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Blood and urinary variables in horses supplemented with electrolytes

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RESEARCH ARTICLE

Abstract

This study was designed to evaluate changes on variables in blood, urine and water balance in horses in response to a single dose of electrolyte supplementation. The essay was conducted on a randomised 3×3 Latin Square design repeated over time, with three animals and three treatments: Treatment 1: control group (without supplementation); Treatment 2: supplementation with a medium dose of electrolytes composed of: 0.25 g of NaCl + 0.125 g of KCl + 0.05 g of CaCl + 0.025 g of MgCl per kg of BW; Treatment 3: supplementation with a high dose of electrolytes composed of: 0.625 g of NaCl + 0.3125 g of KCl + 0.125 g of CaCl + 0.0625 g of MgCl per kg of BW, equivalent to 2.5 times the medium dose of supplementation. The electrolytes were supplied through a nasogastric tube 4 h after the morning meal. The diet provided had a forage:concentrate ratio of 70:30, composed of coastcross hay and commercial concentrate, with an estimated consumption of 2% of body weigth (BW). Horses received 116 mg/kg of BW of commercial mineral salt mixed in the concentrate. Samples of blood, urine and digesta were collected over a 12 h period after supplementation for analysis of sodium, potassium, chloride, calcium and magnesium concentration. Water intake and urine output were also measured. Electrolytic supplementation enhanced (P<0.05) the water intake, water retention and urine output. Blood variables were not altered by electrolyte supplementation (P>0.05). The supplementation also influenced the sodium and chloride excretion in urine (P<0.05). Urine physicochemical characteristics and the concentration of electrolytes excreted with time were significantly altered as a function of the electrolytes supplementation.

Keywords: blood, diets, electrolytes, minerals, supplementation, urine

1. Introduction

The electrolytic supplementation in horse was used to attenuate dehydration and electrolyte losses due to sweating during exercises, as well to help rehydration of dehydrated animals after long transportation (Sampieri *et al.*, 2006). Electrolytes stimulate strategic rehydration which in most cases is more efficient than just providing water (Nyman *et al.*, 1996). Water loss infers electrolyte losses, as sweat in horses, when compared to plasma, is hypertonic for potassium and chloride, and isotonic for sodium. Also, there is excretion of calcium and magnesium in lower concentrations (Bayly *et al.*, 2006; McCutcheon and Geor, 1996). The variation of these electrolytes interferes with several physiological mechanisms and consequently requires close management (Trigo *et al.*, 2010).

Supplementing horses with electrolytes when undergoing situations that could lead to dehydration and electrolyte losses may contribute to a better replenishment of water and ions, and mitigate undesirable changes in their internal homeostasis (Muñoz *et al.* 2010; Van den Berg *et al.*, 1998). Most studies on electrolyte supplementation are performed on horses undergoing water and electrolytes losses, but studying physiological mechanisms of regulation, as well as electrolytic interaction, in horses during rest can contribute to a better understanding of its effects on water intake, urinary excretion and haematological and biochemical variables. This study was carried out to evaluate a single dose of electrolyte supplementation on blood and urine, as well as the water balance in horses.

2. Material and methods

This study was carried out at the Equine Health Laboratory at Universidade Federal Rural do Rio de Janeiro, Brazil. Experimental procedures were approved by the Ethics and Research Committee of UFRRJ, n.138/2011. A completely randomised 3×3 Latin square design repeated over time was used with three animals and three treatments of electrolytic supplementation. Two females and one male healthy adult crossbred horses with a mean age of 16 years and a body weight (BW) of 330±38 kg were used. The horses were cannulated at the right dorsal colon. Each experimental period comprised of three days, totalling nine consecutive days. Electrolytes were supplied at day 1, day 4 and day 7, with a two days wash-out period. The treatments were: Treatment 1: control without supplementation; Treatment 2: supplementation with a medium dose of electrolytes composed of 0.25 g NaCl + 0.125 g KCl + 0.05 g CaCl + 0.025 g MgCl per kg BW; Treatment 3: supplementation with a high dose of electrolytes composed by 0.625 g of NaCl + 0.3125 g of KCl + 0.125 g of CaCl + 0.0625 g of MgCl per kg BW (equivalent to 2.5 times the medium dose).

The diet provided had a forage:concentrate ratio of 70:30 composed of coastcross hay and concentrate. The estimated intake was 2% BW, according to the NRC (2007) recommendations for horses at maintenance (Table 1). All the animals received 116 mg/kg BW of mineral salt mixed in the concentrate.

The medium dose of electrolytes corresponding to electrolytic losses estimated for sweat production in one hour of intense exercise (Sampieri *et al.*, 2006). To formulate the electrolyte supplementation pure salts NaCl, KCl, MgCl and CaCl were used. Salts were weighed to adjust the dose to the animals' BW, diluted in two litres of deionised water, and delivered through a nasogastric tube. One extra litre of deionised water was also delivered by the nasogastric tube with the purpose of rinsing the container used for preparation and the tube itself, totalling three litres. Animals in the control group received three litres of deionised water alone, delivered by nasogastric tube. On the day of the electrolytic supplementation horses were kept in their stalls and the diet was supplied in two equal meals: the first half at 4:00 am, 4 h before the supplementation, and the other half at 22:00 pm, after the last sample collection. Blood and urine samples were collected at the initial time of supplementation (zero) and at 2, 4, 6, 9 and 12 h after that.

Table 1. Diet, feed and water analysis.

Variables	Commercial concentrate[1] (g/kg DM)	Coastcross hay (g/kg DM)	Commercial mineral salt[2] (g/kg DM)	Water (mg/l)	Diet 70:30 [3] (forage:concentrate) (g/kg DM)
Dry matter	926.8	912.7	958.5	7.24	922.6
Ash	183.8	60.8	868.5	–	146.9
Crude protein	130.0	128.7	14.4	–	129.6
Ether extract	35.1	51.9	–	–	40.1
Neutral detergent insoluble fibre (cp)[4]	327.3	593.1	–	–	407.0
Acid detergent insoluble fibre	172.9	344.8	–	–	224.5
Hemicellulose	154.4	248.3	–	–	182.6
Cellulose	121.4	317.9	–	–	180.4
Lignin	51.5	26.9	–	–	44.1
Sodium	0.43	0.10	151.25	0.40	6.08
Potassium	8.36	13.96	5.21	6.00	10.24
Chloride	7.45	9.17	162.93	3.62	14.16
Magnesium	2.72	2.17	14.03	0.55	3.09
Calcium	10.02	2.09	99.67	1.08	11.43

[1] DM = dry matter.

[2] Composition of the commercial mineral salt used during the experimental period (security levels per kg of mineral salt informed by trademark): calcium (max) 150 g; phosphorus (min) 70 g; sulphur 10 g; magnesium 10 g; sodium 150 g; iron 2,500 mg; copper 820 mg; zinc 2,620 mg; manganese 2,124 mg; lysine 10 mg; iodine 20 mg; selenium 12.50 mg; cobalt 20 mg; beta glucans 3,300 mg; chrome 6 mg; vitamin A 60,000 UI/kg; vitamin D3 12,000 UI/kg; vitamin E 450 UI/kg; MOS 2.100 mg; thiamine-vitamin B 150 mg; riboflavin-vitamin B 280 mg; niacin-vitamin B 3,240 mg; pantothenic acid-vitamin B 5,100 mg; pyridoxine-vitamin B6 HCL 20 mg; vitamin B 925.30 mg; vitamin B 12 240 mg; vitamin H-biotin 14 mg; fluorine (max) 700 mg.

[3] Dietetic cation-anion difference (DCAD = [Na] + [K] + [Mg] + [Ca] – [Cl]): 952 mEq/kg of DM.

[4] cp = corrected for ashes and protein

Blood samples were collected in one heparinised EDTA (7.2 mg of EDTA K_2) and in two anticoagulant free tube vacutainer tubes. Afterwards, plasma was separated by centrifugation for ten min at 3,000 rpm, and stored at -18 °C for analysis of sodium, potassium, calcium and magnesium.

Urine samples were collected through a urethral catheter and urine collector on the male and by Foley catheter on the females. Catheters and collectors were prepared and placed 3 h before the supplementation to empty the horses' bladders. Total urine volume was measured with a beaker. Urine samples were stored in plastic containers and frozen for analysis of sodium, potassium, chloride, calcium and magnesium. There was no urine loss around the catheters during the experiment. Digesta samples of approximately 1.2 kg were collected from for chemical analysis and fermentation studies. The samples were weighted and dried to estimate de water balance (unpublished results). The amount of digesta removed had no impact on the results of this study.

Feed analysis was performed on previously dried and ground samples in a Willey mill (Thomas Scientific, Swedesboro, NJ, USA) with an 1 mm screen. Sodium and potassium analysis were performed on a flame photometer (Benfer BFC-300; Benfer, São Paulo, Brazil). Calcium and magnesium analysis were performed in an atomic absorption photometer (Varian Spectra 55B; Agilent Technologies, Santa Clara, CA, USA). Chloride analysis was performed in a spectrophotometer (BTS 310; Applied Biosystems, Foster City, CA, USA) with commercial kits. Water was analysed for sodium, potassium, chloride, calcium and magnesium, performed directly and without dilution. Data was analysed by ANOVA as a split-plot analysis and the mean values were compared by Tukey test at 5% probability using the software Statistics and Genetics Analysis System – SAEG (Universidade Federal de Viçosa, Viçosa, MG, Brazil).

3. Results

Water intake and water balance

Water intake was influenced by the electrolyte supplementation, in the periods from zero to 12 h and from zero to 24 h (Table 2). However, the same effect was not observed ($P>0.05$) for water intake from 12 to 24 h after the supplementation. There were no differences in the intake of water in the 12 to 24 h period.

Note that the influence of the electrolytic supplementation on water intake occurred early in the first 4 h after supplementation (Table 3). Water intake increased by 3.7±4.3, 25.3±9 0 and 24.4±10.2 ml/kg BW in the control, medium and high dose of electrolytes, respectively.

In the 4 to 9 h period after supplementation, the water intake of the horses that had received the medium dose of electrolytes returned to intake of 5.8±8.0 ml/kg BW, close to the control animals (5.2±7.3 ml/kg BW). However, for the horses that had received high dosages the water intake still remained high (28.6±22.8 ml/kg BW). In the following period, 9 to 12 h, the average water intake decreased for all treatments, although the high dose supplementation remained with a higher water intake (14.2±12.6 ml/kg BW) than the other two treatments. In the final interval of 12 to 24 h, there was no difference among electrolytes supplementation.

The water balance for the period up to 12 h after supplementation was significantly influenced by electrolytes supplementation (Table 4). The total volume of water excreted was 19.9±5.0, 30.7±4.4 and 42.4±10.5 ml per kg BW for the control, medium dose and high dose of electrolytes, respectively. Values correspond to the sum of water excreted in urine, faeces and in the digesta samples. Treatment with a high dose of electrolytes showed water retention within 12 h after supplementation of 24.8±10.2 ml/kg BW, which corresponds to approximately 10 l of

Table 2. Water intake (ml/kg of body weight ± standard deviation) on time intervals after electrolytic supplementation.

Time interval (h)	Electrolytic supplementation[1]			VC (%)
	Control	Medium	High	
0 to 12	10.6±7.9^{C}	32.5±6.5^{B}	67.21±14.7^{A}	26.8
12 to 24	28.2±6.0	24.4±8.4	25.8±4.4	22.4
0 to 24	38.7±10.5^{C}	56.9±5.3^{B}	92.0±14.5^{A}	17.7

[1] Mean values in line followed by different uppercase letters differ by Tukey test ($P<0.05$).

Table 3. Water intake (ml/kg of body weight ± standard deviation) on time intervals after electrolytic supplementation.

Time interval (h)	Electrolytic supplementation[1]		
	Control	Medium	High
0 to 4	3.7±4.3^{bB}	25.3±9.0^{aA}	24.4±10.2^{aA}
4 to 9	5.2±7.3^{bB}	5.8±8.0^{bB}	28.6±22.8^{aA}
9 to 12	1.6±1.9^{bA}	1.4±1.2^{bA}	14.2±12.6^{aA}

[1] Coefficient of variation = 37.2%. Mean values in line followed by different uppercase letters differ by Tukey test ($P<0.05$). Mean values in columns followed by different lower case letters differ by Tukey test ($P<0.05$).

Table 4. Water balance (ml/kg of body weight ± standard deviation) in the time interval from zero to 12 hours after electrolytic supplementation.

Water	Electrolytic supplementation[1]			VC (%)
	Control	Medium	High	
Intake	10.6±7.9^{A}	32.5±6.5^{B}	67.2±14.7^{C}	26.8
Excreted in urine	6.3±3.1^{B}	12.4±4.9^{B}	25.1±11.5^{A}	45.2
Lost in digesta	5.5	6.0	5.8	24.7
Excreted in faeces	8.0	12.3	11.5	31.2
Total excreted	19.9±5.0^{C}	30.7±4.4^{B}	42.4±10.5^{A}	20.8
Retained	-9.3±9.8^{B}	1.8±3.6^{B}	24.8±10.2^{A}	34.7

[1] Mean values in line followed by different letters differ by Tukey test (P<0.05).

water for a horse of 400 kg. The medium supplementation, however, did not differ from the control.

Blood variables

The results of the haematocrit and plasma protein measures showed no differences (P>0.05) due to electrolytes supplementation or time after supplementation. The mean haematocrit and plasma protein values were 30% and 6.77 g/dl, with little variation in the results shown by the coefficient of variation of 9.1% and 6.7%, respectively. The mean plasma concentrations of sodium, potassium, magnesium and calcium were not affected by supplementation of electrolytes and time after supplementation (P>0.05).

Urinary variables

The urine output from zero to 12 h after the electrolytes supplementation showed a significant increase in the horses the received the high dose of electrolytes compared to the horses that received a medium dose of electrolytes or only water (control). The urine output in horses that received the medium dose did not differ (P>0.05) from the control animals (Table 5).

Urine output remained stable during the time intervals (Table 6) in horses from the control and the medium electrolyte supplementation, and there was no significant difference between them. However there were differences of urine output in horses at high dose electrolyte supplementation (P>0.05). There was a significant increase in urine output from the 2 to 6 h period after supplementation to the 6 to 9 h period, which was the period with the largest urine production, of 8.9±5.2 ml/kg BW. However, there was a tendency towards a reduction

Table 5. Urine output (ml/kg of body weight ± standard deviation) in the time interval from zero to 12 hours after electrolytic supplementation.

Time interval (h)	Electrolytic supplementation[1]		
	Control	Medium	High
0 to 12	7±3.2^{B}	13±5.1^{B}	27±12.4^{A}

[1] Coefficient of variation = 45.8%. Mean values followed by different letters differ by Tukey test (P<0.05).

Table 6. Mean urine output (ml/kg of body weight ± standard deviation) for the time intervals after electrolytic supplementation.

Time interval (h)	Electrolytic supplementation[1]		
	Control	Medium	High
0 to 2	0.9±0.5^{aA}	1.4±0.5^{aA}	1.4±1.2^{cA}
2 to 4	1.1±0.7^{aA}	2.1±1.4^{aA}	3.5±2.2^{bcA}
4 to 6	1.0±0.5^{aB}	2.7±1.6^{aAB}	5.4±5.1^{bA}
6 to 9	1.0±0.5^{aB}	3.4±1.7^{aB}	8.9±5.2^{aA}
9 to 12	1.3±0.7^{aB}	1.7±0.9^{aB}	5.9±3.0^{abA}

[1] Coefficient of variation = 24.7%. Mean values in line followed by different upper case letters differ by Tukey test (P<0.05). Mean values in columns followed by different lower case letters differ by Tukey test (P<0.05).

in urine production for the following 9 to 12 h period with values of 5.9±3.0 ml/kg BW.

There was a difference of sodium concentration in the urine, which increased from 4 h after the electrolytes supplementation. The highest sodium excretion was observed at 12 h after supplementation. The excretion of potassium was not affected by the electrolyte supplementation (P>0.05), but showed differences after supplementation with a high and low K excretion at 2 h and 9 h, respectively (P<0.05) (Table 7). The urinary excretion of Ca was not affected (P>0.05) by the electrolytes supplementation, however, there were significant differences over time (P<0.05). The highest and the lowest concentrations in urinary calcium were observed at 2 h and 12 h after supplementation, respectively. However, urine output was larger in horses supplied with a high dose of electrolytes than in the control horses, but the calcium concentration was similar. The urinary excretion of chloride was significantly higher in horses that received the medium and high doses of electrolytes than in the control horses: 206.2±25.9, 182.8±35.1 and 144.7±41.4 mmol/l, respectively. The average magnesium excretion

Table 7. Concentrations of sodium, potassium, chloride, magnesium and calcium (mmol/l ± standard deviation) in urine after electrolytic supplementation.

Time (h)	Electrolytic supplementation[1]			VC (%)
	Control	Medium	High	
Sodium				
0	11.31	15.55	13.55	28.3
2	9.00	21.01	13.01	
4	14.50	37.76	42.95	
6	27.91	29.59	47.14	
9	26.91	28.20	43.44	
12	38.62	26.14	67.08	
Mean	21.38±7.63^{B}	26.38±24.16^{AB}	37.86±14.05^{A}	
Potassium				
0	175.93	127.50	136.15	46.2
2	171.06	175.51	152.11	
4	94.80	143.36	88.15	
6	98.08	115.59	39.73	
9	81.94	121.73	44.11	
12	131.21	110.89	51.22	
Chloride				
0	121.20	172.87	167.03	30.6
2	199.00	192.77	192.43	
4	165.53	224.43	207.07	
6	161.30	198.07	201.37	
9	122.52	228.70	181.97	
12	98.36	220.43	146.73	
Mean	144.65±41.38^{B}	206.21±25.88^{A}	182.77±35.08^{A}	
Magnesium				
0	24.34±13.99^{Ba}	39.07±8.90^{Aba}	38.98±10.74^{Aa}	52.5
2	45.02 ±19.56^{Aabc}	29.95±14.70^{Aba}	21.18±6.97^{Bb}	
4	17.47±9.26^{Abc}	16.17±5.25^{ABb}	9.30±7.78^{Bbc}	
6	11.42±8.15^{Abc}	12.33±8.70^{ABb}	7.26±4.03^{Bc}	
9	8.69±9.32^{Abc}	13.89±6.21^{Ab}	6.85±1.88^{Ac}	
12	4.85±9.70^{Ac}	14.42±7.07^{Ab}	5.74±4.07^{Ac}	
Calcium				
0	23.26	57.86	49.59	24.0
2	72.18	55.68	43.47	
4	32.32	31.16	20.90	
6	20.95	29.45	16.18	
9	16.76	44.96	13.20	
12	7.98	38.88	10.77	

[1] Mean values in line followed by different uppercase letters differ by Tukey test (P<0.05). Mean values in columns followed by different lowercase letters differ by Tukey test (P<0.05).

in the urine was not affected (P>0.05) by the electrolyte supplementation, however, there was an effect of time after supplementation of the electrolytes (Table 7). The influence of the electrolytes supplementation was observed in the results, mainly due to increase the urine output, resulting in the dilution of electrolytes in the urine. This effect was more pronounced in the animals that received a high dose of electrolytes.

4. Discussion

Water intake and water balance

The water intake from zero to 12 h after medium and high electrolyte supplementation increased to maintain the hydro-electrolytic balance of the animals. Nyman *et al.* (1996) observed that the endurance horses at exercise that only received water for voluntary rehydration consumed 9.1 ml/kg BW, while the horses that received a saline solution consumed 46 ml/kg BW of water in the 3 h period right after the race. However in this study the horses were kept in stalls with free access to water throughout the study period. This fact should be considered when comparing the results of water intake between this and other studies.

In another study Nyman *et al.* (2005) observed in horses at exercise tests the mean intake of water was 45ml/kg BW in a 24 h period, which is very close to the animals in the control group in this study. Van den Berg *et al.* (1998) evaluated the water intake and electrolytes by horses after a transport of 600 km, with the horses maintained in pens during 12 h and observed values of water intake of 52 ml/kg BW. Muhonen *et al.* (2009) evaluated the water intake of horses after a sudden change in diet, from silage to hay, and observed a value of 23.5 ml/kg BW in a 24 h period. However, this work was carried out in Sweden between January and February, which are the winter months for that country, and consequently there would have been some climatic effects on the physiological responses of these animals. The lower water intake observed in our study could also be related to the weather conditions and time of year. The work was carried out in typical mild winter temperatures, which can induce lower water intake (Robertshaw, 2006).

A negative water balance was observed in horses in the control treatment without supplementation. It must be considered that the water balance was assessed during 12 h after supplementation. Therefore our goal was to evaluate the immediate response to supplementation. Additionally, water intake may be influenced by diet, level of training and animal behaviour (Coenen, 2005).

The physiological mechanism of plasma hyperosmolarity is effective in stimulating thirst. This is mainly regulated by the ability to concentrate the urine. The higher plasma osmolarity is the greater thirst stimulation. Therefore, supplementation with electrolytes directly influences water consumption by altering plasma osmolarity and the ability to concentrate urine, and thereby increasing its output (Toribio, 2007). Thus the electrolytes supplementation before a competition must be thoroughly assessed, including

when to apply them and the dosage to be used. Considering the fast action of the neuroendocrine system to regulate the supplemented electrolytes, any supplementation made several hours before a competition may not enhance the volume of water retained in the body and may even result in an additional physiological stress to the horse to eliminate surplus electrolytes. Additionally, the horse will undergo a physiological stress resulting from exercise during the competition.

Blood variables

In this study, although the supplementation of electrolytes influenced the water intake it did not affect the protein plasma concentration or the haematocrit, as the electrolytes supply was quickly regulated by the urinary system with increased urine output and by the volume of water absorbed into the intestine (Toribio, 2007).

Robert *et al.* (2010) presented a reference for plasma sodium concentration among 130 and 147 mmol/l which is close to that observed in this study. Schott II *et al.* (2002) observed a sodium concentration of 138 mmol/l in horses before exercise, while Nyman *et al.* (1996) and Hess *et al.* (2008) observed values of 136 141 mmol/l, respectively. Robert *et al.* (2010) defined a reference range of plasma chloride concentration from 92 to 100 mmol/l. According to Waller *et al.* (2008) athlete horses presented pre-exercise plasma chloride concentrations of 97 mmol/l. Warren *et al.* (1999) and Bayly *et al.* (2006) observed values of plasma chloride concentrations of 104 and 99.8 mmol/l, respectively. A higher concentration of plasma chloride of 118 mmol/l was observed by Falaschini *et al.* (2005) in horses fed conventional diet. Vervuert *et al.* (2006) observed total calcium plasma concentration of 3.16 mmol/l and total magnesium plasma concentration of 0.77 mmol/l in horses before exercise.

It should be mentioned, however, despite the values plasma sodium, chloride and calcium concentration observed in the present study were in the reference range reported by several authors, inter-instrument variability has an effect on the absolute values reported for electrolytes. Care must be taken when comparing results generated by different laboratories, due to differences in sample collection, laboratory processes and calculation of reference intervals, and most published reference ranges give insufficient information about the reference sample population and methods of calculation to allow accurate comparisons (Lumsden *et al.*, 1980). Prichard and Barwick (2008) observed that reference intervals (RI) require updating because of changes in reference populations and analytical methodology. However, the RI used in this study does accurately reflect the population for which it was used, and therein the interpretations and conclusions of the results should be considered.

Unlike observed for calcium, magnesium and chloride, the plasma concentrations of potassium in our study were below the concentrations observed in other studies. Larsen *et al.* (1996), Schott II *et al.* (2002), Bayly *et al.* (2006), Sampieri *et al.* (2006), and Muñoz *et al.* (2008) observed in horses at rest a potassium plasma concentrations between 3.0 and 4.0 mmol/l; Jansson *et al.* (2002), Falaschini *et al.* (2005), Waller *et al.* (2008), and Muñoz *et al.* (2010) observed values between 4.0 and 4.5 mmol/l; and Warren *et al.* (1999) and Robert *et al.* (2010) observed values above 4.5 mmol/l. The potassium concentration in plasma may vary depending on the uptake capacity of cells to transport potassium from the extracellular to the intracellular environment.

According to Schott II *et al.* (2002) non-contractile tissues have a great ability to capture free potassium and potassium in excess, which contributes, for example, to reduce the risk of muscle fatigue during exercise. According to Aires (2008), although the mechanism is not yet clear, plasma alkalosis, may lead to hypokalemia, stimulating the influx of potassium to the intracellular environment that was observed in this paper. Possibly the high dietetic cation-anion difference contributed to metabolic alkalosis, and consequently, increased the influx of potassium into the intracellular environment intensifying the hypokalemia.

Thus, homeostasis is seen to be efficiently maintained when horses are subjected to an overload of supplemented electrolytes. The maintenance of the acid-base balance, as well as the excretion of the electrolyte overload can be observed in the pH and plasma electrolyte concentrations measures which remained stable for all treatments, as well as the plasma protein and the haematocrit concentrations. Noteworthy is the rapid maintenance of the plasma equilibrium associated with the efficiency of the excretory system, whether urinary or faecal, in contributing to the stability required for the maintenance of cellular integrity.

Urinary variables

According to Toribio (2007) adult horses produce 1 to 2 ml of urine per kg BW/h. Connysson *et al.* (2006) observed a urine output of 21 ml/kg BW/day of horses being fed a diet with adequate levels of protein, and 23 ml/kg BW/day of horses being fed diets with protein levels above the recommended; both values are very close to the production of urine of control animals. Muhonen *et al.* (2009) observed a urine output of 8.3 and 9.6 ml/kg BW per day in animals fed hay and silage, respectively. Ecke *et al.* (1998) observed that the mean sodium concentration in the urine of healthy horses was 25mmol/l, while Robert *et al.* (2010) observed an mean of sodium concentration of 39 mmol/l. Lloyd and Rose (1997) observed urinary potassium concentrations of 250 mmol/l decreasing to 90 mmol/l 12 h after supplementation with $NaHCO_3$, a fact which was associated with an increased urine output. Baker

et al. (1998) observed higher urinary potassium excretion in horses fed a diet with a high percentage of potassium compared with horses fed diets with lower levels of K, demonstrating that the excretion can be larger after the adaptation of the organism to a long-term K supply. That is unlikely to happen when supplying a single dose with high concentrations of electrolytes.

Nielsen *et al.* (1998) observed a calcium urinary concentration of 2.6 g/day from horses fed a diet containing 0.33% calcium. According to King (1994) the maintenance systems that regulate the calcium and magnesium concentrations in the blood work together for the excretion, maintenance and absorption of these electrolytes. Whereas the urine concentration of calcium and magnesium showed no effect of electrolytes supplementation. This study demonstrates that calcium and magnesium urinary output behaved similarly to potassium. However, calcium absorption is also correlated with uptake into bones (Schryver *et al.* 1970). So, the urinary output of these three minerals increase in relation to higher urine output, as was observed in the horses supplied with high doses of electrolytes.

Baker *et al.* (1998) observed higher excretion of chloride in horses fed higher chloride concentration diets when compared to animals fed lower chloride concentration in the diets. The urinary system is extremely efficient in the excretion of chloride, as in the excretion of sodium. According to King (1994) chloride is the urinary anion that is excreted the most, concomitant with sodium and potassium, the two cations excreted in the largest quantities. Nielsen *et al.* (1998) observed a urinary magnesium excretion of 2.9 g/day in horses fed diets with 0.17% magnesium. According to Hintz and Schryver (1972) and Hintz and Schryver (1973) retention capacity of magnesium increases with the increase in magnesium intake. Hintz and Schryver (1973) observed that urinary magnesium concentration is greater in horses fed diets with higher concentration of magnesium. Horses fed diets containing 0.16, 0.31 and 0.86% of magnesium, had 5.6, 10.7 and 20.5 mg Mg/kg BW excreted in urine, respectively.

The results showed indirectly that the urinary system was extremely efficient in the excretion of electrolytes supplied in excess, especially sodium and chloride. The excretion was affected over time due to the increase of water intake as a secondary effect of electrolytes supplementation. The urinary system is in fact largely responsible for maintaining the acid-base balance and the internal electrolyte homeostasis. However, the results confirm the theory that the balance of potassium occurs mainly and more efficiently in the internal, extra and intercellular environment and is excreted over time. This is corroborated by the low plasma concentration of potassium, favouring the cellular uptake of free potassium.

5. Conclusions

Electrolytic supplementation enhanced the water intake, the water retention and the urine output in horses at rest. The electrolytic supplementation has also influenced sodium and chloride concentration in urine. Urine characteristics and the concentration of electrolytes Ca, K and Mg over time were significantly reduced. Blood variables were not altered by electrolyte supplementation.

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