

## Enteral electrolyte solutions with different osmolarities administered in a continuous flow in newborn calves

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**ABSTRACT:** *This study compared the effects of enteral electrolyte solutions with different osmolarities in Holstein cattle. Eighteen newborn calves were evenly divided into three groups and administered the following treatments: hypotonic electrolyte solution (ESHYPO) containing 4g NaCl, 0.5g KCl, 1g sodium acetate, and 7.5g dextrose diluted in 1,000mL water; isotonic electrolyte solution (ESISO) containing 5g NaCl, 1g KCl, 2g sodium acetate, and 10g dextrose diluted in 1,000mL water; and hypertonic electrolyte solution (ESHYPER) containing 6g NaCl, 1g KCl, 3g sodium acetate, and 15g dextrose diluted in 1,000mL water. Solutions were administered at a rate of 15mL kg<sup>-1</sup>hr<sup>-1</sup> for 12 hours in a continuous flow. All three solutions increased the concentration of plasma sodium, but ESHYPO did not alter the serum osmolarity. Both ESISO and ESHYPO resulted in an increase in volemia.*

**Key words:** fluid therapy, calf, hypotonic solution, nasogastric tube.

## Soluções eletrolíticas enteral com diferentes osmolaridade administradas em fluxo contínuo em bezerros recém-nascidos

**RESUMO:** *O presente estudo comparou os efeitos de soluções de eletrolíticas enterais com diferentes osmolaridades administradas em fluxo contínuo. Dezoito animais foram divididos em três grupos: Solução eletrolítica hipotônica (SEHIPO) contendo 4g de NaCl; 0,5g de KCl; 1g de acetato de sódio; 7,5g de dextrose diluídos em 1.000mL de água; Solução eletrolítica isotônica (SEISO) contendo 5g de NaCl; 1g de KCl; 2g de acetato de sódio; 10g de dextrose diluídos em 1.000mL de água; A solução eletrolítica hipertônica (SEHIPER) contendo 6g de NaCl; 1g de KCl; 3g de acetato de sódio; 15g de dextrose diluídos em 1.000mL de água. As soluções foram administradas na dose de 15mLkg<sup>-1</sup>26h<sup>-1</sup> durante 12 horas. Os tratamentos SEHIPO, SEISO e SEHIPER aumentaram a concentração plasmática de sódio, contudo apenas a SEHIPO não ocasionou alteração na osmolaridade sérica. As SEHIPO e SEISO aumentaram a volemia.*

**Palavras-chave:** hidratação, bezerro, solução hipotônica, sonda nasogástrica.

## INTRODUCTION

Electrolyte and acid base imbalances are frequently observed in cattle afflicted with diseases or syndromes (CONSTABLE, 2003), with diarrhea remaining the major cause in calves. These imbalances are treated by means of fluid therapy, which aims to recover volemia and homeostasis.

The infusion of electrolytic solutions in calves is usually performed through enteral or intravenous fluid therapy. Intravenous route allows rapid infusion of a replacement volume, which is

always necessary in cases of severe dehydration and hypovolemic shock. Enteral fluid therapy can be performed using an orogastric or nasogastric tube. The administration of orogastric fluid in a bolus has been used in cattle for decades, while the continuous flow of fluids through a small-caliber nasogastric tube is less common (RIBEIRO FILHO et al., 2011). However, this latter technique is less stressful as it does not require frequent reintroduction of the probe, and allows the animals to be kept in pens without restraint while the fluid therapy is administered slowly and continuously

over a long period of time (RIBEIRO FILHO et al., 2011, 2013).

Many experimental studies have investigated the effects of enteral isotonic and hypertonic electrolyte solutions in cattle (LEVY et al., 1990; NOURI & CONSTABLE, 2006). However, the optimal electrolyte concentration, energy source, and osmolarity of the enteral electrolyte solution remain controversial. Historically, it was presumed that solutions with an osmolarity similar to that of plasma would improve intestinal absorption. However, recent studies have demonstrated that the tonicity of the enteral rehydration solution affects the level of water and electrolyte absorption, with hypotonic solutions resulting in better water absorption than isotonic solutions in children (RAUTANEN et al., 1993), laboratory animals (NISHINAKA et al., 2004), and horses (RIBEIRO FILHO et al., 2014). Low-osmolarity electrolytic solutions have the advantage of a reduced risk of hypernatremia, increased water absorption, and reduced fecal output. However, they also have the capacity to induce hyponatremia in patients, particularly in those decreased serum sodium levels although this has only been reported in patients receiving intravenous fluid therapy (CHOONG et al., 2006).

Experiments using low-osmolarity electrolyte solutions have not yet been performed in cattle. However, considering the results from other species, it is likely that such experiments may provide important data for establishing enteral fluid therapy recommendations and criteria for cattle. Therefore, the aim of the present study was to evaluate the effects of enteral electrolyte solutions with different osmolarities administered in a continuous flow through a small-caliber nasogastric tube over 12 hours on plasma and urinary electrolytes, serum and urinary osmolarity, and volemia in newborn calves. We hypothesized that the use of a hypotonic electrolyte solution would increase volemia without causing hyponatremia or a decrease in serum osmolarity.

## MATERIALS AND METHODS

Six male and 12 female Holstein calves that were an average of 20 days old and had a median body weight of 52kg were used in this study. These calves were clinically healthy with no history of gastrointestinal disease, and underwent a clinical and laboratory evaluation prior to the experiment. Calves remained in individual pens (2 × 3m) and were provided with a wood shavings bed, which was

changed daily. They were fed 1L of milk four times per day and were provided with water *ad libitum* during the experimental period.

Animals were randomly divided into three equal-sized groups. Before starting treatments, a nasogastric tube was introduced and attached to the halter of each animal. The probe was connected to a spiral line, through which the solution flowed from a 20-L recipient positioned above the animal's head. Animals remained in individual stalls and received electrolyte solutions throughout the experimental period. All of the electrolyte solutions contained the same components but in different concentrations, resulting in a hypoosmotic solution (ESHYPO, 200mOsmolL<sup>-1</sup>: 4g sodium chloride, 0.5g potassium chloride, 1g sodium acetate, and 7.5g dextrose), an isosmotic solution (ESISO, 280mOsmolL<sup>-1</sup>: 5g sodium chloride, 1g potassium chloride, 2g sodium acetate, and 10g dextrose), and a hyperosmotic solution (ESHYPER, 350mOsmolL<sup>-1</sup>: 6g sodium chloride, 1g potassium chloride, 3g sodium acetate, and 15g dextrose). Each animal was administered with the appropriate solution for 12 hours at a rate of 15mL kg<sup>-1</sup>hr<sup>-1</sup> via a nasogastric tube<sup>a</sup> (4mm diameter, 150cm long), which was attached to a 20-L container of solution via a spiral line. During the fluid therapy phase, animals remained fasted (both food and water withheld) and were unrestrained.

Plasma concentration of sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), and chloride (Cl<sup>-</sup>), and the volemia and osmolarity of the blood were measured in samples collected at the following times: T0h, immediately prior to the initiation of treatment; T6h, after 6 hours of treatment; T12h, after 12 hours of treatment (end of fluid therapy); and T24h, 12 hours after the end of fluid therapy. In addition, the urinary concentration of sodium (urNa<sup>+</sup>), potassium (urK<sup>+</sup>), and chloride (urCl<sup>-</sup>), and the osmolarity of the urine (urOsm) were measured by collecting samples at T0h, T12h, and T24h.

To measure the osmolarity and volemia of blood, samples were collected in tubes without anticoagulant<sup>b</sup> to obtain the serum. Serum aliquots were kept frozen at -20°C until the laboratory tests were undertaken. Osmolarity of the serum was measured with an osmometer<sup>c</sup>. Concentrations of total protein were then assessed in a multi-biochemical analyzer<sup>d</sup> to calculate the volemia using the formula (BOYD, 1981): %VP = (TP1/TP2) - 1 × 100 where VP is the plasma volume, TP1 is the initial total protein concentration, and TP2 is the final total protein concentration. To examine the sodium, potassium, and chloride concentrations in the blood<sup>e</sup>, samples were collected in 2-mL disposable plastic

syringes that had previously been heparinized with lithium heparin.

Urine samples were collected by massaging the preputial region in males and the region below the vulva in females, which were cleaned before each collection. The aliquots of urine were also kept frozen at  $-20^{\circ}\text{C}$  until biochemical analysis. Sodium and potassium contents of the urine were measured using a B462 flame photometer<sup>f</sup>; chloride was measured with a multi-biochemical analyzer<sup>d</sup>; and osmolarity was measured using an osmometer<sup>e</sup>.

The statistical program SAEG 9.1 (SAEG, 2007) was used for data analysis. Data were analyzed using repeated measures analysis of variance (ANOVA), which evaluated the effects of treatment and time, and the interaction between these. When the analysis was significant for one or more factor, Tukey's test was used to compare the mean values. Where the data did not meet the assumptions of ANOVA, nonparametric analysis was employed and the mean values were compared using the Kruskal-Wallis test. All analyses were interpreted considering a significance level of 5% ( $P<0.05$ ).

## RESULTS AND DISCUSSION

There was significant variation in plasma sodium levels of animals from all treatment groups over time ( $P<0.05$ ; Table 1), with all three groups exhibiting an increase in plasma sodium concentration during the rehydration period (T6h and T12h). The finding that an enteral electrolytic solution containing  $4\text{gL}^{-1}$  NaCl (ESHYPO) did not cause a decrease in plasma levels, but rather a small increase, indicated that ESHYPO could be employed in cases of mild hyponatremia to correct imbalance. However, it should be emphasized that hypotonic solutions containing less than  $5\text{gL}^{-1}$  NaCl should be used with caution in animals with moderate to severe hyponatremia, with constant patient monitoring.

Findings of the present study are in accordance with those of CHAKRABARTI et al. (2005), who compared the capacity of rats to absorb water, sodium, potassium, chloride, and glucose from a hypotonic electrolyte solution with a low concentration of sodium ( $60\text{mMolL}^{-1}$ ), a hypotonic electrolyte solution with a low glucose concentration ( $60\text{mMolL}^{-1}$ ), and an isotonic electrolyte solution (control). They reported that all of the substances mentioned above were more effectively absorbed from the hypotonic solution, and that this also did not cause the onset of hyponatremia.

Although the ESISO and ESHYPER treatment groups exhibited the highest plasma sodium levels at T12h (Table 1), there was no significant difference between these and ESHYPO ( $P>0.05$ ). Intravenous infusion of maintenance hypotonic solutions in humans is considered to be one cause of hyponatremia in patients who received large volumes of these solutions or who are hydrated over long periods of time (CHOONG et al., 2006). Based on the literature and the findings of the present study, it is believed that maintenance of enteral electrolyte solutions should contain less sodium, and that the 0.9% saline solution should only be used in patients with hyponatremia and more pronounced hypochloreaemia.

There was no significant difference in the serum osmolarity of animals in the ESHYPO treatment group over time (T0h-T12h) ( $P>0.05$ ), despite the slight increase in value at T12h (Table 1). By contrast, there was a significant increase in the serum osmolarity of the ESISO and ESHYPER treatment groups at T12h ( $P<0.05$ ), which was correlated with the greater amounts of electrolytes they contained, particularly sodium and chloride. It has previously been shown that the sodium ion is mainly responsible for such an increase, accounting for approximately 85% of serum osmolarity (ANDREWS & GRINDEM, 2000), but that the chloride ion also contributes to osmolarity, despite being present at a lower concentration in the blood (MORAIS & BIONDO, 2012). It must be highlighted, however, that despite these increases, the serum osmolarity remained in the normal range for cattle (NAYLOR et al., 1990).

The optimal concentration of electrolytes, energy source, and osmolarity of enteral electrolyte solutions in animals remain controversial. However, most scientists advocated for the use of isotonic electrolyte solutions in calves (LEVY et al., 1990; NAYLOR et al., 1990; CONSTABLE et al., 2001; NOURI & CONSTABLE, 2006), arguing that the use of hypertonic solutions can generate electrolytic imbalances and result in lower abomasal emptying rates in calves than isotonic electrolyte solutions. Furthermore, hypertonic solutions with high glucose content can accentuate diarrhea. However, experimental studies in children (RAUTANEN et al., 1993), laboratory animals (NISHINAKA et al., 2004), and horses (RIBEIRO FILHO et al., 2014) demonstrated that hypotonic enteral electrolyte solutions improve water absorption, reduce the risk of hypernatremia, and decrease fecal output compared with isotonic enteral electrolyte solutions.

Table 1 - Plasma concentrations of sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), and chloride ( $\text{Cl}^-$ ), and the osmolarity and volemia of the blood in healthy newborn calves treated with hypotonic (ESHYPO), isotonic (ESISO), and hypertonic (ESHYPER) electrolyte solutions administered by nasogastric tube in a continuous flow for 12 hours. Values are means  $\pm$  standard deviations.

Treatment	T0h	T6h	T12h	T24h
----- $\text{Na}^+$ (mMol L <sup>-1</sup> )-----				
ESHYPO	136 $\pm$ 2.99 <sup>Ac</sup>	139 $\pm$ 2.10 <sup>Aab</sup>	142 $\pm$ 2.14 <sup>Aa</sup>	137 $\pm$ 1.63 <sup>Abc</sup>
ESISO	137 $\pm$ 2.10 <sup>Ab</sup>	140 $\pm$ 2.64 <sup>Ab</sup>	145 $\pm$ 2.88 <sup>Aa</sup>	140 $\pm$ 2.71 <sup>Ab</sup>
ESHYPER	137 $\pm$ 0.90 <sup>Ac</sup>	141 $\pm$ 2.06 <sup>Aab</sup>	144 $\pm$ 1.41 <sup>Aa</sup>	139 $\pm$ 2.71 <sup>Abc</sup>
-----Osmolarity (mMol L <sup>-1</sup> )-----				
ESHYPO	289.67 $\pm$ 4.38 <sup>Aa</sup>	292.17 $\pm$ 3.71 <sup>Aa</sup>	298.33 $\pm$ 5.01 <sup>Aa</sup>	292.17 $\pm$ 2.93 <sup>Aa</sup>
ESISO	292.00 $\pm$ 3.29 <sup>b</sup>	297.83 $\pm$ 4.96 <sup>Aab</sup>	302.17 $\pm$ 5.31 <sup>Aa</sup>	296.50 $\pm$ 7.34 <sup>Ab</sup>
ESHYPER	289.50 $\pm$ 4.32 <sup>b</sup>	297.67 $\pm$ 6.53 <sup>Aab</sup>	300.17 $\pm$ 6.43 <sup>Aa</sup>	293.67 $\pm$ 4.88 <sup>Aab</sup>
-----Volemia (%)-----				
ESHYPO	0 $\pm$ 0 <sup>Ab</sup>	2.0 $\pm$ 0.8 <sup>Aa</sup>	4.0 $\pm$ 1.4 <sup>Aa</sup>	2.0 $\pm$ 1.3 <sup>Aa</sup>
ESISO	0 $\pm$ 0 <sup>Ab</sup>	3.0 $\pm$ 1.5 <sup>Aa</sup>	3.0 $\pm$ 1.4 <sup>Aa</sup>	1.3 $\pm$ 2.1 <sup>Aa</sup>
ESHYPER	0 $\pm$ 0 <sup>Ab</sup>	0.5 $\pm$ 1.6 <sup>Aa</sup>	0.0 $\pm$ 2.8 <sup>Aabc</sup>	-2.2 $\pm$ 0.7 <sup>Bc</sup>
----- $\text{K}^+$ (mMol L <sup>-1</sup> )-----				
ESHYPO	4.18 $\pm$ 0.43	4.33 $\pm$ 0.41	44.02 $\pm$ 0.54	4.18 $\pm$ 0.26
ESISO	4.22 $\pm$ 0.68	4.22 $\pm$ 0.56	4.05 $\pm$ 0.32	4.20 $\pm$ 0.69
ESHYPER	3.95 $\pm$ 0.35	4.12 $\pm$ 0.33	3.90 $\pm$ 0.18	4.08 $\pm$ 0.30
----- $\text{Cl}^-$ (mMol L <sup>-1</sup> )-----				
ESHYPO	95 $\pm$ 2.83 <sup>Ac</sup>	100 $\pm$ 1.50 <sup>Ab</sup>	103 $\pm$ 1.72 <sup>ABa</sup>	97 $\pm$ 2.88 <sup>Abc</sup>
ESISO	96 $\pm$ 2.33 <sup>Ac</sup>	101 $\pm$ 2.61 <sup>Ab</sup>	107 $\pm$ 3.15 <sup>Aa</sup>	98 $\pm$ 3.19 <sup>Abc</sup>
ESHYPER	96 $\pm$ 1.72 <sup>Ab</sup>	101 $\pm$ 1.55 <sup>Aa</sup>	103 $\pm$ 1.50 <sup>Ba</sup>	97 $\pm$ 3.54 <sup>Ab</sup>

Mean values followed by different capital letters in the same column or by different lowercase letters in the same row differ according to Tukey's Test ( $P < 0.05$ ).

As can be seen from table 1, ESHYPO and ESISO treatments resulted in an increase in volemia at T6h and T12h, while the ESHYPER treatment caused only a slight increase in volemia at T6h, which had decreased by T12h. This demonstrated that the ESHYPO and ESISO treatments were more effective in increasing the plasma volume, with the effects lasting until T24h (12 hours after the hydration period).

The osmolarity of electrolyte solutions influences how effectively their water and electrolyte contents are absorbed by the intestine, which, in turn, will affect the level of volemia expansion. The close relationship between the osmolarity of the solution and volemia expansion has been confirmed in several experimental studies (RAUTANEN et al., 1993; RIBEIRO FILHO et al., 2014), which demonstrated that the lower the osmolarity of the electrolyte solution, the higher the absorption, supporting the results of the present study. Furthermore, the animals that received the ESHYPO treatment showed no adverse effects from the low osmolarity of the electrolyte solution. However, it must be emphasized that patients with severe hyponatremia must have their serum or plasma

sodium and osmolarity levels monitored if being treated with hypotonic enteral electrolytic solutions, as a severe reduction in these variables could lead to adverse effects (RIBEIRO FILHO et al., 2014).

There was no significant variation between treatment groups or times in the potassium levels ( $P > 0.05$ ; Table 1). The amount of potassium contained in the electrolytic solutions did not cause imbalances in its plasma concentrations, which remained within the reference range for bovine species (KANEKO et al., 2008).

Plasma chloride levels showed a similar behavior to sodium (Table 2). During the rehydration period (T0h-T12h), animals from all treatment groups exhibited a gradual increase in plasma chloride levels over time ( $P < 0.05$ ) due to it being present in all three of the enteral electrolyte solutions. However, despite this increase, it should be emphasized that the values remained in the normal range for bovine animals (KANEKO et al., 2008), showing that none of the tested enteral electrolyte solutions caused an imbalance in the animals' plasma chloride concentrations, despite the ESHYPER treatment containing approximately 55% more chloride than the ESHYPO treatment. This

Table 2 - Urinary concentrations of sodium (urNa<sup>+</sup>), potassium (urK<sup>+</sup>), and chloride (urCl<sup>-</sup>), and the osmolarity of the urine in healthy newborn calves treated with hypotonic (ESHYPO), isotonic (ESISO), and hypertonic (ESHYPER) enteral electrolyte solutions administered by nasogastric tube in a continuous flow for 12 hours. Values are means ± standard deviations.

Treatment	T0	T12	T24
-----urNa <sup>+</sup> (mMolL <sup>-1</sup> )-----			
ESHYPO	40.0±33.55 <sup>Ab</sup>	55.5±28.90 <sup>Bb</sup>	119.5±58.23 <sup>Aa</sup>
ESISO	22.28±4.49 <sup>Ac</sup>	71.5±30.67 <sup>Bb</sup>	172.17±53.3 <sup>Aa</sup>
ESHYPER	25.0±5.68 <sup>Ab</sup>	110.0±20.34 <sup>Aa</sup>	171.0±65.9 <sup>Aa</sup>
-----urK <sup>+</sup> (mMolL <sup>-1</sup> )-----			
ESHYPO	9.6±7.38 <sup>Aa</sup>	1.17±0.4 <sup>Ab</sup>	10.1±0.63 <sup>Aa</sup>
ESISO	12.63±11.33 <sup>Aa</sup>	5.53±4.82 <sup>Aa</sup>	9.8±5.54 <sup>Aa</sup>
ESHYPER	9.58±7.11 <sup>Aa</sup>	3.58±3.04 <sup>Aa</sup>	7.32±3.48 <sup>Aa</sup>
-----urCl <sup>-</sup> (mMolL <sup>-1</sup> )-----			
ESHYPO	54.17±14.55 <sup>Aab</sup>	33.83±15.3 <sup>Bb</sup>	78.08±33.58 <sup>Aa</sup>
ESISO	57.33±37.71 <sup>Aa</sup>	46.0±17.98 <sup>Ba</sup>	71.17±40.38 <sup>Aa</sup>
ESHYPER	39.17±21.07 <sup>Aa</sup>	66.5±13.56 <sup>Aa</sup>	72.83±36.21 <sup>Aa</sup>
-----Urinary osmolarity (mMolL <sup>-1</sup> )-----			
ESHYPO	573.0±133.67 <sup>Aa</sup>	146.33±64.17 <sup>Bb</sup>	599.0±263.66 <sup>Aa</sup>
ESISO	700.67±167.4 <sup>Aa</sup>	193.33±54.74 <sup>Bb</sup>	678.83±269.95 <sup>Aa</sup>
ESHYPER	704.5±195.64 <sup>Aa</sup>	282.00±65.66 <sup>Ab</sup>	522.0±274.62 <sup>Aab</sup>

Mean values followed by different capital letters in the same column or by different lowercase letters in the same row differ according to Tukey's Test (P<0.05).

indicated that the animals were able to excrete the excess chloride via their kidneys, keeping its plasma concentration within the normal range.

Animals in the ESISO and ESHYPER treatment groups exhibited higher sodium levels in their urine at T12h and T24h (P<0.05; Table 2), due to the higher content of sodium in these solutions. The ESHYPO treatment also caused the concentration of sodium in the urine to increase at T12h, although this was not significant (P>0.05; Table 2). These findings combined with those for serum sodium values (Table 1) indicated that treatment with ESHYPO may not accentuate the deficit of this electrolyte in animals with slight hyponatremia. However, animals with severe hyponatremia may respond differently, and so the use of this kind of solution should be monitored in such patients.

Animals in the ESHYPO treatment group exhibited a significant decrease in potassium levels in their urine at T12h (P<0.05), though these had returned to basal levels by 12 hours after the end of fluid therapy. This decrease may have been associated with the dilution of potassium in the urine, indicating that the amount of potassium in the electrolyte solution was not excessive. By contrast, animals that received the ESISO and ESHYPER treatments showed a less marked and non-significant decline in potassium urine levels at T12h (P>0.05; Table 2), likely due to

the increased amount of potassium in these solutions. Therefore, serum potassium should also be monitored in calves with marked hypokalemia.

There was a significant difference in the chloride content of urine between treatments at T12h (P<0.05), with animals in the ESHYPER treatment group exhibiting the highest values due to the higher chloride content of this solution (Table 2). By the end of the fluid therapy period (T12h), the amount of urinary chloride in animals in the ESHYPO treatment group had significantly decreased (P<0.05) due to the lower content of this electrolyte in this solution. However, no similar decrease in chloride levels was detected in the serum (Table 1).

The osmolarity of the urine exhibited significant differences between treatments (P<0.05), with the ESHYPER treatment group having higher values than the other treatment groups at T12h (Table 2). This behavior reflected urinary sodium and chloride levels (Table 2) and was caused by the hypertonic electrolyte solution containing more sodium and chloride than the other solutions. In addition, the urinary osmolarity varied over time (P<0.05), with all treatment groups experiencing a significant decrease in urOsm at T12h. This was more evident in animals that received ESHYPO; however, possibly due to the lower electrolyte contents of this solution (Table 2).

## CONCLUSION

Hypotonic and isotonic enteral electrolyte solutions are effective in expanding volemia without causing plasma hyponatremia or a decrease in serum osmolarity. These findings open up new possibilities for the use of hypotonic electrolyte solutions in calves.

## ACQUISITION SOURCE

<sup>a</sup>Provar Commercial LTDA, São Paulo, SP, BRA;

<sup>b</sup>Vacuntainer BD, Juiz de Fora, MG, BRA;

<sup>c</sup>Advanced Micro-Osmometer Model 3320; Advanced Instruments Inc., Norwood, MA, USA;

<sup>d</sup>HumaStar 300; Human GmbH, Wesbaden, DEU;

<sup>e</sup>ABL80 FLEX-Radiometer Copenhagen; Radiometer Medical 248 ApS, Brønshøj - Copenhagen, DNK;

<sup>f</sup>Flame photometer model B462; Micronal, São Paulo, Brazil.

## BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the Ethics Committee of the Veterinary Department of the University of Viçosa (Process no. 031/2012) in accordance with the Veterinary Professional Ethics Code, the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation, and current Brazilian legislation.

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