melhorar o desempenho dos cães e evitar complicações. Foram estudados seis cães adultos saudáveis, que participaram de operações de busca e salvamento há pelo menos um ano. Os animais não apresentavam qualquer evidência de doença clínica, nem receberam qualquer suplemento ou medicação antes da avaliação. Os cães foram avaliados antes, durante e após o exercício e submetidos à reposição volêmica com água mineral (T1) e suplementação hidroeletrolítica (T2). Temperatura corporal (TC), parâmetros de hidratação (peso corporal, eritrograma e proteína total (PT)), indicadores energéticos (glicose, lactato), eletrólitos (K^+, Na^+, Cl^-, P^+, $Ca^{2+,}$ Mg^{2+}) e níveis hormonais (cortisol, aldosterona, insulina) foram determinados. Após o exercício, foi detectada desidratação isotônica em ambos os tratamentos, acompanhada de eritrocitose e perda de peso. Durante a fase de recuperação, em ambos os tratamentos, os cães apresentaram aumento significativo de TC e lactato e diminuição significativa de insulina, PT e P^+. A TC e o lactato aumentaram após o exercício e retornaram ao valor basal após a recuperação. A insulina diminuiu após o exercício, sem alterações na glicemia; a manutenção do cortisol indicou a adaptabilidade dos cães aos estímulos ambientais e resistência ao estresse, e a aldosterona não alterou durante o exercício. Tanto a reposição volêmica com água quanto a suplementação hidroeletrolítica e energética são capazes de corrigir a desidratação isotônica apresentada pelos cães.

Palavras-chave: Desidratação. Eletrólitos. Exercício. Hormônio. Cães militares.

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Introduction

Search and rescue dogs carry out crucial social work by locating victims. These animals require adequate physical conditioning to perform such an activity since they cover different dimensions and distinct types of terrain (Rovira et al., 2008).

With physical exercise, homeostasis undergoes alterations due to the increase in total energy expenditure (Monteiro et al., 2004), which may lead to hormonal changes (Helton, 2009; Pösö & Hyyppä, 1999) and electrolytic imbalances (Assenza et al., 2014). Moreover, these dogs may exhibit dehydration, with reduced water supply for body cooling and raising body temperature, resulting in inferior physical and olfactory performance and heat intolerance (Otto et al., 2017).

Knowledge of the physiological demands and hydroelectrolytic and energetic changes exercise causes to these dogs is crucial in assisting the teams during exercise and actual search events. This information fosters improvements in the physical aptitude of these animals for more extended periods and reduces the risks of alterations that may occur with physical exhaustion. Thus, the present study aimed to evaluate the hydration parameters and electrolyte, hormone, glucose, and lactate concentrations in search and rescue dogs treated with mineral water or hydroelectrolytic and energetic supplementation.

Materials and Methods

Animals

This study was approved by the Ethics Committee in the Use of Animals (CEUA) of the Federal University of Espírito Santo in January 2018, under protocol No. 56/2017, under Brazilian laws for procedures and scientific use of animals. The Espírito Santo Military Fire Brigade commander responsible for the dogs signed a consent form.

A non-randomized clinical trial was conducted at the Military Fire Brigade in Brazil. Six healthy adult dogs, which have participated in search and rescue operations for at least one year, were used in this study. The animals consisted of two Belgian Malinois Shepherds, three German Shepherds, and one mixed-breed between Belgian Malinois Shepherd and German Shepherd; three neutered males and three spayed females, aged 2 to 6 years old, weighing between 25.6 and 34.6 kg (31.0 ± 3.0 kg).

Animal inclusion was carried out with previous anamnesis and a complete physical examination. A blood sample was also collected for the hematological and biochemical analyses of the following parameters: alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), urea, and creatinine. None of the animals showed clinical disease nor received any supplement or medication before the assessment.

Procedures

No dogs had exercised in the last three days prior to the start of the study. The same dogs performed activities in T1 and T2. The dogs were subjected to physical exercise for 60 min and evaluated seven times in two treatments. In the first treatment (T1), dogs received volume replacement just with mineral water, while in the second treatment (T2), they received mineral water supplemented with a commercial electrolytic and energetic supplement. Both forms of liquid replacement were offered 5 min after the exercise.

The evaluation times were divided considering exercise and recovery for sample collection (Figure 1). Body temperature (BT), body weight (BW), erythrogram (hematocrit (Ht), red blood cell count (He), hemoglobin (Hb), concentrations of glucose, lactate, total protein content (TP), electrolytes (K^+, Na^+, Cl^-, P^+, Ca^{2+}, Mg^{2+}) and hormones (cortisol, aldosterone, insulin) were determined in different times (Figure 1).

The ambient temperature and relative air humidity were monitored during each exercise in the woods. The dogs conducted the search and rescue exercise in a forest area of approximately 5 ha (20°13'23.0" S 40°15'29" W) in the State of Espírito Santo.

The exercise was similar in both treatments. An extra staff member ('victim') was hidden in the woods to simulate an actual disappearance, and the dogs searched with their handlers for 60 min. During exercise, there was no control regarding the variables of speed and gait of the animals

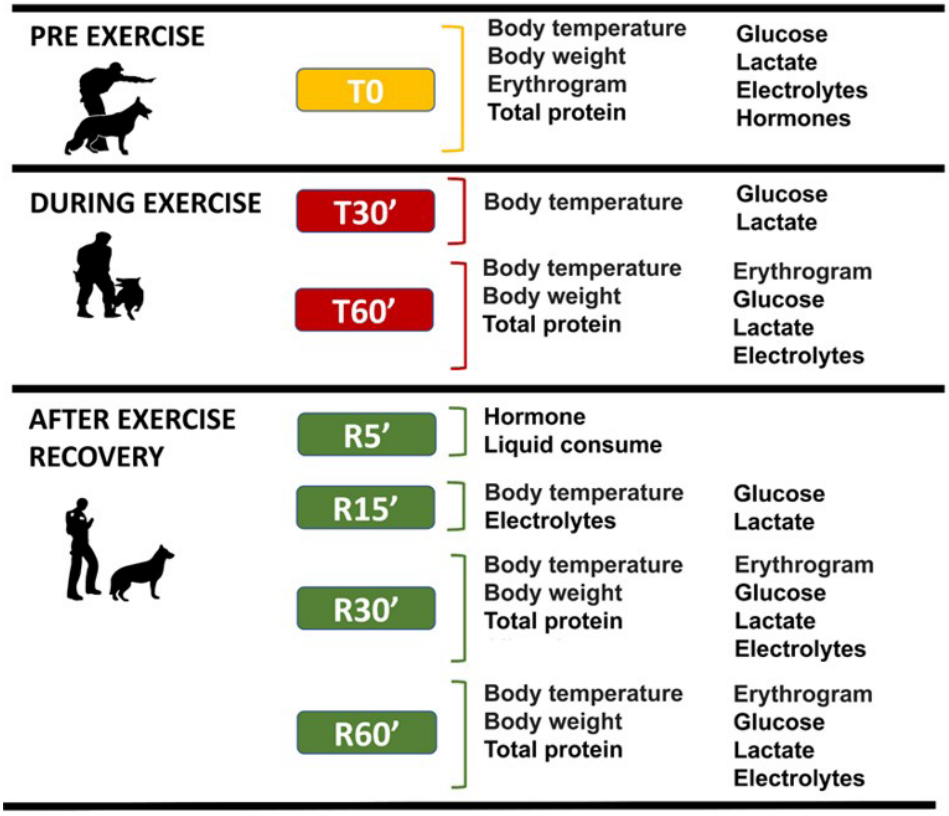

Figure 1 – Evaluation and collection times for search and rescue dogs. Legend: T0, pre-exercise with dogs at rest; T30', during exercise with 30 min of activity; T60', after 60 min of exercising; in the recovery period, evaluations were made 5 min after the end of the exercise (R5'); 15 min after (R15'); 30 min later (R30'); and 60 min later (R60').

since each one's rhythm is unique, alternating treatments of walking, trotting, running, and going through obstacles.

Volume replacement was carried out in T1 with mineral water only, containing bicarbonate (31.3 mg/L), nitrate (8.7 mg/L), Na^+ (7.8 mg/L), Ca^{2+} (6.5 mg/L), Cl^- (6.2 mg/L), Mg^{2+} (1.9 mg/L), K^+ (1.8 mg/L), sulfate (1.62 mg/L) and fluoride (0.03 mg/L). The water supply for each dog was, on average, 2.0 liters, and the intake at R5' was *ad libitum*. Liquid consumption was measured by weighing the volume that each animal ingested.

Hydroelectrolytic and energetic replacement was conducted at T2 using a commercial supplement (Eletrolítico Pet®, Vetnil, Brazil), diluting a sachet of 10 g for each 250 mL of mineral water, as recommended by the manufacturer. Each kilogram of supplement contains the following compounds: Ca^{2+} (max. 5,300 mg; min. 4,222 mg), Cl^- (min. 318 g), Mg^+ (min. 11 g), K^+ (min. 59.6 g), Na^+ (min. 171 g), maltodextrin (min. 150 g). After product reconstitution in mineral water, 2.0 liters, on average, were provided for each animal, and the intake at R5' was *ad libitum*. The amount ingested by each animal was also weighed.

For blood sample collection, the animals fasted for 3 h without food before the first sampling (T0). Water intake before exercise was *ad libitum*. The blood measurements were collected from the cephalic vein, with previous trichotomy and antisepsis. On average, volumes sampled at each treatment were 10 mL of whole blood, except T30', where the sample volume was 1.0 mL.

Part of the collected blood was added to tubes without anticoagulant, except for the sample taken at T30'. The blood was then centrifuged to obtain serum for the insulin, cortisol, aldosterone, TP, Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- dosages. Another part of the sample was placed in a tube containing ethylenediaminetetraacetic acid (EDTA) to conduct the erythrogram, in which Ht, He, and Hb indices were evaluated. The samples were cooled between 2 °C and 8 °C for transport to the laboratory. A portable Accu-Check® Performa glucometer and Accutrend Plus – Lactate 10® device were used for glucose and lactate determination, respectively.

The BT was verified rectally with a digital thermometer, respecting the minimum measurement time. The amount of liquid ingested by dogs during volume replacement and BW was obtained using an electronic scale.

Statistics

The Wilcoxon test was used to evaluate BW and liquid volume consumed by dogs in the treatments. The Friedman

test evaluated all other parameters between times (T0, T30', T60', R5', R15', R30', and R60'). The data were analyzed using BioEstat® 5.0 Software, assuming a significance level of 5 percent for all tests, and the graphs were generated using the GraphPad Prism 7® program.

Results

Body weight, mineral water, and hydroelectrolytic supplementation

Immediately after the exercise (T60'), the BW was significantly lower than the weight obtained at T0 in both treatments (T1: P= 0.0042; T2: P = 0.0274). After 30 and 60 min of recovery (at R30' and R60'), the BW did not differ significantly from the other times (P >0.05). When treated with mineral water, a mean loss of 0.97 Kg was observed in dogs from T0 to T60', equivalent to 3.13 percent of the initial BW. When treated with a commercial supplement, on the other hand, an average loss of 0.63 Kg was verified, equal to 2.06 percent of the initial BW.

The dogs that ingested only mineral water consumed significantly more liquid (1.0 ± 0.51 liters) than those who ingested electrolytic-energetic supplementation (0.55 ± 0.27 liters) (P =0.043).

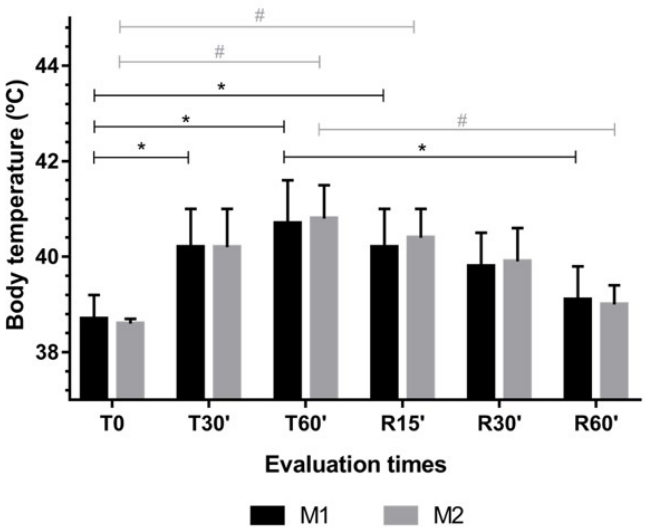

Figure 2 – Means and standard deviation of body temperature during and after the 60-min exercise in search and rescue dogs (n = 6), submitted to two different treatments: with water replacement (T1) and with hydroelectrolytic and energetic replacement (T2). Legend: M1 - water replacement; M2 - hydroelectrolytic and energetic replacement; *indicates a significant difference between the times for T1 (P < 0.05); #indicates a significant difference between the times for T2 (P < 0.05); T0, pre-exercise; T30', during the physical activity, after 30 min of exercise; T60', end of the 60-min exercise period; R15', 15 min of recovery; R30', 30 min of recovery, and R60', 60 min of recovery.

Body temperature

In T1, the BT increased gradually with exercise, and significant differences were observed between the values obtained at baseline (T0), T30', T60', and R15'. At R60', the BT was significantly lower than the value recorded immediately after exercise (T60'). In turn, the BT of the animals in T2 was significantly higher at T60' and R15' when compared to T0. At R60', the BT was also significantly lower than the values recorded immediately after exercise (T60') (Figure 2).

Energy indicators (glucose and lactate) and hormone levels

The mean values and standard deviation of the evaluated parameters are shown in Table 1. In the energy assessment, glucose did not present a significant difference between the evaluation times (T0 – R60') in both treatments. In T1, lactate levels increased significantly after 60 min of exercise (T60') and remained high until R15', differing from the baseline values (T0). In T2, lactate concentrations at R60' were significantly lower than those observed shortly after the end of the exercise period (T60'). The mean insulin concentrations 5 min after the end of the exercise period (R5') were significantly lower (10.1 ± 2.4 µUI/mL) than those observed during pre-exercise (T0) (23.3 ± 3.4 µUI/mL) in the treatment with mineral water and also in the treatment with hydroelectrolytic and energetic replacement (T0 = 11.8 ± 1.7 µUI/mL; R5´= 8.3 ± 2.1 µUI/mL).

Cortisol concentration did not differ statistically between pre-exercise time and 5 min of recovery when dogs received only mineral water (T0 = 1.8 ±1.0 µg/dL; R5´= 2.9 ± 2.0 µg/dL) or hydroelectrolytic and energetic replacement (T0 = 2.3 ± 0.9 µg/dL; R5´= 2.1 ± 1.2 µg/dL). Aldosterone concentration did not differ statistically between pre-exercise time and 5 min of recovery when dogs received only mineral water (T0 = 35.0 ± 6.6 pg/mL; R5´= 27.4 ± 10.1 pg/mL) or hydroelectrolytic and energetic replacement (T0 = 36.0 ± 7.0 pg/mL; R5´= 22.9 ± 5.5 pg/mL).

Discussion

The results obtained in the present study evidence that dogs exhibited dehydration, characterized by weight loss and an increment in erythrocyte parameters. The animals did not show indicative clinical signs of exercise intolerance or exhaustion.

The dogs' BW decreased immediately after the 60-min exercise period and returned to baseline values after

Table 1 – Means and standard deviation of the erythrogram, total protein content, and parameters electrolytes, glucose, and lactate levels in the SAR dogs (n = 6), evaluated in two different groups: with water replacement (M1) and with hydroelectrolytic and energetic replacement (M2); and at distinct evaluation times performed before, during, and after the 60-min search, rescue, and salvage training exercise

ANALYSIS (unit)	T	T0	T30′	T60′	R5′	R15′	R30′	R60′
Hematocrit (%)	1	$51.7^{ac} \pm 0.7$	---	$54.4^{ab} \pm 0.8$	---	---	$52.0^{ac} \pm 2.4$	$50.9^{ac} \pm 2.1$
	2	$53.5^{a} \pm 3.3$	---	$55.8^{ab} \pm 1.6$	---	---	$54.1^{a} \pm 2.9$	$50.8^{ab} \pm 3.9$
Red Blood Cell (x10⁶/µL)	1	$7.6^{ab} \pm 0.4$	---	$8.0^{a} \pm 0.3$	---	---	$7.7^{ab} \pm 0.5$	$7.5^{b} \pm 0.5$
	2	$8.4^{a} \pm 0.3$	---	$8.5^{ab} \pm 0.3$	---	---	$8.4^{ab} \pm 0.5$	$7.9^{abc} \pm 0.4$
Hemoglobin (g%)	1	$17.3^{ab} \pm 1.2$	---	$18.5^{a} \pm 0.8$	---	---	$17.6^{ab} \pm 1.1$	$17.1^{b} \pm 1.5$
	2	$18.4^{a} \pm 0.9$	---	$18.7^{a} \pm 0.6$	---	---	$17.8^{ab} \pm 1.7$	$17.0^{b} \pm 0.9$
Total protein (g/dl)	1	$7.4^{a} \pm 0.4$	---	$7.2^{a} \pm 0.4$	---	---	$6.6^{ab} \pm 0.4$	$6.1^{b} \pm 0.5$
	2	$7.9^{a} \pm 0.3$	---	$7.3^{a} \pm 0.2$	---	---	$7.2^{a} \pm 0.4$	$7.0^{b} \pm 0.5$
K^+ (mEq/L)	1	$4.5^{a} \pm 0.2$	---	$4.4^{a} \pm 0.2$	---	$4.4^{a} \pm 0.2$	$4.3^{a} \pm 0.2$	$4.3^{a} \pm 0.3$
	2	$5.0^{a} \pm 0.2$	---	$4.8^{a} \pm 0.4$	---	$4.6^{a} \pm 0.5$	$4.6^{a} \pm 0.4$	$4.8^{a} \pm 0.4$
Na^+ (mEq/L)	1	$137.5^{a} \pm 2.6$	---	$137.3^{a} \pm 3.0$	---	$137.0^{a} \pm 2.6$	$136.0^{a} \pm 2.3$	$136.0^{a} \pm 3.0$
	2	$141.8^{a} \pm 4.4$	---	$141.1^{a} \pm 2.1$	---	$139.5^{a} \pm 3.9$	$140.2^{a} \pm 2.8$	$143.7^{a} \pm 4.4$
Cl^- (mEq/L)	1	$100.2^{a} \pm 3.0$	---	$99.2^{a} \pm 1.3$	---	$104.0^{ab} \pm 2.4$	$96.6^{ac} \pm 3.9$	$98.2^{ac} \pm 1.7$
	2	$102.5^{ab} \pm 3.9$	---	$104.0^{ab} \pm 2.6$	---	$105.3^{ab} \pm 3.9$	$107.0^{ab} \pm 3.6$	$110.6^{b} \pm 4.7$
P^+ (mg/dL)	1	$5.9^{a} \pm 0.8$	---	$4.0^{b} \pm 0.8$	---	$4.4^{ab} \pm 1.1$	$3.7^{ab} \pm 1.0$	$4.2^{ab} \pm 1.0$
	2	$5.8^{a} \pm 1.2$	---	$3.7^{b} \pm 1.3$	---	$3.9^{b} \pm 1.2$	$3.9^{b} \pm 1.0$	$5.0^{ab} \pm 1.7$
Ca^{2+} (mg/dL)	1	$8.4^{a} \pm 0.9$	---	$7.9^{a} \pm 1.0$	---	$8.0^{a} \pm 1.0$	$7.5^{a} \pm 0.6$	$8.7^{a} \pm 2.1$
	2	$8.1^{a} \pm 1.0$	---	$7.4^{a} \pm 0.8$	---	$7.3^{a} \pm 0.8$	$7.8^{a} \pm 0.9$	$7.9^{a} \pm 0.6$
Mg^{2+} (mg/dL)	1	$2.1^{a} \pm 0.1$	---	$1.8^{a} \pm 0.1$	---	$1.8^{a} \pm 0.2$	$1.7^{ab} \pm 0.2$	$1.6^{ab} \pm 0.1$
	2	$2.2^{a} \pm 0.9$	---	$1.9^{a} \pm 1.4$	---	$1.4^{a} \pm 0.2$	$2.2^{a} \pm 1.2$	$2.3^{a} \pm 1.4$
Glucose (mg/dL)	1	$78.8^{a} \pm 5.0$	$75.2^{a} \pm 2.0$	$80.2^{a} \pm 2.6$	---	$74.3^{a} \pm 3.4$	$77.3^{a} \pm 2.9$	$77.0^{a} \pm 3.8$
	2	$78.7^{a} \pm 3.4$	$79.7^{a} \pm 3.6$	$76.8^{a} \pm 1.2$	---	$84.5^{a} \pm 2.7$	$79.7^{a} \pm 5.9$	$79.2^{a} \pm 5.3$
Lactate (mmol/L)	1	$1.5^{a} \pm 0.9$	$3.2^{a} \pm 1.1$	$3.6^{ab} \pm 1.5$	---	$3.3^{ab} \pm 1.0$	$3.0^{a} \pm 1.0$	$2.5^{a} \pm 0.9$
	2	$3.1^{a} \pm 0.6$	$3.6^{a} \pm 0.7$	$3.7^{a} \pm 1.3$	---	$3.4^{a} \pm 1.6$	$2.5^{a} \pm 0.9$	$1.8^{ab} \pm 0.3$
Cortisol (µg/dL)	1	$1.8^{a} \pm 1.0$	---	---	---	---	---	---
	2	$2.3^{a} \pm 0.9$	---	---	---	---	---	---
Aldosterone (pg/mL)	1	$35.0^{a} \pm 6.6$	---	---	---	---	---	---
	2	$36.0^{a} \pm 7.0$	---	---	---	---	---	---
Insulin (µUI/mL)	1	$23.3^{a} \pm 3.4$	---	---	---	---	---	---
	2	$11.8^{a} \pm 1.7$	---	---	---	---	---	---

Legend: Means followed by the same lowercase letter in a row do not differ statistically. M refers to the volume replacement group: T0, pre-training; T60', end of the 60-min training period; R30', 30 min of recovery; and R60', 60 min.

30 min of recovery. Such weight loss was associated with fluid loss during exercise, which occurs due to an elevation in the respiratory rate and evaporation of water through exhaled air (McNicholl et al., 2016) and increased salivation (Johnson et al., 2006). Both processes dissipate heat produced during exercise and reduce BT (McNicholl et al., 2016; Rizzo et al., 2017).

The animals in T1 and T2 lost an average of 30 mL/kg/hour and 20 mL/kg/hour of water, respectively. However, another study reported a much lower loss (7 mL/kg/hour) in dogs that participated in recreational activities (O'Connor & Potts, 1969). Such discrepancy between studies may be due to the type and intensity of physical activity performed.

The dogs' weight loss was equivalent to 2.06 percent of their body weight in T2 and 3.13 percent in T1, characterizing subclinical dehydration, with losses inferior to 5 percent BW. In a study on dogs physically conditioned for exercise, BW was used to assess water loss and estimate dehydration after 30 min of exercise on three distinct days. It is well known that weight losses of up to 2 percent in physically active people can compromise physical performance, causing fatigue and reduced alertness (Benton & Young, 2015). If the exercise period had extended beyond 60 min in the present study, the loss of bodily fluid could have influenced the dogs' performance. Therefore, replacing bodily fluid as soon as the loss occurs is essential.

Dogs in T2 ingested significantly less liquid volume than those in T1 (P=0.043). This may be due to the lower percentage of observed weight loss, thus reducing water replacement requirements. Moreover, the electrolytic and energetic supplement in the water may have influenced water intake due to the altered taste. The ambient temperature and relative air humidity were similar in both treatments and did not affect the amount of water ingested.

According to Figure 2, BT increased gradually after the beginning of the activity in both treatments, reaching values above average for the species (Rizzo et al., 2017) after 30 min of exercise, returning to baseline values after 60 min of recovery. The increase in BT can be explained by increased metabolic activity during muscular effort (López et al., 2006). Recovery periods are essential to dissipate heat and reduce BT and may influence the performance of these animals (Rizzo et al., 2017; Zanghi et al., 2018). In the case of search and rescue dogs, which perform prolonged physical activities during actual search operations, it is crucial to monitor BT during exercise. When subjected to extremely high temperatures, a 30-min BT-recovery period is recommended, as is rehydration, since, with dehydration, the supply of water for cooling (salivation, circulation, and evaporation) is reduced (Otto et al., 2017).

The increment in erythrocyte parameters (Ht, He, and Hb), observed immediately after the end of the exercise period (T60') in both treatments (Table 1), may have been due to an increase in circulating catecholamines and activation of the sympathetic nervous system (SNS), which take place with exercise. As a result, splenic contractions occur, with the consequent increase in erythrocyte release into the circulation to assist in the transport of O_2 (Richardson et al., 2020).

Additionally, the reduction in plasma volume that occurred in the dehydration of the dogs can also cause hemoconcentration, leading to increased erythrocyte parameters (Rovira et al., 2007a), as observed in agility dogs (López et al., 2006; Rovira et al., 2007b). In contrast, no difference was found in the Ht in the search and rescue dogs before or after the exercise period, and the non-observation of hemoconcentration may have been due to the provision of rest periods with water supply between the exercise simulations (Spoo et al., 2015). The comparison between these studies also corroborates that water supplementation during search and rescue exercises can reduce dehydration.

Although increased TP is also found in cases of hemoconcentration, the dogs exhibited a significant reduction in TP values immediately after the end of the exercise (T60') (Table 1). Protein reduction after physical activity may be due to the displacement of fluids and substances from the vascular compartment to the interstitial. During such activity, increases in osmolarity due to lactate accumulation cause movement between compartments (Angle et al., 2009; McKenzie et al., 2007). Changes in serum TP concentration with exercise may indicate extracellular fluid volume expansion.

The energetic utilization of glucose may decrease during physical activity in conditioned dogs (*e.g.*, sled dogs), which use other energy substrates, including proteins and free fatty acids. It is likely that endogenous protein sources, such as plasma proteins, are used as a substrate. Short, maximal, and submaximal exercise can cause significant increases in TP by changing compartment fluids (Rovira et al., 2007b). On the other hand, the reduction of TP may occur during prolonged exercises, as observed in sled dogs, due to the catabolism of plasma proteins for energy production (McKenzie et al., 2007).

The concentrations of Na^+ did not change after the exercise (Table 1), as observed in agility dogs (Spoo et al., 2015; Zanghi et al., 2018). However, other studies verified reduced sodium levels post-exercise due to greater water intake than required (Spoo et al., 2015). Even after the ingestion of liquid in both treatments, the concentrations of Na^+ did not decrease, indicating that the amount of Na^+ present in both mineral water and commercial supplements was sufficient to maintain its concentration. After physical activity, the replacement of Na^+ in people is essential to avoid plasma reduction and blood osmolarity, which, thus, prevents diuresis by maintaining plasma renin activity and aldosterone levels, as well as the thirst mechanism and voluntary intake of water (Meyer & Perrone, 2009).

The increase in Cl^- levels during recovery in T1 (Table 1) may be due to respiratory alkalosis caused by the tachypnea presented by the dogs (Angle et al., 2009; Rovira et al., 2007b; Zanghi et al., 2018). When the partial pressure of carbon dioxide (PCO_2) is reduced, CO_2 leaves the cells to reach a new equilibrium point. Cl^- exits the red blood cells in exchange for bicarbonate, causing a reduction in plasma bicarbonate concentration and an increase in the levels of Cl^- (Guillaumin & Dibartola, 2017).

The mean concentrations of K^+ did not change between the evaluated times (Table 1). However, an increase in K^+ levels may occur due to the release of this ion from muscle cells during intense exercises, such as in agility dogs (López et al., 2006), or in cases of rhabdomyolysis resulting from intense physical activity (Hinchcliff et al., 2004). The absence of alterations in K^+ concentration in the dogs of the present study may be due to their adaptation to activity, constant exercise frequency, and lower exercise intensity compared to agility dogs (López et al., 2006). Nonetheless, this finding does not guarantee that intense days or hours in actual search operations will not cause changes in this ion since the intensity, duration, and time of such occurrences are pretty variable.

In T1, the mean concentrations of Mg^{2+} and P^+ decreased after exercise (Table 1). During exercise, there is a greater demand for Mg2+ and P+ as cofactors in skeletal muscle

metabolic processes (Davenport et al., 2001). In T2, the concentrations of Mg2+ did not decrease after exercise, possibly due to the supplementation performed.

The dogs in the present study did not exhibit alterations regarding the concentrations of Ca^{2+} (Table 1), which may indicate that the conducted activity did not require using this mineral's reserves. A reduction in concentrations of this ion could be found due to increased muscle contractions during exercise, which enhances the use of this mineral's reserves and leads to the subsequent drop in serum levels (Rosenstein et al., 2018).

When analyzing the electrolytic alterations exhibited by the dogs in this study, isotonic-type dehydration was verified, given that liquid was lost without changes in Na+ and Cl—concentration. Due to the type of dehydration presented by the animals herein, it is recommended that volume replacement be carried out with hypertonic solutions, such as the supplementation provided in T2.

Lactate levels increased during the exercise and declined after recovery (Table 1). The 60-min exercise-induced hyperlactatemia and maintained the glycemic threshold, consuming lactate as a way to obtain energy. The lactate released by muscle cells in metabolic exercise situations is used as an energy source via gluconeogenesis (Rovira et al., 2007b). The observed results suggest that search and rescue dogs are trained animals that demonstrate an adequate physical aptitude index.

Due to increased oxygen demand, exercise-induced hyperlactatemia is highly variable (Rosenstein et al., 2018). The metabolic or anaerobic lactate threshold is 4.0 mmol/L, which indicates the transition between aerobic exercise and anaerobic metabolism (Rovira et al., 2007b). The evaluated dogs herein did not present mean values equal to or greater than 4.0 mmol/L, inferring that during the period of activity and recovery, the aerobic metabolism was efficient in maintaining the physiological demand of the exercise. This study's observed increment in erythrocyte indices may have increased oxygen transport capacity to the active musculature, providing higher aerobic capability without exceeding the anaerobic threshold (Rovira et al., 2008).

Although dogs in T2 consumed commercial supplements that contained maltodextrin in their composition and were used as an energy source, the glycemia of the animals remained constant throughout the evaluation period in both treatments (Table 1). Some studies in dogs reported no alteration in the glucose values (Haverbeke et al., 2008; Rovira et al., 2008), and others revealed hyperglycemia after exercise (Angle et al., 2009; Hinchcliff et al., 2004). The discrepancy between the values found in these studies and the present assessment may be due to the duration of each type of activity, breed peculiarities, or differences in the resting and pre-exercise periods.

The hormones cortisol and insulin are glycoregulators, and their concentrations may change according to the type of exercise to control the input of muscle energy (Pösö & Hyyppä, 1999). The cortisol levels of the dogs in the present study did not show alterations after the exercise (Table 1), which could be indicative of good physical conditioning since cortisol can also indicate changes in the physiological state, such as reduction of well-being and stress during physical activity (Angle et al., 2009).

The environmental stimuli the animals were exposed to during the simulation were probably insufficient to trigger increased cortisol concentrations. The continuous exercise performed with these working dogs assists in habituating the most diverse environments and social interactions. One study substantiated that after submitting military dogs to a second round of stimuli (social, visual, and auditory), the cortisol levels of the animals did not increase when compared to the first contact with such challenges, evidencing environmental adaptation (Haverbeke et al., 2008).

After the exercise, the levels of insulin decreased in both treatments. During physical activity, the pancreas reduces the secretion of insulin. It increases the secretion of counterregulatory hormones (glucagon, epinephrine, and noradrenaline), aiding in the synthesis of glucose, with more significant mobilization of glycogen in the musculature and triglycerides in adipose tissue (Marliss et al., 2000).

The concentrations of aldosterone did not change with exercise (Table 1), nor did the levels of some aldosterone-related electrolytes. Since the levels of Na^+, K^+, and Ca^{2+} in the dogs of the present study did not change with exercise, there was probably no stimulus for aldosterone release, unlike findings reported in another study, where sled dogs presented increased plasma concentrations of aldosterone and elevated levels of Na^+ (McKenzie et al., 2007).

Another study evaluated three volume replacement strategies in military working dogs: one with free access to water, the second supplemented with an oral electrolyte solution, and the third with subcutaneous administration of fluids. No significant differences were detected in electrolyte concentrations, although the animals supplied with the oral electrolyte solution ingested larger volumes than those receiving only water, possibly due to palatability (meat flavor), avoiding the effects of dehydration (Otto et al., 2017). In our study, ingesting water and supplementation corrected dehydration; the *ad libitum* supply probably contributed to sufficient intake. The supplement given to

the animals was not palatable, and the ingested volume was smaller, a factor that could explain the difference in consumption compared to other studies.

Conclusion

It can be concluded that both the water replacement or the hydroelectrolytic and energetic supplementation provided orally during the immediate recovery phase of the exercise can correct the isotonic dehydration presented by the animals after 60 min of exercise. Thus, it is recommended that dogs be allowed 30-min rest intervals for every 60 min of physical activity in actual search operations. During these breaks, water and supplements should be provided *ad libitum* to correct the losses due to dehydration, enabling the physical recovery and continuance of the animals for more extended periods at work with lower risks of activity-related diseases.

As a limitation of the study, we point out the need for a larger number of animals to increase the significance of the statistical methods. Standardizing the intensity of physical effort in the search activities could bring more homogeneous results. However, we considered standardizing the search type, activity time, place, and environmental conditions.

Conflict of interest

None.

Ethics Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The experimental procedures were reviewed and approved by the Ethics Committee in the Use of Animals (CEUA) of the Federal University of Espírito Santo in accordance with the Protocol 056/2017. Those responsible for the animals signed a Term of Free and Informed Consent, authorizing their participation in the study.

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