http://dx.doi.org/10.1590/1678-4162-12730

Arq. Bras. Med. Vet. Zootec., v.75, n.1, p.1-13, 2023

Effectiveness of oral electrolyte solutions with different compositions for the treatment of neonatal calves with induced osmotic diarrhea

[*Eficácia de soluções eletrolíticas orais com diferentes composições no tratamento de bezerros neonatos com diarreia osmótica induzida*]

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ABSTRACT

In a randomized controlled trial, we evaluated the effects of five oral electrolyte solutions (OESs) with different compositions of water, electrolyte, and acid-base balances of diarrheal neonatal calves. Osmotic diarrhea and dehydration were induced with sucrose in milk, spironolactone, and hydrochlorothiazide for 48 h in thirty 10-day old Holstein healthy calves with 43.5 ± 3.80 kg BW who were fed with natural milk. They were allocated to five treatment groups (n=6) based on the administered OES (commercial: OES A, B, C, D; and non-commercial: OES UEL). On the day of treatment, the calves received 6L of OES in two doses apart from milk intakes. Venous blood samples were collected at -48h (start of induction), -24h, 0h (start of the treatment day), 8h, 16h, 24h, 48h, and 72h. TPP, glucose, D-lactate, L-lactate, pH, pCO_2, HCO_3^-, BE, Na^+, K^+, Cl^-, SID_3, SIG, AG, A_{tot} and percentage change in plasma volume (%PV) were measured or calculated and analyzed by two-way repeated measures ANOVA. All the calves developed osmotic diarrhea, mild to moderate dehydration, hyponatremia, relative hyperchloremia, and moderate to severe metabolic acidosis. The tested OESs were well accepted by the calves and effective in reversing dehydration, electrolyte imbalances, and metabolic acidosis. OES D did not completely correct hyponatremia, and SEO B caused transient hyperglycemia. It has been concluded that all the tested OESs are safe and effective for the treatment of diarrhea in calves with moderate degrees of dehydration and metabolic acidosis, which indicates that they have proper compositions for this purpose.

Keywords: dehydration, fluid therapy, hyponatremia, neonatal calf diarrhea, strong ion acidosis

RESUMO

Em um ensaio clínico controlado e aleatório, foram comparados os efeitos de cinco soluções eletrolíticas orais (SEOs) sobre os equilíbrios hídrico, eletrolítico e ácido-base de bezerros neonatos diarreicos. Diarreia osmótica e desidratação foram induzidas com sacarose no leite, espironolactona e hidroclorotiazida, por 48h, em 30 bezerros Holandeses com 10 dias de idade, 43,5 ± 3,8kg de peso, e alimentados com leite natural. Eles foram distribuídos em cinco grupos de tratamento (n=6) de acordo com a SEO administrada (comercial: SEO A, B, C, D; e não comercial: SEO UEL). No dia do tratamento, os bezerros receberam 6L de SEO em duas doses além da ingestão de leite. Amostras de sangue venoso foram coletadas em -48h (início da indução), -24h, 0h (início do dia de tratamento), 8h, 16h, 24h, 48h e 72h. PPT, glicose, lactatos L e D, pH, pCO_2, HCO_3^-, BE, Na^+, K^+, Cl^-, SID_3, SIG, AG, A_{tot} e variação percentual no volume plasmático (%VP) foram mensurados ou calculados, e analisados por meio de ANOVA de duas vias de medidas repetidas. Todos os bezerros desenvolveram diarreia osmótica, desidratação leve a moderada, hiponatremia, hipercloremia relativa e acidose metabólica moderada a grave. As SEOs testadas foram bem aceitas pelos bezerros e eficazes para a reversão da desidratação, dos desequilíbrios eletrolíticos e da acidose metabólica. A SEO D não corrigiu completamente a hiponatremia, e a SEO B causou hiperglicemia transitória. Conclui-se que todas as SEOs são eficazes para o tratamento da diarreia em bezerros com graus moderados de desidratação e de acidose metabólica, o que indica que possuem composições adequadas para esse fim.

Palavras-chave: acidose por íons fortes, desidratação, diarreia neonatal em bezerros, hiponatremia, terapia com fluidos

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Submitted: March 28, 2022. Accepted: November 7, 2022.

INTRODUCTION

The main cause of mortality in calves during the first weeks of life is neonatal diarrhea, which accounts for the economic losses in dairy herds in different countries (Smith 2012; Uetake, 2013; Reiten *et al*., 2018). In addition to dehydration, the most frequent imbalances observed in diarrheal calves are hyponatremia, normo- or hyperchloremia, potassium depletion accompanied by normo- or hyperkalemia, hyper-L-lactatemia, hyper-D-lactatemia, hypoglycemia, and metabolic acidosis (Sayers *et al*., 2016; Trefz *et al*., 2017; Gomez *et al*., 2017).

Oral electrolyte solutions (OESs) should be the first option for the treatment of diarrheal calves (Smith and Berchtold, 2014; Doré *et al*., 2019). The OESs indicated for the treatment of dehydrated and acidotic diarrheal calves should provide enough sodium to reverse hyponatremia, adequate potassium to correct depletion in the body, agents that facilitate the intestinal absorption of sodium and water (glucose, acetate, propionate, or glycine), an alkalizing agent (bicarbonate, acetate, or citrate) to facilitate the correction of metabolic acidosis, and an energy source to supply the calf's demand (Smith and Berchtold, 2014; Constable *et al*., 2021).

In North American and European countries, the use of OESs is well consolidated, and various products are available on the market (Smith and Berchtold, 2014). In Brazil, few formulations are commercially available (Bregadioli *et al*., 2017). In addition, there is little scientific information regarding its effects. Studies carried out with some of these OESs evaluated their effects in isolation, administering them to healthy (Bachmann *et al*., 2009; Constable *et al*., 2009) or diarrheal calves (Miqueo *et al*., 2018). A single study compared the effects of these OESs in healthy calves (Bregadioli *et al*., 2018). It was important to know if the same effects would occur in diarrheal calves and whether OESs would reverse the imbalances. The authors are unaware of previous results of controlled studies that had proven the therapeutic efficacy of three of the four commercial OESs included in the present study (OES B, C, and D).

The OESs tested in the present study have different compositions, and the accepted hypothesis is that this promotes different capabilities related to the reversal of the imbalances caused by diarrhea. The purpose of this study was to compare the effectiveness of four commercial OESs and one non-commercial OES, with different compositions, in correcting the water, electrolyte, and acid-base imbalances of neonatal calves with induced osmotic diarrhea.

MATERIALS AND METHODS

The present study was approved by the Ethics Committee on the Use of Animals of the Universidade Estadual de Londrina (CEUA/UEL protocol n. 9847.2017.10). Thirty 10-days old healthy male Holstein calves with body weight (BW) of 43.5±3.8kg were used in this randomized controlled trial. The calves were housed in individual pens lined with shavings. During the adaptation period that lasted 7 or 8 days (from 2- or 3-days old), they were fed with natural whole milk in a volume corresponding to 12% of the BW divided into two daily feedings through a bottle. They had free access to water, and after 5 days of life, they received commercial pre-starter ration and coast-cross grass hay (*Cynodon dactylon*) at will.

Osmotic diarrhea and dehydration were induced in all the calves using a standardized protocol (Leal *et al*., 2008, 2012). The protocol lasted for 48h with oral administration every 8h. It was based on the ingestion of 16.5mL/kg BW of natural whole milk plus 4g/kg BW of sucrose diluted to 20% in warm water and two types of diuretics: 2mg/kg BW of spironolactone (Espironolactona 50 mg; Eurofarma Laboratórios SA, Itapevi, SP, Brazil) and 2mg/kg BW of hydrochlorothiazide (Hidroclorotiazida 50 mg; EMS SA, Hortolândia, SP, Brazil). During the 48h of the diarrhea induction period, the water was kept free in the daytime and withdrawn during the 12h of the night.

The calves were randomly assigned to five treatment groups (n=6) according to the OES administered. The tested OESs were prepared using four commercial products currently available on the Brazilian market and indicated for the oral hydration of neonatal calves: OES A - Glutellac® (Bayer Saúde Animal Ltda; São Paulo, SP, Brazil), OES B - Nurture Lyt® (Nutron Alimentos Ltda., Itapira, SP, Brazil), OES C - Rehydion® Gel (Ceva Saúde Animal

Ltda., Paulínia, SP, Brazil), and OES D - Hydrafeed® (Hypred S.A., Dinard, France), in addition to a non-commercial electrolyte solution (OES UEL). The composition and osmolarity of each OES are presented in Table 1. All solutions were prepared immediately before being administered using warm water (38°C) according to the manufacturer's instructions. The OES UEL was prepared by dissolving 7g of NaCl, 3g of KCl, 10g of sodium acetate trihydrate, and 40g of anhydrous D-glucose in 2L of water.

Table 1. Composition of oral electrolyte solutions (OESs) used in the study

	OES A[1]	OES B[2]	OES C[3]	OES D[4]	OES UEL
Na^+ (mEq/L)	100.1	124.6	120.0	95.0	100.0
K^+ (mEq/L)	18.5	27.7	22.6	8.0	20.6
Cl^- (mEq/L)	60.7	96.8	82.0	65.0	86.0
HCO_3^- (mEq/L)	-	55.5	-	38.0	-
$acetate^-$ (mEq/L)	58.0	-	60.0	-	35.0
glucose (mmol/L)	46.0	109.0	23.3	0[a]	100.0
$SID_{effective}$ (mEq/L)[5]	58.0	55.5	60.6	38.0	34.6
osmolarity (mOsm/L)	283.0	402.7	305.6	335	326
Na^+:glucose	2.1	1.1	5.1	-	1.0
Na^+:Cl^-	1.6	1.3	1.4	1.4	1.1
pH	6.232	7.952	6.606	6.415	7.502

[1] Glutellac®; [2] Nurture Lyt®; [3] Rehydion Gel®; [4] Hydrafeed®;
[5] effective strong ion difference ($SID_{effective} = [Na^+] + [K^+] - [Cl^-]$);
[a] contains lactose.

The calves were treated with OES in a single day, and the volume administered totaled 6L. The OESs were offered in a bottle, and administrations through an esophageal tube were only performed to complete the volume of 3L when the voluntary intake was not adequate. On the day of treatment, they continued to receive natural whole milk (16.5mL/kg BW) plus sucrose (4g/kg BW, diluted to 20% in warm water) every 8h; however, the administration of diuretics had been suspended. The sucrose intake was intended to ensure that the diarrhea persisted in the animals throughout the treatment day. The following schedule was followed on the day of treatment, establishing a four-hour interval between the milk and OES administrations: intake of milk mixed with sucrose solution (36.5mL/kg BW) at 8:00am, 16:00pm, and 0:00am; and intake of 3L of OES (approximately 70mL/kg BW) at 12:00pm and 20:00pm. A total volume of approximately 250mL/kg BW of fluids was ingested on the day of treatment. From the day after the treatment, the sucrose intake was suspended, and the calves began to receive natural whole milk in a volume corresponding to 12% of the BW divided into three daily meals.

Physical examinations were performed every 8 hours by a single trained individual, who did not know which treatment group each calf belonged to. The color and humidity of the mucous membranes, hydration status, degree of enophthalmia, skin turgor, capillary refill time, appetite, stool characteristics, posture, and behavior were evaluated. The scoring systems proposed by Walker *et al*. (1998a) and Smith (2009) with some modifications, were used to assess fecal consistency, degree of dehydration, and behavior, posture, and sucking reflex (Tab. 2). Adding the defined scores, the general disease score was determined on a scale from 0 to 10, where 0 represented health and 10 represented the highest disease score.

Weighing was performed immediately before meals at -48h (before the start of the diarrhea induction protocol), -24h, 0h (start of treatment), 24h (day after treatment), 48h, and 72h. The volume of water voluntarily ingested was measured at the beginning of each day (-24h, 0h, 24h, 48h, and 72h).

Blood samples were obtained eight times: -48h, -24h, 0h, 8h, 16h, 24h, 48h, and 72h, always before meals. The jugular vein was catheterized (Catheter 18G) and the catheter was sealed with a closing Luer connector throughout the experimental period. Blood samples were collected directly from the catheter using a

hypodermic needle coupled with a plastic syringe, and they were packed in vacuum flasks containing EDTA anticoagulant with and without sodium fluoride. To obtain fluoridated plasma, centrifugation (1,500 × *g* for 10min) was performed, and the sample was kept frozen (-20°C) until the time of analysis. For blood gas analysis, the blood samples were collected using heparinized plastic syringes coupled with 21G hypodermic needles. The analyses were performed immediately after collection.

Table 2. Disease score based on fecal consistency, hydration status, and behavior, posture, and sucking reflex

Score	Criteria
	Fecal consistency
0	Firm: well-formed
1	Semi-pasty: tending to pasty, but still retain their shape
2	Pasty: mild diarrhea, without definite form and with solid components
3	Semi-liquid: moderate diarrhea, with few solid components
4	Liquid: severe diarrhea, with virtually no solid components
	Dehydration degree
0	Absent: moist mucous membranes, skin turgor for up to 1 sec and absent enophthalmos
1	Mild (5 to 8%): moist mucous membranes, skin turgor 1-2 sec and enophthalmos 2-4 mm
2	Moderate (8 to 10%): sticky mucous membranes, skin turgor 2-5 sec and enophthalmos 4-6 mm
3	Severe (10 to 12%): dry mucous membranes, skin turgor 5-10 sec and enophthalmos 6-8 mm
	Behavior, posture, and sucking reflex
0	Alert, standing position and vigorous sucking
1	Lethargic, standing position, sucking present but not vigorous
2	Depressed, preferential or permanent sternal recumbency, slow and disorganized sucking
3	Comatose, permanent lateral recumbency, absent sucking

The concentration of the total plasma proteins (TPP) was measured in non-fluoridated plasma using the refractometry method after centrifugation in a microhematocrit centrifuge. Blood measurements of pH, pCO_2, HCO_3^-, BE, Na^+, K^+, Cl^-, L-lactate, and glucose were performed using a blood gas analyzer (RAPIDpoint 500 System, Siemens Healthcare Diagnostics Inc., Deerfield, Illinois, USA). D-lactate was measured in the fluoridated plasma by a colorimetric method (D-Lactate Colorimetric Assay Kit; BioVision Inc., Milpitas, California, USA) with readings in a plate reader (iMark; Bio-Rad Laboratories, Inc., Tokyo, Japan).

The following variables were calculated using the respective formulas:

a) Anion gap (AG): $AG = (Na^+ + K^+) – (Cl^- + HCO_3^-)$

b) Strong ion difference (SID): $SID_3 = (Na^+ + K^+) – (Cl^-)$

c) Total plasma concentration of non-volatile weak acids (A_{tot}): A_{tot} = TPP (g/dL) × 3.43 (Constable *et al*., 2005)

d) Strong ion gap (SIG): $SIG = [A_{tot}/(1+10^{(7,08 – pH)})] – AG$ (Constable *et al*., 2005)

e) Percentage change in plasma volume (%PV): $\%PV = [(TPP_1/TPP_2) – 1] \times 100$

where TPP_1 is the TPP value observed before induction and TPP_2 is the TPP values of the subsequent moments (Carlson e Bruss, 2008).

SigmaPlot for Windows 13.0 (Systat Software Inc., San Jose, California, USA) was used to perform the statistical analysis. The Shapiro-Wilk and Brown-Forsythe tests were used to verify the Gaussian distribution and equality of variance, respectively. The disease scores were analyzed at the same time as the blood or plasma variables (-48h, -24h, 0h, 8h, 16h, 24h, 48h, and 72h). Two-way repeated measures ANOVA was used to test the effect of the time factor (different times before and after OES administration), the effect of the treatment factor (different OESs), and the interaction between these two factors. When the F statistic was significant, the Tukey's test was used to compare the means. Statistical significance was set at $P<0.05$.

RESULTS

The induction protocol used was effective in causing osmotic diarrhea and dehydration. All the calves had liquid feces from -24h until 16h. The feces maintained a light-yellow color and a slightly foul odor. Dehydration ranged from mild (12/30) to moderate (18/30) at 0h. Most of the calves (22/30) remained alert in a standing position, with a vigorous sucking reflex until 0h. Six calves were lethargic, of which four presented non-vigorous sucking, and two calves were depressed with preferential sternal recumbency and slow sucking. The disease score gradually increased until 0h, and the percentage loss in BW varied between 5.7% and 9.1%, with an average of 7.06% (Table 3 and Fig. 1). There were no differences between the groups (P>0.05).

Table 3. Mean and standard deviation (mean ± SD) of body weight (kg), difference in the body weight (kg), water intake (L), and disease score measured over time in calves with induced osmotic diarrhea treated with different oral electrolyte solutions (OESs). Diarrhea induction period: -48h to 0h; treatments with 3L of OES: 4h and 12h

OES	-48h	-24h	0h	8h	16h	24h	48h	72h
				Body weight (kg)				
A	43.18 ± 4.32^{Aa}	40.25 ± 4.63^{Ab}	39.22 ± 4.53^{Ab}	-	-	42.43 ± 4.45^{Aa}	42.23 ± 5.03^{Aa}	42.13 ± 4.95^{Aa}
B	44.37 ± 2.75^{Aa}	41.52 ± 1.84^{Ab}	41.35± 2.54^{Ab}	-	-	43.67 ± 2.46^{Aa}	43.50 ± 2.72^{Aa}	43.77 ± 2.42^{Aa}
C	43.03 ± 2.51^{Aa}	40.53 ± 2.24^{Ab}	40.05 ± 1.57^{Ab}	-	-	42.50 ± 2.65^{Aa}	42.27 ± 3.21^{Aa}	42.83 ± 2.96^{Aa}
D	43.37 ± 6.78^{Aab}	41.72 ± 6.27^{Abc}	40.38 ± 6.13^{Ac}	-	-	41.92 ± 4.85^{Abc}	42.92 ± 4.96^{Aab}	43.60 ± 5.63^{Aa}
UEL	41.65 ± 1.94^{Aa}	39.27 ± 1.97^{Ab}	39.25 ± 2.55^{Ab}	-	-	40.77 ± 2.47^{Aab}	40.78 ± 2.48^{Aab}	41.97 ± 2.08^{Aa}
				Difference in the body weight (kg)				
A	0^{Aa}	-2.93 ± 0.76^{Ab}	-3.97 ± 1.73^{Ab}	-	-	-0.75 ± 0.51^{Aa}	-0.95 ± 1.39^{Aa}	-1.05 ± 1.25^{Aa}
B	0^{Aa}	-2.85 ± 1.35^{Ab}	-3.02 ± 1.28^{Ab}	-	-	-0.70 ± 1.12^{Aa}	-0.87 ± 0.96^{Aa}	-0.60 ± 1.36^{Aa}
C	0^{Aa}	-2.50 ± 1.12^{Ab}	-2.98 ± 1.13^{Ab}	-	-	-0.53 ± 0.67^{Aa}	-0.77 ± 0.71^{Aa}	-0.20 ± 0.73^{Aa}
D	0^{Aab}	-1.65 ± 1.08^{Abc}	-2.98 ± 1.17^{Ac}	-	-	-1.45 ± 3.46^{Aabc}	-0.45 ± 1.86^{Aab}	0.23 ± 1.21^{Aa}
UEL	0^{Aa}	-2.38 ± 1.19^{Ab}	-2.40 ± 2.06^{Ab}	-	-	-0.88 ± 1.75^{Aab}	-0.87 ± 1.73^{Aab}	0.32 ± 1.21^{Aa}
				Water intake (L)				
A	-	3.19 ± 2.08^{Aab}	4.56 ± 0.98^{Aa}	-	-	1.90 ± 1.94^{Ab}	1.74 ± 1.90^{Ab}	2.10 ± 2.52^{Ab}
B	-	3.77 ± 1.20^{Aab}	4.97± 1.25^{Aa}	-	-	3.19 ± 1.15^{Ab}	1.37 ± 0.78^{Ac}	1.33 ± 1.47^{Ac}
C	-	3.10 ± 1.36^{Aab}	3.67 ± 1.43^{Aa}	-	-	2.56 ± 1.30^{Aabc}	1.05 ± 0.85^{Ac}	1.75 ± 1.76^{Abc}
D	-	3.40 ± 1.17^{Aa}	4.19 ± 0.92^{Aa}	-	-	2.53 ± 4.46^{Aab}	0.87 ± 0.89^{Ab}	0.91 ± 0.88^{Ab}
UEL	-	3.46 ± 1.16^{Aab}	4.02 ± 0.70^{Aa}	-	-	2.16 ± 1.09^{Abc}	1.30 ± 1.18^{Ac}	0.97 ± 1.05^{Ac}
				Disease score (0 – 10)				
A	2.50 ± 0.84^{Ac}	4.83 ± 0.75^{Ab}	6.17 ± 0.98^{Aa}	5.00 ± 0.89^{Aab}	4.33 ± 0.52^{Ab}	4.00 ± 0.00^{Ab}	2.50 ± 0.55^{Ac}	2.17 ± 0.75^{Ac}
B	2.50 ± 0.84^{Ac}	4.50 ± 0.84^{Ab}	5.83 ± 0.75^{Aa}	4.83 ± 0.98^{Aab}	4.50 ± 0.55^{Ab}	4.33 ± 0.52^{Ab}	3.17 ± 0.75^{Ac}	2.67 ± 0.52^{Ac}
C	3.00 ± 0.63^{Ac}	5.17 ± 0.41^{Aab}	6.33 ± 0.82^{Aa}	5.17 ± 1.17^{Aab}	4.83 ± 1.17^{Ab}	3.67 ± 0.82^{Ab}	2.83 ± 0.75^{Ac}	2.17 ± 0.75^{Ac}
D	2.50 ± 0.55^{Ac}	4.50 ± 0.55^{Aab}	5.67 ± 0.82^{Aa}	4.67 ± 1.63^{Aab}	4.67 ± 1.63^{Aab}	4.17 ± 0.98^{Ab}	3.50 ± 1.05^{Abc}	2.50 ± 0.55^{Ac}
UEL	2.50 ± 0.84^{Ac}	5.00 ± 0.00^{Aab}	5.83 ± 0.75^{Aa}	5.17 ± 1.17^{Aab}	4.33 ± 0.52^{Ab}	3.83 ± 0.98^{Abc}	3.33 ± 1.97^{Ac}	3.00 ± 2.10^{Ac}

$^{A, B, C}$ Comparison between the OESs at each time point (P<0.05). $^{a, b, c, d}$ Comparison between the time points in each OES (P<0.05).

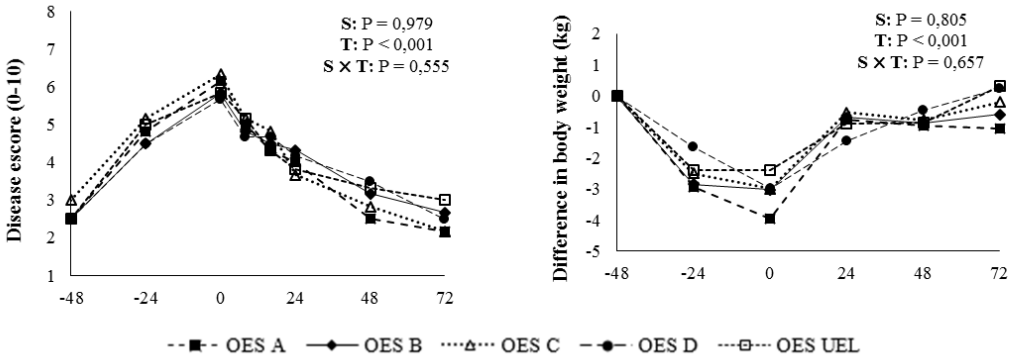

Figure 1. Variations (mean values) in disease score and difference in the body weight of neonatal calves with induced osmotic diarrhea treated with different oral electrolyte solutions (OESs). Diarrhea induction period: -48h to 0h; treatments with 3L of OES: 4h and 12h. Effects of the electrolyte solution (S) and time (T) factors and the interaction between them (S × T).

The induced changes were characterized by a continuous decrease in pH, HCO_3^-, BE, Na^+, SID_3 (Fig. 2), %PV, and SIG (Fig. 3); a continuous increase in TPP, A_{tot}, and AG (Fig. 3); and the maintenance of the values of Cl^-, glucose and L-lactate (Fig.2 and 3) until 0h. These variations did not differ between the groups (P>0.05). The pCO_2 values also did not differ between the groups, and the decrease lasted for up to 8h (Fig. 2). The K^+ values did not change until 0h, and, at this time, they were higher in the OES A group and lower in the OES C group (Fig. 2). The D-lactate increased at 0h, but this was not significant in the OES C and OES D groups (Fig. 3).

In general, the treatments caused similar effects in the groups. The disease score of the calves was restored within 48h, and the calves regained their original BW at 24h (Fig. 1 and Table 3). The feces of most calves (20/30) remained liquid until 24h and normalized up to 72h. The correction of dehydration occurred in a considerable number of calves (14/30) at 8h, and almost all the calves (28/30) were well hydrated at 24h. At 48h, all the calves showed restored physiological behavior, posture, and milk intake.

The treatments promoted the correction of pH, HCO_3^-, and BE from 8h, and the correction of pCO_2 from 16h. In the OES UEL group, the restoration of HCO_3^- and BE to baseline values were late (48h) (Fig. 2). The restoration of the Na^+ concentration was gradual for up to 72h and did not occur in the OES D group, despite the increase. The K^+ concentration temporarily decreased in the OES A (24h and 48h) and OES D (16h and 24h) groups. The Cl^- levels decreased after the first OES administration (8h) and returned to baseline values after 16h or 24h, except in the OES D group, where it remained low until 72h despite the gradual increase. The correction of SID_3 occurred after 8h, being later in the OES UEL group (72h) (Fig. 2). The corrections for %PV, TPP, A_{tot}, AG, and SIG occurred after 8h (Fig. 3). In the OES A, OES B, and OES UEL groups, D-lactate returned to baseline values after 48h. The glucose levels varied over time only in the OES B group with higher values than the baseline values at 8h and 16h (Fig. 3).

Few differences were observed between the groups during and after the treatment. The K^+ values were higher in the OES B group than in the OES D group at 8h and 16h, and higher than in the OES A, OES C, and OES D groups at 24h. The Cl^- values remained higher in the OES UEL group than in the OES D group between 16h and 48h (Fig. 2). L-lactate was higher in the OES B group than in the OES C group at 8h, and glucose was higher in the OES B group at 16h (Fig. 3).

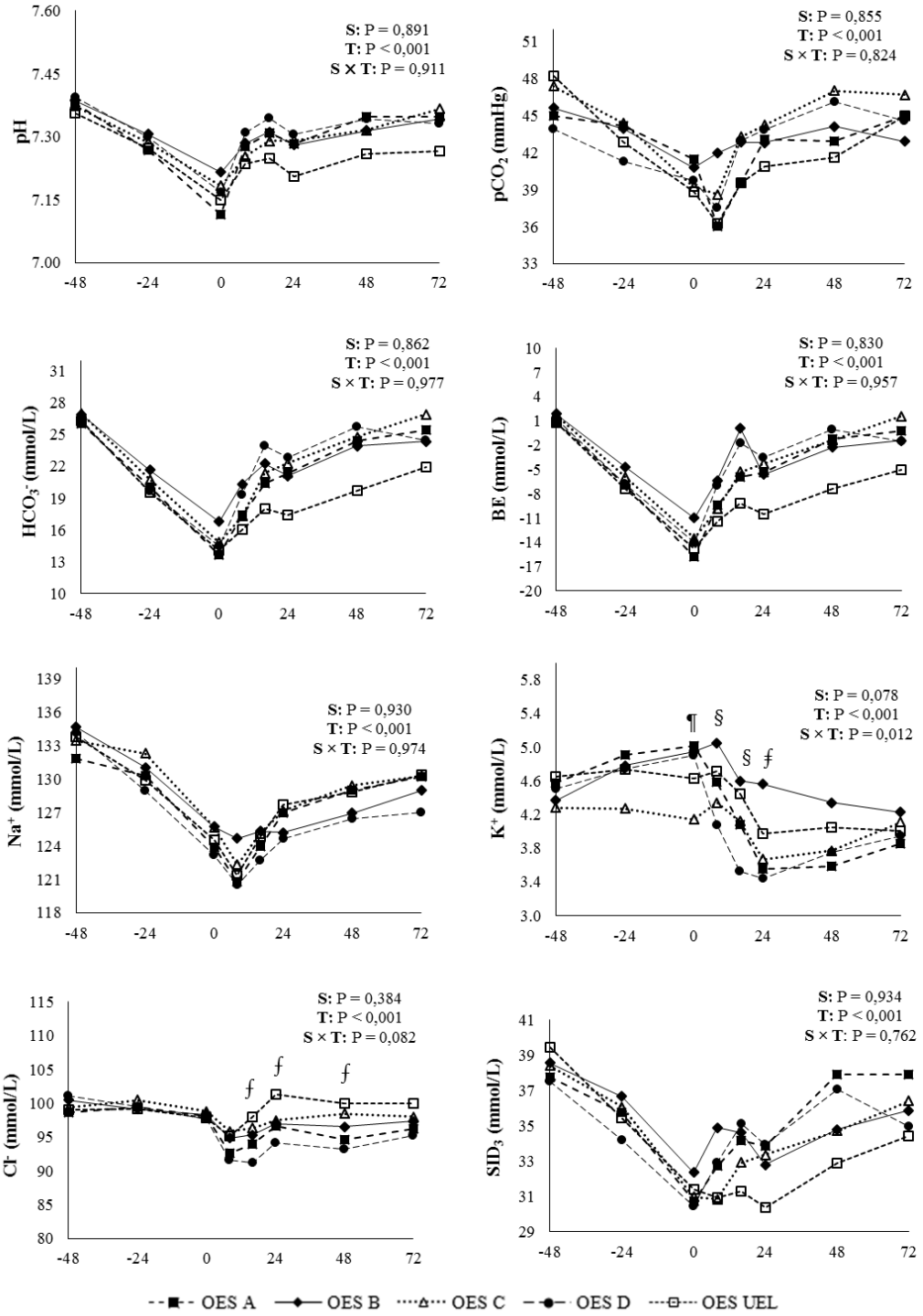

Figure 2. Variations (mean values) in pH, pCO_2, HCO_3^-, BE, Na^+, K^+, Cl^-, and SID_3 in venous blood of neonatal calves with induced osmotic diarrhea treated with different oral electrolyte solutions (OESs). Diarrhea induction period: -48h to 0h; treatments with 3L of OES: 4h and 12h. Effects of the electrolyte solution (S) and time (T) factors and the interaction between them (S × T). ¶ OES A differs from OES C at 0h. § OES B differs from OES D at 8h and 16h. ƒ OES B differs from OES A, C, and D at 24h. ƒ OES D differs from OES UEL at 16h, 24h, and 48h.

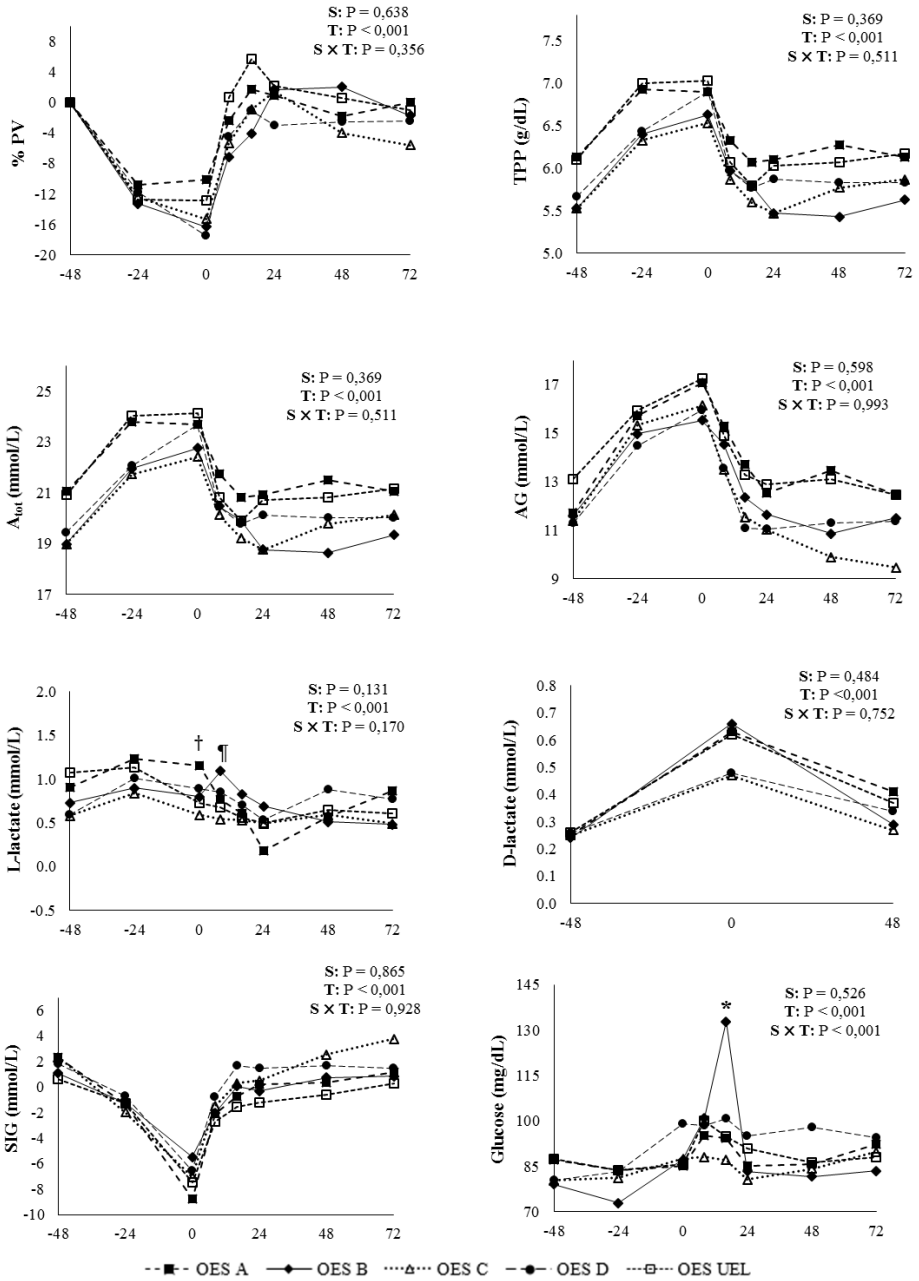

Figure 3. Variations (mean values) in the percentage change in plasma volume (%PV), TPP, A_{tot}, AG, L-lactate, D-lactate, SIG, and glucose in the plasma of neonatal calves with induced osmotic diarrhea treated with different oral electrolyte solutions (OESs). Diarrhea induction period: -48h to 0h; treatments with 3L of OES: 4h and 12h. Effects of the electrolyte solution (S) and time (T) factors and the interaction between them (S × T). † OES A differs from OES C at 0h. ¶ OES B differs from OES C at 8h. * OES B differs from the others at 16h.

The daily voluntary water intake varied with time (P<0.001), but it was not influenced by the type of OES administered (P=0.788). High volumes were ingested during the days of induction (-24h and 0h), and there was a drop after 24h, mainly in the OES B, OES C, and OES UEL groups (Table 3).

DISCUSSION

The protocol for inducing osmotic diarrhea was effective in causing water, electrolyte, and acid-base imbalances consistent with those found in natural cases of diarrhea (Trefz *et al*., 2015, 2017; Gomez *et al*., 2017). The calves presented with profuse diarrhea, mild to moderate dehydration, hyponatremia, and relative hyperchloremia, judging by the low SID_3 values, and strong ion (metabolic) acidosis, with acidemia and compensatory hypocapnia. Metabolic acidosis ranged from moderate to severe, with an average drop of 8.20 to 12.25mmol/L in the BE values. Although there was a decrease in the SIG values and an increase in the AG values, there was no hyper-L- or -D-lactatemia. Despite the severity of the induced metabolic acidosis, few calves exhibited depression. This is consistent with the observation that hyperchloremic acidosis does not cause behavioral changes in calves (Gentile *et al*., 2008), while metabolic acidosis due to the accumulation of organic acids, especially D-lactate, is strongly accompanied by signs of depression (Lorenz, 2009; Lorenz *et al*., 2005; Trefz *et al*., 2012; Lorenz e Gentile, 2014).

The decrease in SIG and the increase in AG indicate that metabolic acidosis was not exclusively hyperchloremic, but it was partly due to the accumulation of organic acids (Ewaschuk *et al*., 2003; Constable, 2014; Trefz *et al*., 2015). In natural cases of calf diarrhea, the accumulation of D-lactate has been identified as the main cause of metabolic acidosis (Lorenz, 2009; Lorenz *et al*., 2005; Lorenz e Gentile, 2014). Since hyper-L- and -D-lactatemia were not present in the studied calves, it should be admitted that other unmeasured strong anions may have accumulated in addition to the relative hyperchloremia and contributed to the magnitude of metabolic acidosis.

The percentage reduction in plasma volume varied from 10% to 17% in the different groups, with an average of 14%. The decreases in plasma volume observed in previous studies that induced osmotic diarrhea and dehydration with sucrose and diuretics were more pronounced, ranging from 20% to 26% (Constable *et al*., 1996; Walker *et al*., 1998a, 98b; Leal *et al*., 2008, 2012; Kirchner *et al*., 2014; Doré *et al*., 2019). This difference can be explained by the fact that the calves in the present study were not completely deprived of water. Voluntary water intake partially reversed the water imbalance.

The treatments with the studied OESs gradually restored the health status until the end of the experimental period. They reversed dehydration, hyponatremia, relative hyperchloremia, and metabolic acidosis within a short period, and these effects were, in general, similar with only a few specific distinctions between the groups. This proves that all the tested OES were effective in correcting imbalances, which indicates that they have an adequate composition for the treatment of diarrheal calves. Previous studies with the tested OES evaluated their effects in isolation: OES A in healthy calves (Bachmann *et al*., 2009) and diarrheal calves (Miqueo *et al*., 2018) and OES C in healthy calves (Constable *et al*., 2009). In addition, these OESs were prepared by dilution in milk (Constable *et al*., 2009) or in milk replacer (Miqueo *et al*., 2018), which limited the comparison with the results of the present study. The only previous investigation that compared the effects of OES A, B, C, and UEL used healthy calves and found that these solutions had similar effects on the electrolyte and acid-base balance (Bregadioli *et al*., 2018).

The compositions of the OESs used in the present study fit what is considered suitable for the treatment of neonatal calves with diarrhea (Constable *et al*., 2001, 2021; Smith, 2009). The rapid correction of dehydration, confirmed by the restoration of %PV, TPP, and A_{tot} to baseline values 4h after the first intake of OES, indicates that all the tested OESs were effectively absorbed. The hemodilution intensified throughout the treatment day because of the sum of the effects of the second OES ingestion. Although osmotic diarrhea was maintained throughout the day of treatment, the calves recovered their lost weight and were clinically hydrated on the following day (24h). These results contrast previous evidence that the expansion of plasma volume did not occur when OES C was ingested by healthy calves (Constable *et al*., 2009; Bregadioli *et al*., 2018), which demonstrates that the verification of the effects in unbalanced calves provides more reliable results on the properties of the OESs.

Regarding the compositions of the studied OESs, it can be highlighted that the Na^+ concentrations

were similar, the K^+ concentrations were differentiated by the low value in the OES D, and the Cl^- concentrations differed in them, with a higher value in OES B and lower values in OESs A and D (Table 1). The alkalizing agent differed in them − bicarbonate$^-$ in OESs B and D, and acetate$^-$ in OESs A, C, and UEL − as well as the $SID_{effective}$, which reflects the relationship between the electrolytes present, notably the relationship between Na^+ and Cl^-, and, therefore, resembles the concentration of the alkalizing agent. OESs A, B, and C had high $SID_{efective}$, while OESs D and UEL had low $SID_{efective}$, with values closer to those of calf plasma SID_3 (Constable *et al*., 2005). Based on the $SID_{efective}$ values, it was logical to assume that OESs A, B, and C would have higher alkalizing effects than OESs D and UEL when administered in similar volumes (Constable *et al*., 2005, 2021; Constable, 2014). Previous studies comparing OES administered to calves have shown that the OES with a higher $SID_{efective}$ has a higher alkalizing capacity (Constable *et al*., 2005, 2009; Bachmann *et al*., 2009, 2012; Stämpfli *et al*., 2012; Sayers *et al*., 2016) and are therefore recommended for the treatment of diarrheal calves. This was partially observed in the studied calves. The alkalinization promoted by OES UEL was, in fact, later; however, the alkalizing effects of OES D were comparable to those of OESs A, B, and C (Fig. 2).

The effects of OESs on plasma electrolytes were characterized by a gradual increase in the Na^+ concentrations, which corrected hyponatremia, and a decrease in the Cl^- concentrations, which reversed relative hyperchloremia and, consequently, elevated the plasma SID_3. These changes explain the correction of metabolic acidosis, notably hyperchloremic acidosis, confirming that the restoration to electrolyte balance promotes the correction of the acid-base balance (Constable *et al*., 2005; Constable, 2014). The later alkalizing effect of OES UEL can be explained by the higher concentration of Cl^- relative to Na^+ in its composition, which determines a lower $SID_{effective}$ value and less impact on plasma Cl^- and SID_3 (Fig. 2). Unlike the other studied OESs, OES D did not reverse hyponatremia in calves at the end of the experimental observation period, and chloremia remained low. This probably occurred because OES D has reduced concentrations of Na^+ and Cl^-, and these are not sufficient to replace Na^+ losses at the same speed as the other tested OESs. The effects of the OESs on plasma K^+ were, in general, insignificant, and transient: hypokalemia was caused by OES D, which has a very low concentration of K^+; slightly higher plasma values were associated with the intake of OES B, which has a higher concentration of K^+ than the others, on the day of treatment and the following. Compared with the studied calves, the effects of OESs A, B, C, and UEL on plasma electrolytes were less pronounced in the healthy calves (Bregadioli *et al*., 2018).

The low SIG and high AG values were promptly corrected with the administration of OESs, which could be attributed to the correction of hemoconcentration. Just as plasma D-lactate levels returned to baseline values 48h after the start of treatment, other unmeasured anions that accumulated during the development of dehydration decreased again. The effect should not be attributed directly to the composition of the OES, but to hemodilution, the better distribution among the fluid compartments of the body, and the optimized urinary excretion resulting from the restoration of the physiological hemodynamic condition. The reduction of D-lactate is proof that glomerular filtration has been restored, since the metabolism of this isomer is less efficient and renal elimination is a mandatory condition (Ewaschuk *et al*., 2004; Lorenz and Vogt, 2006; Lorenz and Gentile, 2014). Once the metabolic acidosis and dehydration are corrected, renal perfusion improves, increasing the volume of urine produced, and the tubular reabsorption of D-lactate decreases, reducing its concentration in plasma (Ewaschuk *et al*., 2004; Lorenz d Vogt, 2006; Trefz *et al*., 2012). It can be assumed that the same occurs with other accumulated organic acids. Its reduction in plasma is added to the correction of hyperchloremic acidosis, which determines the magnitude of the alkalizing effect and guarantees the successful correction of metabolic acidosis. Similar effects on SIG and AG have been observed by other authors in diarrheal calves treated with OESs (Constable *et al*., 2005; Stämpfli *et al*., 2012; Sayers *et al*., 2016).

Regarding the plasma levels of glucose, OES B had the highest concentration of glucose in its composition (109mmol/L), which determined the occurrence of hyperglycemia in the calves in the

present study after ingesting it, differentiating it from the other OESs. When tested in healthy calves, OES B caused lasting hyperglycemia (Bregadioli *et al*., 2018). The high concentration of glucose accounts for the higher osmolarity than the other OESs (Bregadioli *et al*., 2017). The studied calves did not present with hypoglycemia, but this can be an important imbalance in natural cases of diarrhea in calves (Trefz *et al*., 2017; Tsukano *et al*., 2018). Thus, the use of OES B is recommended for diarrheal calves with anorexia, ingesting a lower daily volume of milk, as a strategy likely to be effective in mitigating the negative energy balance.

Finally, the volume of OES ingested on the day of treatment (6L divided into two administrations) was greater than the volume usually administered for the treatment of diarrheal calves (4L divided into two administrations) and commonly used in controlled studies to verify the therapeutic efficacy of this procedure (Constable *et al*., 2009; Bachmann *et al*., 2009, 2012; Stämpfli *et al*., 2012; Sayers *et al*., 2016). The option to use a larger volume than the traditional was based on the idea of guaranteeing the daily fluid maintenance volume (150mL/kg) and correcting the expected dehydration of 10% of BW (100mL/kg). Ignoring the volume of water voluntarily ingested (58mL/kg), the studied calves received a total fluid volume of approximately 250mL/kg throughout the treatment day. As dehydration was below expectations (average of 7% of BW), the calves received an excess volume of 30mL/kg. However, this cannot be considered unnecessary, as fluid feces were eliminated throughout the day of treatment.

CONCLUSION

It can be concluded that all the OESs used in the present study are effective for the treatment of diarrheal calves with moderate degrees of dehydration and metabolic acidosis, which indicates that they have an adequate composition for this purpose. Regardless of the differences in their compositions, all the OESs promoted the expansion of the plasma volume, correcting dehydration, and reestablished the acid-base balance of the calves. All the OESs reversed electrolyte imbalances, except OES D, which did not completely correct hyponatremia. OES B caused hyperglycemia and should be used with caution in normoglycemic calves.

ACKNOWLEDGMENTS

The study was funded by the CNPq/INCT-Leite under Grant Number 465725/2014-7 and by PROEX/CAPES 10943/2017. We would like to acknowledge Bayer Saúde Animal, CEVA Saúde Animal, Hypred, and Nutron Alimentos for providing free samples of the commercial products used in the treatments. Júlio A.N. Lisbôa is recipient of the National Council of Technological and Scientific Development (CNPq) fellowship.

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