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Enteral fluid therapy administered in continuous flow by naso-ruminal route using three maintenance electrolyte solutions: effects on physiological biomarkers and the hemogram of bovines

Pedro Ancelmo Nunes Ermita[1*] José Dantas Ribeiro Filho[1] Rinaldo Batista Viana[2] Micheline Ozana da Silva[1] Samuel Rodrigues Alves[1] Lorena Chaves Monteiro[1] Caio Monteiro Costa[1] Lucas Drumond Bento[1] Artur Neves Passos[1] Felipe Sperandio Mattos[3]

[1]Departamento de Veterinária, Universidade Federal de Viçosa (UFV), Av. PH Rolfs, s/n, Campus universitário, 36570-900, Viçosa, MG, Brasil. E-mail: pedro.ermita2@gmail.com. *Corresponding author.

[2]Universidade Federal Rural da Amazônia (UFRA), Belém, PA, Brasil.

[3]União de Ensino Superior de Viçosa (UNIVIÇOSA), Viçosa, MG, Brasil.

ABSTRACT: *The aim of this study was to evaluate the effects of three enteral electrolyte solutions, each with different energy sources, administrated as continuous flow on the physiological parameters and blood count of healthy Holstein heifers. Six Holstein heifers were used in a crossover design. All animals received all three treatments: solution with calcium propionate, 4g of NaCl, 0.5g of KCl, 0.3g of* $MgCl_2$ *and 10g of calcium propionate diluted in 1000mL of water (measured osmolarity: 299mOsm/L); solution with glycerol, 4g of NaCl, 0.5g of KCl, 0.3g of* $MgCl_2$*, 1g of calcium acetate, and 10mL of glycerol in 1000mL of water (measured osmolarity: 287mOsm/L); solution with propylene glycol, 4g of NaCl, 0.5g of KCl, 0.3g of* $MgCl_2$*, 1g of calcium acetate, and 15mL of propylene glycol in 1000mL of water (measured osmolarity: 378mOsm/L). Physical evaluations and blood samples were collected immediately before the initiation of fluid therapy, at 3-hour intervals over the 12-hour period of fluid therapy, and 12 hours after the end of fluid therapy. Animals presented no signs of stress or discomfort. All solutions resulted in a significant decrease in erythrocyte concentration, hemoglobin concentration, and hematocrit, without affecting the leukogram. Enteral fluid therapy administered as continuous flow via the naso-ruminal route was well-tolerated by animals with minimal effects on animal welfare, even when administered for 12 hours. This technique is indicated as an alternative route for parenteral maintenance fluid therapy. Electrolyte solutions proposed here were able to significantly expand blood volume.*

Key words: *ruminants, polyionic solutions, physiologic parameters, volemia, hemogram.*

Hidratação enteral via nasorruminal em fluxo continuo utilizando três soluções eletrolíticas de manutenção: efeitos sobre os biomarcadores fisiológicos e o hemograma de bovinos

RESUMO: *Objetivou-se avaliar os efeitos de três soluções eletrolíticas enterais de manutenção com diferentes fontes de energia administradas em bovinos adultos por via nasorruminal em fluxo contínuo sobre parâmetros fisiológicos e hematológicos. Foram utilizadas seis novilhas holandesas em um delineamento crossover. Os animais foram submetidos a três tratamentos: Solução contendo Propionato de cálcio - 4g de NaCl, 0,5g de KCl, 0,3g de* $MgCl_2$ *e 10g de propionato de cálcio para cada 1000mL (Osmolaridade mensurada: 299mOsm/L); Solução contendo Glicerol - 4g de NaCl, 0,5g de KCl, 0,3g de* $MgCl_2$*, 1g de acetato de cálcio e 10mL de glicerol para cada 1000mL (Osmolaridade mensurada: 287mOsm/L); Solução contendo Propilenoglicol - 4g de NaCl, 0,5g de KCl, 0,3g de* $MgCl_2$*, 1g de acetato de cálcio e 15mL de propilenoglicol para cada 1.000mL (Osmolaridade mensurada: 378mOsm/L). Foi realizado exame físico e colheita de sangue para os hemogramas imediatamente antes do início da hidratação e a cada três horas durante 12h de tratamento e mais uma colheita 12h após o final do período experimental, perfazendo seis colheitas ao total. Todas soluções promoveram ao longo das 12 horas de tratamento hemodiluição com redução nos valores de hemácias, concentração de hemoglobina e volume globular, sem, contudo, alterar o leucograma. A hidratação enteral em fluxo contínuo via nasorruminal, mostrou-se uma técnica bem tolerada pelos animais, como mínimos efeitos sobre o bem-estar, mesmo quando administrada por 12 horas, sendo, portanto, uma técnica indicada como uma opção à hidratação parenteral na terapia de manutenção de fluidos. As três soluções eletrolíticas aqui propostas são capazes de expandir significativamente a volemia.*

Palavras-chave: *ruminantes, soluções poliônicas, parâmetros fisiológicos, volemia, hemograma.*

INTRODUCTION

The prevalence of hydroelectrolyte and acid-base disorders is common in variable bovine diseases; therefore, fluid therapy has become an indispensable part of the buiatric routine (RIBEIRO FILHO et al., 2013). In this way, accurate evaluation of dehydration and acid-base disorders is necessary for optimal hydroelectrolyte replacement.

In bovine medicine, fluid therapy is most commonly administered by intravenous and oro-ruminal routs (RIBEIRO FILHO et al., 2013). Due to the large volumes administered, professional

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supervision is required during fluid therapy, and the final cost of administration via the parenteral route is sometimes unviable (RIBEIRO FILHO et al., 2011). Consequently, this route of fluid therapy is only used under critical scenarios, such as hypovolemic shock.

Enteral fluid therapy, like the oro-ruminal route, allows a large volume of electrolyte fluid to be administered directly into the rumen in cases of mild and moderate dehydration (ROUSSEL, 2014), and is used careful in cases with motility disorder. Although, this technique is simple, when performed over several days, successive tube passages increase the risk of laryngeal and esophagus lesions. In addition, large volumes of fluid can cause some discomfort to the animal when administered quickly into the rumen.

An alternative method that has been used and studied in Brazil for more than 10 years, is enteral fluid therapy with continuous flow, using a naso-gastric or naso-ruminal tube with a small diameter. This technique has been used successfully in horses (AVANZA et al., 2009; RIBEIRO FILHO et al., 2012; RIBEIRO FILHO et al., 2015); owing to the small gastric capacity of this species, several doses or slow and continuous administration is required throughout the day.

In goats (ATOJI-HENRIQUE et al., 2012), calves (RIBEIRO FILHO et al., 2017), and bubaline calves (ERMITA et al., 2016) continuous flow via the naso-ruminal route has been tested and promising results have been reported. Some studies have already been performed in adult cattle (RIBEIRO FILHO et al., 2009; RIBEIRO FILHO et al., 2011; RIBEIRO FILHO et al., 2013); however, many questions remain unanswered, including osmolarity, the correct electrolyte composition, energy precursor, and infusion rate for these animals. This is because this technique is not widespread in this species, and animals need to remain in an individual stall during therapy.

One important characteristic of enteral fluid therapy is the possibility of creating new solutions with altered compositions that meet the needs of the animal and increase the therapeutic efficiency (RIBEIRO FILHO, 2011). When used in continuous flow, the deleterious effects described above are minimized, besides not preventing the animal from walking and laying inside the stall and feeding during fluid therapy.

According to CONSTABLE (2003), solutions for enteral use should contain sodium, potassium, chloride, calcium, phosphate, and a glycemic precursor, such as propionate. In addition, RIBEIRO FILHO et al. (2014) stated that by maintaining volemia and electrolyte balance, the solution should not cause any adverse events.

The objective of this study was to evaluate the effects of enteral fluid therapy administered as continuous flow by the naso-ruminal route with three enteral electrolyte solutions, containing different energy precursors, on the physiological biomarkers and the hemogram of clinically healthy cattle.

MATERIALS AND METHODS

This experiment included six healthy Holstein heifers from the flock of the Experimental Research and Extension Unit in Dairy Cattle of the Federal University of Viçosa (Unidade Experimental de Pesquisa e Extensão em Gado de Leite da Universidade Federal de Viçosa), aged 16 and 18 months and with a mean body weight of 300kg. Animals were adapted to the experimental environment for 10 days before the beginning of the study, during which they were restricted in an individual stall in a *Tie Stall* system. Animals were fed with corn silage as a balanced ration and water was provided *ad libitum.*

A *crossover* 6×3 design was adopted (six animals × three treatments) and all animals received all treatments. To avoid an overlap effect, there was a 7-day interval between treatments. The composition of the electrolyte solutions was as follows: Solution with calcium propionate solution (SEPCa), 4g of NaCl[a], 0.5g of KCl[a], 0.3g of $MgCl_2$[a]and 10g de calcium propionate[b] in 1000mL of water (measured osmolarity: 299mOsm/L); glycerol solution (SEGly), 4g of NaCl[a], 0.5g of KCl[a], 0.3g of $MgCl_2$[a], 1g of calcium acetate[a], and 10mL of glycerol[c] in 1000mL of water (measured osmolarity: 287mOsm/L); and a solution with propylene glycol, 4g of NaCl, 0.5g of KCl, 0.3g of $MgCl_2$, 1g of calcium acetate, and 15mL of propylene glycol in 1000mL of water (measured osmolarity: 378mOsm/L).

A naso-ruminal tube with a small diameter (4mm diameter and 1.8m length) was used to administer fluid. The tube was attached to the halter and connected to a gallon with a 20L capacity with a spiral hydration system[d], set 1.5m above the head of the animal. The continuous flow rate was 15mL/kg/h, over 12 hours, and animals did not receive food or water during the hydration period. After the hydration period, the animals were in water and food fasting for 12 hours, for a 24-hour experimental cycle. Samples were collected and evaluated at the following times: T0h, just before the beginning of the experiment; T3h, 3 hours after the beginning of hydration; T6h, 6 hours after the beginning of hydration; T9h, 9 hours after the beginning of hydration; T12h, 12 hours after the beginning of hydration and the end of hydration period; and T24h, 12 hours after the end of hydration (observation period).

The following physiological parameters were analyzed: heart rate (HR, bpm), respiratory rate (RR, mpm), rectal temperature (RT, °C), ruminal movements (RM in 3 minutes), abdominal girth (AG, cm), and fecal humidity (FH%). Blood samples were collected via jugular venipuncture using a vacuum system[e] in K_2EDTA tubes, and blood counts were determined in an automatic blood counter device[f]. White blood cell counts were obtained by blood scrubbing as recommended by STOCKHAM & SCOTT (2011). Abdominal girth was calculated using a measuring tape across the flank of the bovine. Feces were collected directly from the rectal ampulla and were immediately weighed and dried in a drying oven at 60°C for several days. The feces were weighed until a constant weight was achieved. Humidity was determined using the following equation: [(initial weight - final weight) × 100/initial weight].

Results were analyzed descriptively to obtain the mean and the standard deviation. The normality of the data was evaluated with the Shapiro-Wilk test and the sphericity of the variances was determined with the Mauchly test. When the pre-requisites of normality and sphericity were achieved, analysis of variance (ANOVA) based on the factorial design of repetitive measures was used to evaluate the main effects across time, treatments, and in the time*treatment effect. Multiple comparisons were compared using the Tukey *post hoc* test. When it was not possible to use ANOVA, the Friedman test with the Wilcoxon *post hoc* test associated with a Bonferroni correction was used. All analyses were performed with the statistical package SPSS 20 (IBMD, Statistic). Significance was considered when P<0.05.

RESULTS AND DISCUSSION

Physiological variables have been used to evaluate the welfare and adaptation of cattle under variable edaphoclimatic and management conditions (DINIZ et al., 2017). In this study, a decrease (P<0.05) in the heart rate of animals in the SEPCa group was observed during the hydration phase, and the respiratory rate remained the same (P>0.05) at baseline in the three groups. Notably, under all situations, these biomarkers remained within the reference values suggested by FEITOSA (2004). In this way, the data observed in the present study (Table 1), confirmed that enteral fluid therapy administered as continuous flow under the proposed conditions (solution type and fluid therapy technique), does not cause stress to the animals, demonstrating that the use of naso-ruminal tubes and the administration of these solutions did not cause discomfort to animals. This was clearly observed during the hydration period when the heifers were mostly laying down, quiet, and ruminating, as previously described by RIBEIRO FILHO et al. (2011, 2013).

Several factors can alter body temperature, including exercise, environmental temperature, digestion, water intake, disease, and time of day (circadian or nictemeral variation), which can cause variation of 1.5°C in 24 hours (FEITOSA, 2004). During the fluid therapy period between T0h and T3h, the rectal temperature of animals in the SEPCa group, was not correlated with the technique used or experimental time; this variation was probably caused by the circadian variation and, at all time points observed, the temperature remained inside the reference interval for the species (REECE, 2015). Stability in rectal temperature was also observed by RIBEIRO FILHO et al. (2015) and ERMITA et al. (2016).

The ruminal movements were not affected over time with either treatment, as described by ATOJI-HENRIQUE et al. (2012). Effects of enteral fluid therapy on gastrointestinal tract motility seem to be associated with the dose and solution used, as described by RIBEIRO FILHO et al. (2012) who used enteral fluid therapy in healthy horses and observed an increase in intestinal motility. Although, using the same dose in the present study (15mL/kg/h), solution used by authors contained different concentrations of electrolytes and other energy sources. This suggested that in addition to physiological differences between species, the solution used could be a major factor for the effects observed in the intestinal tract.

Abdominal girth has been utilized in studies investigating enteral fluid therapy to verify the degree of abdominal distention, as some energy sources can sustain ruminal microbiota

Table 1 - Mean ± standard deviation of the heart rate (HR – bpm), respiratory rate (RR - mpm), rectal temperature (RT - °C), ruminal movement/3 min. (RM), abdominal girth (AG - cm) and feces humidity (FH - %) of heifers under enteral fluid therapy in continuous flow with three different electrolyte solutions.

		------Fluid therapy period------					Observation
	Groups	T0h	T3h	T6h	T9h	T12h	T24h
HR	SEPCa	70.7±11.4^{Aa}	67.5±9.6^{Aab}	63.8±10.1^{Aab}	62.0±10.7^{Ab}	63.2±7.6^{Bab}	66.5±7.53^{Aab}
	SEGlyc	67.7±22.4^{A}	62.0±13.8^{A}	55.8±12.8^{A}	58.2±7.7^{A}	55.8±9.2^{B}	57.3±8.9^{B}
	SEProp	71.5±15.4^{Aab}	70.0±10.7^{Aab}	63.5±11.3^{Ab}	61.7±10.8^{Ab}	74.7±6.7^{Aa}	67.5±5.8^{ABab}
RR	SEPCa	20.2±6.8^{abc}	18.3±5.8^{bc}	17.3±5.9^{bd}	19.7±5.3^{c}	19.0±4.5^{cd}	23.7±2.7^{a}
	SEGlyc	21.0±10.6^{ab}	18.7±5.4^{ab}	17.5±5.2^{b}	17.5±6.2^{bc}	19.7±5.9^{ac}	21.5±4.7^{a}
	SEProp	17.0±4.6^{bd}	17.3±6.0^{bc}	20.3±7.6^{ad}	20.3±5.3^{acd}	21.3±5.1^{bcd}	22.2±3.7^{a}
RT	SEPCa	38.6±0.5^{ac}	38.4±0.4^{bd}	38.4±0.5^{cd}	38.6±0.5^{ad}	38.7±0.6^{ab}	38.6±0.3^{ad}
	SEGlyc	38.7±0.4^{cd}	38.4±0.2^{ac}	38.5±0.3^{c}	38.8±0.2^{bd}	38.9±0.3^{bd}	38.9±0.4^{ab}
	SEProp	38.7±0.3^{ab}	38.5±0.4^{b}	38.5±0.2^{ab}	38.7±0.3^{a}	38.6±0.3^{ab}	38.8±0.2^{ab}
RM	SEPCa	3.8±0.4	3.8±0.4	4.0±0.0	4.0±0.6	4.0±0.0	3.7±0.5
	SEGlyc	4.0±0.9	4.2±0.4	4.0±0.6	4.0±0.0	3.8±0.4	4.0±0.0
	SEProp	3.8±0.4	3.7±0.5	3.7±0.8	4.0±0.6	3.7±0.5	3.7±0.5
AG	SEPCa	179.7±24.4	175.2±15.9	182.7±18.8	183.8±20.2	183.2±16.2	179.2±17.1
	SEGlyc	178.7±15.6	177.3±14.5	176.5±15.4	176.2±16.6	177.3±18.2	177.5±15.7
	SEProp	173.0±16.5	179.3±7.5	184.0±11.8	182.7±11.8	178.8±11.8	178.8±13.2
FH	SEPCa	85.2±2.2	86.6±1.1	85.5±1.0	86.33±2.4	86.5±2.4	-
	SEGlyc	86.9±2.8	86.6±2.3	85.9±2.3	86.3±2.4	86.5±2.4	-
	SEProp	87.7±3.0	86.6±3.7	86.3±1.3	86.4±3.5	86.5±3.9	-

Means followed of lower case letters are different in the same line and capital letters are different in the same column (P<0.05).

fermentation. This can result in gas production and accumulation inside the gut, causing discomfort or conditions such as a bloat. This kind of change was not observed in the present study, as there was no significant change in abdominal girth (P>0.05) across the whole fluid therapy period under all treatments, corroborating the findings of RIBEIRO FILHO et al. (2013). The choice of energy precursor directly affects these findings, because calcium propionate, glycerol, and propylene glycol can be oxidized by the ruminal microbiota or absorbed in the integral form, not over-stimulating gas production.

There was no variation in fecal humidity (P>0.05) over time or between treatments. This result confirmed that the electrolyte solutions proposed were readily absorbed by the intestinal tract with minimal losses through feces. Osmolarity is an important characteristic for the efficacy of an electrolyte solution, as hypo or isotonic solutions are absorbed quickly and in large amounts, as suggested by AVANZA et al. (2009). Hypertonic enteral solutions (osmolarity>320mOsm/L) could have decreased volemic effects, remaining longer in the intestinal lumen, increasing the risk of osmotic diarrhea; however, this was not observed in the present study, even for the SEProp group with 378mOsm/L, which was well tolerated by the animals.

The concentrations of red blood cells and hemoglobin (Table 2) decreased significantly after 6 hours of fluid therapy (T6h) with all treatments, and remained low over 12 hours of fluid therapy (T12h) in the SEGly and SEProp groups, returning to basal levels at 24 hours (T24h). These results confirmed that the solutions were well absorbed by the gastrointestinal tract and that the osmolarity of SEProp did not limit its use. The expanse capacity of the solutions (hemodilution) is demonstrated by the decreased hematocrit concentration (P<0.05) observed following 3 hours of fluid therapy (T3h) in all groups. After being absorbed in the rumen and gut, the solutions dilute the solid components of the blood and proteins, explaining the results of this study, as described by RIBEIRO FILHO et al. (2011) and RIBEIRO FILHO et al. (2013). These findings showed that enteral fluid therapy

Table 2 - Mean ± standard deviation of erythrogram (red blood cells (RBC - 10^6 cells/µL), hemoglobin (Hg – g/dL), packed cell volume (PVC - %), mean corpuscular volume (MCV – fL), mean corpuscular hemoglobin (MCH – pg), mean corpuscular hemoglobin concentration (MCHC - %), Red Cell Distribution Width (RDW - %) and platelets (PLT - 10^3 cells/µL) of heifers under enteral fluid therapy in continuous flow with three different electrolyte solutions.

		----------Fluid therapy period----------					
	Groups	T0h	T3h	T6h	T9h	T12h	T24h
RBC	SEPCa	5.6±2.3^{a}	5.3±2.3^{ab}	5.1±2.1^{bc}	4.9±2.1^{c}	5.3±2.2^{abc}	5.1±1.9^{abc}
	SEGlyc	4.8±1.6^{b}	4.4±1.3^{c}	4.5±1.5^{c}	4.4±1.4^{bc}	4.5±1.7^{bc}	5.2±1.7^{a}
	SEProp	4.4±1.3^{a}	4.1±1.1^{b}	4.0±1.1^{b}	3.9±1.3^{b}	4.0±1.3^{b}	4.7±1.3^{a}
Hg	SEPCa	9.2±1.0^{a}	8.5±0.9^{abc}	8.2±0.5^{b}	8.0±0.4^{c}	8.4±0.7^{abc}	9.2±1.3^{abc}
	SEGlyc	9.1±1.2^{b}	8.6±1.5^{c}	8.5±1.3^{c}	8.3±1.0^{c}	8.5±1.^{2bc}	10.0±1.5^{a}
	SEProp	9.3±2.0^{b}	8.5±1.7^{c}	8.6±2.1^{c}	8.3±2.1^{c}	8.4±1.8^{c}	10.5±2.2^{abc}
PVC	SEPCa	32.3±5.2^{a}	27.3±4.5^{b}	26.2±5.2^{b}	25.5±3.4^{b}	26.3±2.6^{b}	26.2±3.0^{ab}
	SEGlyc	29.2±4.3^{a}	26.5±5.2^{b}	26.0±4.7^{b}	25.2±2.9^{b}	26.2±3.8^{b}	27.3±2.5^{ab}
	SEProp	29.0±3.5^{a}	26.2±1.9^{bc}	25.3±3.1^{c}	24.7±3.4^{c}	25.3±3.3^{c}	30.3±4.1^{ab}
MCV	SEPCa	43.4±5.4	43.7±5.3	43.4±5.6	43.2±5.3	43.2±5.4	46.0±5.4
	SEGlyc	44.8±7.1	44.7±7.2	44.6±6.7	44.6±6.7	44.5±7.1	45.4±7.1
	SEProp	43.0±2.1	42.6±2.4	42.9±2.1	43.0±2.2	42.6±2.0	43.3±1.8
MCH	SEPCa	19.9±11.1	19.7±11.0	19.3±10.4	19.7±10.7	19.1±10.0	20.6±9.2
	SEGlyc	20.7±7.3	21.1±7.2	21.0±7.7	20.8±7.4	20.8±7.3	20.7±7.0
	SEProp	23.0±9.9	23.1±9.9	23.4±10.0	23.6±10.3	23.2±10.4	24.2±9.5
MCHC	SEPCa	46.2±26.2	45.7±26.2	44.7±24.6	42.6±18.5	44.6±24.0	45.8±23.2
	SEGlyc	47.0±17.0	48.0±17.0	47.9±18.3	47.5±17.8	47.5±17.3	48.6±18.1
	SEProp	53.4±23.0	54.2±23.4	54.6±23.7	55.1±24.6	54.5±25.2	56.0±22.3
RDW	SEPCa	18.3±1.9	18.4±2.0	18.8±1.7	18.6±2.3	18.3±2.5	18.2±2.4
	SEGlyc	17.3±3.2	16.9±2.5	17.0±2.5	17.2±2.7	17.1±2.7	17.0±2.6
	SEProp	18.0±3.6	18.2±3.8	17.8±4.0	17.9±4.1	17.9±3.9	16.2±2.0
PLT	SEPCa	336.5±213.5	352.0±227.1	425.4±219.4	302.3±197.6	490.8±340.7	396.5±292.5
	SEGlyc	429.2±341.1	440.3±341.5	318.2±303.5	329.4±262.1	309.0±250.8	333.2±259.2
	SEProp	363.5±246.8	358.8±190.9	386.0±173.3	428.2±177.9	368.0±195.5	392.5±146.6

Means followed of lower case letters are different in the same line and capital letters are different in the same column (P<0.05).

as continuous flow is an effective alternative to intravenous fluid therapy for adult cattle. All other parameters of the erythrogram remained unchanged and within the reference intervals for this species.

There were no changes in the leucogram profile throughout the experimental period or between treatments (Table 3). This result clearly shows that intubation with a naso-ruminal tube for 12 hours, and all experimental management, had no significant excitatory or stressful effects on the animals, which would result in relative polycythemia following the release of adrenaline (THRALL et al., 2012).

CONCLUSION

Enteral fluid therapy given as a continuous flow via the naso-ruminal route is well-tolerated by animals with minimal effects on welfare, even when administered for 12 hours, and is indicated as an alternative route for parenteral maintenance fluid therapy. The three electrolyte solutions proposed here are able to significantly expand blood volume.

Table 3 - Mean ± standard deviation of leucogram (leucocytes (Leu - 10^3 cels./µL), lymphocytes (Lym - cels./µL), neutrophils (Neut - cels./µL), band neutrophils (BN - cels./µL), monocytes (Mon - cels./µL), eosinophils (Eos - cels./µL) and basophils (Bas - cels./µL)) of heifers under enteral fluid therapy in continuous flow with three different electrolyte solutions.

		Fluid therapy period					
	Group	T0h	T3h	T6h	T9h	T12h	T24h
Leu	SEPCa	9.9±4.1	11.0±4.6	12.4±4.4	12.6±5.4	9.1±3.1	10.0±3.6
	SEGlyc	10.3±4.8	10.8±5.6	11.8±6.0	12.8±4.8	10.4±2.1	10.0±3.4
	SEProp	9.9±0.3	10.5±1.8	11.4±2.1	11.5±10.3	10.9±2.7	9.8±1.9
Lym	SEPCa	6515±3201	7189±4254	8714±4100	8851±5335	6061±2726	6324±2382
	SEGlyc	6963±2900	6614±3070	8508±5206	8305±2853	7099±1841	6538±49
	SEProp	7155±439	6846±1802	7509±2195	7556±1087	7138±1853	6462±1920
Neut	SEPCa	3139±1077	3470±1239	3170±1455	3350±1887	2813±788	3149±1321
	SEGlyc	2987±1880	3837±2473	3958±2403	4015±1600	2872±434	3412±2088
	SEProp	2311±298	3340±197	3542±887	3454±1057	3224±1339	3055±673
BN	SEPCa	73±64	64±74	88±91	89±65	109±84	41±55
	SEGlyc	105±49	63±51	73±64	134±126	118±129	83±143
	SEProp	125±50	97±60	63±58	139±96	168±51	45±62
Mon	SEPCa	109±125.9	122±79	224±163	89±65	36±43	174±171
	SEGlyc	75±67	136±156	215±180	62±86	162±85	219±206
	SEProp	76±50	101±108	152±134	99±169	158±120	168±128
Eos	SEPCa	64±102	155±89	147±138	192±160	68±88	261±105
	SEGlyc	334±427	160±211	307±361	284±319	180±86	312±273
	SEProp	272±124	136±126	85±91	232±179	222±193	46±63
Bas	SEPCa	0.0±0.0	0.0±0.0	16±32	0.0±0.0	14±28	0.0±0.0
	SEGlyc	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	SEProp	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	15±33

Means followed of lower case letters are different in the same line and capital letters are different in the same column ($P<0.05$).

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[a]Sulfal Química Ltda., Belo Horizonte, MG, Brazil;
[b]Adicel Ltda., Belo Horizonte, MG, Brazil;
[c]Biobrotas Olequímica, Brotas, SP, Brazil;
[d]Enterequi System, Viçosa, MG, Brazil;
[e]Vacuteiner BD, Juiz de Fora, MG, Brazil;
[f]HumanCount Plus, Belo Horizonte, MG, Brazil.

BIOETHICS AND BIOSSECURUTY COMMITTE APPROVAL

The project was previously approved for the Ethical Committee in the Use of Animals of the Universidade Federal de Viçosa (CEUA/UFV) with the protocol number 44/2017.

CONFLICTS OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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