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Comparison between the dilution of electrolyte concentrate in milk and water for oral rehydration of diarrheal calves

Comparação entre a diluição de concentrado de eletrólitos em leite ou em água para a hidratação oral de bezerros diarreicos

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Highlights

Milk and water with electrolytes were compared in the treatment of diarrheal calves.
Both rehydration methods effectively corrected dehydration and metabolic acidosis.
Dehydration was corrected more rapidly when electrolytes were diluted in water.

Abstract

Administration of oral electrolyte solution (OES) is the most commonly used method to correct dehydration and electrolyte and acid-base imbalances in calves with diarrhea. To prepare OES, the electrolyte concentrate (EC) is diluted in water. Alternatively, it can be diluted in milk and the correction of dehydration depends on voluntary water intake. Although milk dilution has been used, its efficacy has not been consistently proven. This study compared the effectiveness of two rehydration methods for correcting imbalances in diarrheal calves. Twenty-four neonatal calves with induced osmotic diarrhea were distributed into two treatment groups using a commercial EC: GM with EC diluted in the milk at meals, and GW with EC diluted in water (volumes of 5% BW, at 4 and 12 h). All calves were fed with milk (volumes of 4% BW, at 0, 8, and 16 h) and had free access to water. Clinical and laboratory variables were monitored for 48 h. Calves presented with moderate dehydration, hyponatremia, relative hyperchloremia, and moderate strong ion metabolic acidosis. Both rehydration methods were effective in correcting imbalances and re-establishing plasma SID3 levels by the end of the day of treatment.

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Plasma volume expansion was faster in the GW. In conclusion, electrolyte-enriched milk is as effective as OES in treating non-depressed diarrheal calves.
Key words: Neonatal diarrhea. Strong ion acidosis. Fluid therapy. Electrolyte-enriched milk. Oral electrolyte solution.

Resumo

A administração de soluções eletrolíticas orais (SEO) é o método mais empregado para a correção da desidratação e dos desequilíbrios eletrolíticos e ácido base em bezerros com diarreia. Para preparar a SEO, o concentrado de eletrólitos (CE) é diluído em água. De maneira alternativa, o CE pode ser diluído no leite e a correção da desidratação depende da ingestão voluntária de água. Apesar da diluição em leite ser utilizada, sua eficiência não foi totalmente comprovada. Este estudo comparou a eficácia dos dois métodos de hidratação para corrigir os desequilíbrios de bezerros diarreicos. Vinte e quatro bezerros neonatos com diarreia osmótica induzida foram distribuídos por dois grupos de tratamento utilizando um CE comercial: GM com o CE diluído no leite das refeições; e GW com o CE diluído na água (volumes de 5% PC, nas horas 4 e 12). Todos foram alimentados com leite (volumes de 4% PC, nas horas 0, 8 e 16) e tiveram acesso livre à água. Variáveis clínicas e laboratoriais foram acompanhadas ao longo do tempo até 48 h. Os bezerros apresentaram desidratação moderada, hiponatremia, hipercloremia relativa e acidose metabólica por íons fortes de intensidade moderada. Ambos os métodos de hidratação foram efetivos para a correção dos desequilíbrios e reestabelecimento da SID3 plasmática ao término do dia de tratamento. A expansão do volume plasmático foi mais rápida no GW. A ingestão de leite enriquecido com eletrólitos é tão eficiente quanto a ingestão de SEO para o tratamento dos bezerros diarreicos não deprimidos.
Palavras-chave: Diarreia neonatal. Acidose por íons fortes. Terapia com fluidos. Leite enriquecido com eletrólitos. Solução eletrolítica oral.

Introduction

Diarrhea is crucially important among the diseases that affect neonatal calves, as it increases production costs and is the leading cause of mortality (Svensson et al., 2003; Lorenz et al., 2011; Windeyer et al., 2014; Gomes et al., 2021). The intense loss of fluids and electrolytes results in changes such as hemoconcentration, azotemia, hyponatremia, relative hyperchloremia, metabolic acidosis, potassium depletion, hyper-L- or -D-lactatemia, and hypoglycemia (Constable et al., 2005; Gomez et al., 2013, 2017; Trefz et al., 2017).

During the course of diarrhea, rehydration should be performed with oral or intravenous administration of electrolyte solutions. The oral route should be chosen for calves that present with mild to moderate dehydration, unchanged aboral transit of the ingesta, and an active sucking reflex (Smith, 2009; Smith & Berchtold, 2014). To be effective in correcting imbalances in diarrheal calves, oral electrolyte solutions (OES) must contain electrolytes, an alkalinizing agent, such as acetate or bicarbonate, glucose, a high effective strong ion difference (SID_3: 60-80 mmol L^{-1}), and osmolarity close to

300 mOsm L^{-1} (Smith & Berchtold, 2014; Constable et al., 2021).

Traditionally, OES is prepared by diluting commercial or non-commercial electrolyte concentrate (EC) in water and is recommended to administer it 2-3 h after feeding with milk or milk replacer (Smith & Berchtold, 2014). Alternatively, EC can be diluted directly in milk or milk replacer and is therefore administered during routine feeding without the need for additional administration, which can increase adherence to treatment (Goodell et al., 2012).

The dilution of EC in milk or milk replacer results in a hypertonic solution, with a high concentration of sodium, which increases plasma osmolarity and causes thirst. Therefore, calves must have free access to water for this treatment to be successful (Bachmann et al., 2012; Wenge et al., 2014; Wilms et al., 2020). Despite the ease of application of this dilutional method, few studies have compared the efficiency of the two rehydration methods in healthy (Bachmann et al., 2009, 2012) and diarrheal calves (Kirchner et al., 2014; Wenge et al., 2014; Wenge-Dangschat et al., 2020). These studies have some methodological limitations, such as water deprivation (Kirchner et al., 2014), lack of measurement of blood gases and electrolytes (Wenge et al., 2014), pre-treatment with EC causing partial correction of imbalances (Wenge-Dangschat et al., 2020), and the use of different ECs diluted in water or milk replacer (Miqueo et al., 2018). Furthermore, the experimental follow-up period was short, comprising only 4 (Bachmann et al., 2009, 2012) or 6 h (Kirchner et al., 2014; Wenge-Dangschat et al., 2020) after the ingestion of OES or electrolyte-enriched milk or milk replacer.

Despite its practicality and some manufacturers already recommending its use, the effectiveness of this alternative treatment method remains not consistently proven. As the result depends on the voluntary intake of water in an adequate volume by the calves, we hypothesized that the dilution of EC in milk is not as effective as the administration of OES in correcting the imbalances caused by diarrhea. The objective of this study was to compare the effectiveness of the oral administration of a commercial EC diluted in water and milk to correct water, electrolyte and acid-base imbalances in neonatal calves with induced osmotic diarrhea and dehydration.

Materials and Methods

This study was characterized as a randomized controlled clinical trial with repeated measures design and was approved by the Ethics Committee on the Use of Animals of the Sociedade Cultural e Educacional de Garça – Faculdade de Ensino Superior e Formação Integral (FAEF), under the protocol CEUA-FAEF 007/2021.

Animals and experimental procedures

The study included 24 healthy newborn Jersey calves, females (n=19) and males (n=5), belonging to the FAEF herd where the experiment was conducted. The calves were separated from their dams and fed colostrum immediately after birth. They received colostrum with a Brix degree of > 25% in a volume corresponding to 15% of their BW. After 24 h of life, the successful passive transfer of immunity was confirmed

in all calves, which had a total plasma protein concentration of 8.58 ± 1.21 g dL-1. The calves remained in individual pens, cleaned, and covered with wooden shavings throughout the experimental period.

For ten days, which corresponded to the adaptation period, the calves received whole milk in a bottle at a volume corresponding to 12% BW, divided into two daily feedings. Water, Tifton hay (*Cynodon dactylon*) and pelleted commercial starter feed (Geramilk Bezerra, Neovia Nutrição e Saúde Animal Ltda., Paulínea, SP, Brazil) were provided ad libitum.

The induction of osmotic diarrhea and dehydration was carried out over a period of 48 h in calves that remained healthy, 10 to 15 d old and with a BW of 26.71 ± 2.30 kg. The protocol used was previously described (Leal et al., 2008, 2012), and consisted of the administration of whole milk (16.5 mL kg^{-1}) with sucrose (4 g kg^{-1}) diluted at 20% in warm water every 8 h. After each feeding, oral administration of spironolactone (Espironolactona 25 mg; Eurofarma Laboratórios S.A., Itapevi, SP, Brazil) and hydrochlorothiazide (Hidroclorotiazida 25 mg; EMS S.A., Hortolândia, SP, Brazil), both at a dose of 2 mg kg^{-1}, was performed every 8h. Water deprivation was maintained for 12 h at night (Bregadioli et al., 2022).

After induction of osmotic diarrhea and dehydration, correction of water, electrolyte and acid-base imbalances was performed for a period of one day using a commercial EC (Hidralac®, JA Saúde Animal Ltda., Patrocínio Paulista, SP, Brazil). The calves were randomly distributed into two treatment groups (n=12) according to the dilution of the EC: diluted in milk (GM) or in water (GW). In both groups, EC was diluted as recommended by the manufacturer, that is 25 mL of EC per each liter of water or milk. In both groups, natural whole milk was offered at a volume corresponding to 12% BW and divided into three daily meals (at 0, 8 and 16 h). In the GM, EC was diluted in the milk of the meals. In the GW, OES was administered in a bottle at a volume corresponding to 5% BW at 4 and 12 h. From the beginning of the treatment, the calves had guaranteed free access to water.

After dilution, the OES had the following composition: 102 mEq L^{-1} sodium, 23 mEq L^{-1} potassium, 48 mEq L^{-1} chloride, 76 mEq L^{-1} acetate, 32 mmol L^{-1} glucose, a calculated osmolarity of 279 mOsm L^{-1}, pH 6.5, and an effective SID_3 of 76 mEq L^{-1}.

Clinical examinations

During the experimental period, including the induction (−48-0 h), treatment (0-24 h), and follow-up (24-48 h) phases, the calves were examined every 8 h by a single trained and qualified individual who was unaware of the treatment group to which the calves belonged. The color and humidity of the mucous membranes, state of hydration, degree of enophthalmos, skin turgor, capillary refill time, appetite, fecal characteristics, attitudes, posture, and behavior were evaluated.

Scores were established for stool consistency, degree of dehydration, and characteristics of behavior, posture, and sucking reflex, based on the system proposed by Walker et al. (1998) and Smith (2009), with some modifications. The final disease scores ranged from 0 to 10, with 0 representing healthy and 10 representing the greatest degree of health change (Table 1).

Table 1
Scores for stool consistency, hydration status, and characteristics of behavior, posture, and sucking reflex used to evaluate calves

Score	
	Fecal consistency
0	Firm: well formed;
1	Semi-pasty: tending to pasty but maintaining its shape;
2	Pasty: mild diarrhea, without a defined shape and with solid components;
3	Semi-liquid: moderate diarrhea with few solid components;
4	Liquid: severe diarrhea, practically without solid components;
	Degree of dehydration
0	Absent: moist mucous membranes, skin turgor up to 1 s, enophthalmos absent;
1	Mild (5–8%): pasty mucous membranes, skin turgor 1–2 s, enophthalmos 2–4 mm;
2	Moderate (8–10%): sticky mucous membranes, skin turgor 2–5 s, enophthalmos 4–6 mm;
3	Severe (10–12%): dry mucous membranes, skin turgor 5–10 s, enophthalmos 6–8 mm;
	Behavior, posture, and sucking reflex
0	Alert, quadrupedal position, vigorous sucking;
1	Lethargic, quadrupedal position, sucking present but not vigorous;
2	Depressed, preferential or permanent sternal recumbency, slow and disorganized sucking;
3	Comatose, permanent lateral recumbency, sucking absent.

Sample collection and processing

Sample collection and weighing were carried out immediately before feedings, at eight defined time points: −48, −24, 0, 8, 16, 24, and 48 h. The volume of water voluntarily ingested was measured at the beginning of each day (−24, 0, 24, and 48 h).

Venous blood samples were collected by puncturing the jugular vein, using a disposable needle (30 × 0.8 mm) attached to a plastic syringe and placed in vacuum vials containing EDTA anticoagulant. For the blood gas analysis, venous blood samples were collected using heparinized syringes (A-Line Luer Lock, Becton Dickinson Company, BD Brasil, São Paulo, SP, Brazil).

The analyzes were performed immediately after collection. The packed cell volume (PCV) was measured using a microhematocrit centrifuge and the concentration of total plasma proteins (TPP) was measured by refractometry. Blood gas analysis consisted of blood determinations of pH, partial pressure of carbon dioxide (pCO_2), bicarbonate concentration (HCO_3^-), base excess (BE), and concentrations of sodium (Na^+), potassium (K^+), chloride (Cl^-), creatinine, and L-lactate (Epoc®; Epocal Inc., Siemmens Healthcare Diagnostics, Ottawa, Canada).

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 concentration of non-volatile weak acids (A_{tot}): A_{tot} = TPP (g dL^{-1}) x 3.43 (Constable et al., 2005)

d) 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 value at subsequent time points (Carlson & Bruss, 2008).

Statistical analysis

Shapiro-Wilk and Brown-Forsythe tests were used to verify the Gaussian distribution and equality of variance, respectively. Two-way repeated measures ANOVA was used to test the effects of the time factor (different time points before and after EC administration), the EC dilution method factor (M × W), and the interaction between these two factors. When the F statistic was significant, Tukey's test was used for multiple comparisons. Data are presented as means and standard deviations. The SigmaPlot for Windows 13.1 (Systat Software Inc., San Jose, CA, USA) was used for all analyses and an error probability of 5% was assumed.

Results and Discussion

All calves studied presented with osmotic diarrhea, dehydration, and electrolyte and acid-base imbalances. From 8 h after the beginning of induction (−40 h) until the moment of treatment (0 h), the feces were liquid without solid components, yellowish in color and without an unpleasant odor. BW reduced in both groups until −24 h, and remained until 0 h, with a percentage loss of −6.09 ± 2.90% BW in GM and −7.83 ± 3.26% BW in GW without distinction between groups (P = 0.227). The disease score increased until 0 h, reaching values of 6.00 ± 0.95 in GM and 6.50 ± 0.52 in GW (P = 0.163). The estimated degree of dehydration increased continuously in both groups until 0 h, reaching values of 9.16 ± 0.83% in GM and 9.00 ± 0.85% in GW (P = 0.757). Voluntary water intake was highest on the second day of the induction period (Table 2). During the 48h osmotic diarrhea induction period, all calves remained alert, in a quadrupedal position, with an active and vigorous sucking reflex.

The induced osmotic diarrhea caused a continuous decrease in the values of pH, pCO_2, HCO_3^-, BE, Na^+, SID_3, and %PV, and an increase in the values of Cl^-, PCV, TPP, A_{tot}, and creatinine. K^+, AG, and L-lactate levels did not vary until 0 h. No differences were observed between the groups during the induction period (P > 0.05) (Tables 3 and 4).

Variation was observed over time for all variables studied (P < 0.001), except AG, however, the effect of EC dilution was observed only for Cl^- (P < 0.028). The interaction between the factors time and dilution was significant only for Na^+ (P = 0.011), Cl^- (P = 0.004), and %PV (P = 0.003) (Tables 2, 3, and 4).

On the day of treatment, EC was well accepted by all calves, regardless of whether they were diluted in water or milk, and feeding was spontaneous, with a vigorous and active

sucking reflex. The BW and percent change in BW returned to baseline values at 8 h and 16 h, respectively. The degree of dehydration and the disease score gradually decreased and returned to the baseline values at 24 h and 48 h, respectively (Table 2).

Regardless of the treatment used, the EC promoted the correction of pH, pCO_2, HCO_3^-, and BE, returning to baseline values after 16 h or 24 h. Regarding electrolytes, the concentration of Na^+ increased with treatment, however, it did not return to baseline values in GW. In the GM, this occurred only at 24 h. The concentration of K^+ varied slightly over time and the concentrations of Cl^- remained reduced after 8 h. SID_3 values gradually increased and returned to baseline values at 16 h (Table 3).

The increased PCV, TPP, and A_{tot} values were corrected and returned to baseline values at 8 h. The reduced %PV increased with treatment, maintaining higher values than the baseline from 16 h in the GW and 24 h in the GM. Creatinine gradually decreased, however, returned to baseline values only at 48 h. The L-lactate levels varied slightly (Table 4).

Of all the variables studied, differences were observed between the groups only for Na^+, Cl^-, PCV, and %PV. Na^+ concentrations were higher in the GM at 16 and 24 h, and Cl^- concentrations were higher in the GM between 8 and 24 h (Table 3). PCV was lower in the GW between 8 and 24 h and %PV was higher in the GW at 8 and 16 h (Table 4).

All calves remained healthy after the end of the experimental monitoring period. In most calves (18/24), feces recovered to a normal consistency (firm or semi-pasty) at 24 h.

The osmotic diarrhea induction protocol used was effective in causing dehydration and electrolyte and acid-base imbalances. Dehydration was moderate, there was hyponatremia, relative hyperchloremia with a reduction in SID3, and, consequently, strong ion metabolic acidosis of moderate intensity. The imbalances were similar to those observed in previous studies on the induction of osmotic diarrhea, in which dehydration (Constable et al., 1996, 2001; Taylor et al., 2017; Bregadioli et al., 2022) and metabolic acidosis (Bregadioli et al., 2022) occurred at moderate intensity.

The imbalances observed in the calves studied were similar, for the most part, to those found in cases of natural diarrhea (Constable et al., 2005; Gomez et al., 2017; Trefz et al., 2015, 2017). However, no increase was observed in AG, and the calves studied remained alert with appetite and a vigorous sucking reflex. The induction method used causes metabolic acidosis due to relative hyperchloremia (Bregadioli et al., 2022) and this type of imbalance does not cause behavioral changes in calves (Gentile et al., 2008). Calves with naturally occurring diarrhea commonly present with depression, a decreased or absent sucking reflex, and postural changes due to hyper-D-lactatemia (Lorenz, 2004). In the induction protocol used, sucrose was expected to undergo fermentation in the large intestine together with lactose and other unabsorbed substrates, generating D-lactic acidosis (Ewaschuk et al., 2004). However, this probably did not occur, as has already been demonstrated in a previous study using the same induction protocol (Bregadioli et al., 2022).

Table 2

Values (mean ± SD) of body weight, change in body weight, voluntary water intake, disease score, and degree of dehydration measured in neonatal calves with osmotic diarrhea and dehydration induced for 48 h and treated with commercial electrolyte concentrate diluted in milk (M), ingested at 0, 8, and 16 h, or diluted in water (W), administered orally, in a volume equivalent to 5% of body weight at 4 and 12 h. T = effect of time; D = effect of the dilution method; T × D = interaction between time and dilution

	-48	-24	0	8	16	24	48	P		
								T	D	T × D
Body weight (kg)										
M	26.60±2.2^{Aab}	25.09±2.51^{Ac}	25.00±2.52^{Ac}	25.91±2.51^{Abc}	26.67±2.66^{Aab}	27.07±2.57^{Aa}	27.24±2.34^{Aa}	<0.001	0.857	0.582
W	26.83±2.47^{Aab}	25.27±2.81^{Ac}	24.73±2.45^{Ac}	26.23±2.92^{Ab}	27.28±3.14^{Aa}	27.04±3.16^{Aab}	27.60±4.34^{Aa}			
Change in body weight (%)										
M	0±0^{Aab}	-5.7±2.08^{Acd}	-6.09±2.90^{Ad}	-2.65±2.04^{Abc}	0.17±2.29^{Aab}	1.72±2.68^{Aa}	2.44±3.83^{Aa}	<0.001	0.855	0.571
W	0±0^{Aab}	-5.89±3.88^{Ac}	-7.83±3.26^{Ac}	-2.36±3,15^{Ab}	1.54±4.3^{Aa}	0.62±3.84^{Aab}	2.46±7.55^{Aa}			
Voluntary water intake (L)										
M		1.38±0.57^{Aab}	2.08±0.74^{Aa}			1.39±0.72^{Ab}	0.88±1.02^{Ab}	<0.001	0.445	0.717
W		1.20±0.88^{Ab}	2.08±0.61^{Aa}			1.07±0.72^{Ab}	0.57±0.53^{Ab}			
Disease score (0–10)										
M	0.5±0.79^{Ae}	5.25±0.62^{Aa}	6.00±0.95^{Aa}	3.66±0.77^{Ab}	2.25±1.28^{Ac}	1.41±1.16^{Acd}	0.66±0.88^{Ade}	<0.001	0.665	0.180
W	0.16±0.38^{Af}	5.16±0.93^{Ab}	6.50±0.52^{Aa}	3.83±1.19^{Ac}	1.58±0.79^{Ad}	1.16±0.71^{Ade}	0.66±0.65^{Aef}			
Degree of dehydration (%)										
M	0±0^{Ac}	7.08±0.66^{Ab}	9.16±0.83^{Aa}	5.80±2.24^{Ab}	1.41±2.60^{Ac}	0.41±1.44^{Ac}	0±0^{Ac}	<0.001	0.397	0.781
W	0±0^{Ad}	7.33±1.23^{Ab}	9.00±0.85^{Aa}	5.00±1.75^{Ac}	0.83±1.94^{Ad}	0.16±0.38^{Ad}	0±0^{Ad}			

^{A,B} Different capital letters represent differences between treatments ($P < 0.05$)

^{a,b,c,d} Different lowercase letters represent differences between time points ($P < 0.05$).

Table 3

Values (mean ± SD) of pH, partial pressure of carbon dioxide (pCO2), bicarbonate ion (HCO3−), base excess (BE), sodium (Na+), potassium (K+) , chloride (Cl−), and strong ion difference (SID3) measured in venous blood of neonatal calves with osmotic diarrhea and dehydration induced for 48 h and treated with commercial electrolyte concentrate diluted in milk (M), ingested at 0, 8, and 16 h, or diluted in water (W), administered orally, in a volume equivalent to 5% of body weight at 4 and 12 h. T = effect of time; D = effect of the dilution method; T × D = interaction between time and dilution

	-48	-24	0	8	16	24	48	P		
								T	D	T × D
				pH						
M	7.42±0.03^{Aa}	7.33±0.04^{Ab}	7.27±0.08^{Ac}	7.34±0.06^{Ab}	7.38±0.05^{Aab}	7.40±0.04^{Aa}	7.42±0.05^{Aa}	<0.001	0.774	0.100
W	7.46±0.02^{Aa}	7.31±0.08^{Ac}	7.24±0.10^{Ad}	7.36±0.08^{Abc}	7.41±0.05^{Aa}	7.41±0.04^{Aab}	7.41±0.04^{Aab}			
				pCO_2 (mmHg)						
M	46.60±5.97^{Aa}	44.58±6.58^{Aab}	42.15±4.85^{Ab}	41.44±5.73^{Ab}	44.37±6.57^{Aab}	47.16±6.20^{Aa}	44.88±5.16^{Aab}	<0.001	0.851	0.053
W	47.80±4.47^{Aa}	44.95±1.97^{Aab}	42.99±5.74^{Ab}	41.85±6.74^{Ab}	42.75±6.84^{Ab}	44.20±6.23^{Aab}	44.18±5.68^{Aab}			
				HCO_3^- (mmol L^{-1})						
M	27.91±2.16^{Aa}	23.22±3.04^{Ab}	19.03±4.24^{Ac}	22.68±5.04^{Ab}	26.78±5.90^{Aa}	29.08±5.72^{Aa}	28.02±5.66^{Aa}	<0.001	0.789	0.508
W	27.38±2.30^{Aa}	23.35±3.37^{Ab}	19.23±4.56^{Ac}	23.29±6.08^{Ab}	26.49±6.63^{Aa}	26.76±4.47^{Aa}	26.85±5.28^{Aa}			
				BE (mmol L^{-1})						
M	2.67±1.88^{Aa}	-2.84±2.94^{Ab}	-7.9±4.86^{Ac}	-2.94±5.53^{Ab}	1.64±6.41^{Aa}	4.46±5.91^{Aa}	3.5±5.34^{Aa}	<0.001	0.689	0.561
W	1.94±2.17^{Aa}	-4.55±6.07^{Acd}	-8.05±5.64^{Ad}	-2.01±6.83^{Abc}	1.60±7.46^{Aab}	2.26±6.49^{Aa}	1.91±5.28^{Aa}			
				Na^+ (mmol L^{-1})						
M	138.33±1.72^{Aa}	137.83±3.40^{Aa}	132.00±5.16^{Abc}	130.83±4.36^{Ac}	132.25±3.76^{Abc}	135.41±3.26^{Aab}	133.75±6.73^{Abc}	<0.001	0.063	0.011
W	138.08±1.83^{Aa}	137.75±3.81^{Aa}	130.83±3.15^{Abc}	127.83±4.28^{Abc}	127.08±7.05^{Bc}	129.08±6.09^{Bbc}	131.91±5.03^{Ab}			
				K^+ (mmol L^{-1})						
M	4.86±0.49^{Aa}	4.96±0.52^{Aa}	5.04±0.71^{Aa}	4.90±0.74^{Aa}	4.57±0.74^{Aa}	4.43±0.89^{Aa}	4.83±0.89^{Aa}	0.046	0.88	0.958
W	4.82±0.45^{Aa}	5.00±0.69^{Aa}	4.87±0.91^{Aa}	4.80±0.84^{Aa}	4.72±1.11^{Aa}	4.49±1.21^{Aa}	4.65±0.79^{Aa}			
				Cl^- (mmol L^{-1})						
M	102.66±1.87^{Aab}	106.33±2.49^{Aa}	104.25±5.17^{Aa}	99.91±4.20^{Abc}	96.41±4.73^{Ac}	97.25±4.47^{Ac}	97.83±3.24^{Ac}	<0.001	0.028	0.004
W	102.08±2.02^{Ab}	106.00±6.74^{Aa}	103.25±4.04^{Aab}	94.83±4.58^{Bc}	90.41±2.96^{Bd}	93.16±3.01^{Bcd}	95.91±3.80^{Ac}			
				SID_3 (mmol L^{-1})						
M	40.53±2.35^{Aa}	36.46±2.13^{Ab}	32.79±3.74^{Ac}	35.82±4.70^{Abc}	40.40±6.18^{Aa}	42.60±5.41^{Aa}	40.75±5.58^{Aa}	<0.001	0.938	0.210
W	40.82±2.57^{Aab}	36.75±4.13^{Ac}	32.45±4.12^{Ad}	37.80±4.99^{Abc}	41.39±5.77^{Aa}	40.40±6.49^{Aab}	40.65±5.04^{Aab}			

^{A,B} Different capital letters represent differences between treatments (P < 0.05)
^{a,b,c,d} Different lowercase letters represent differences between time points (P < 0.05).

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Table 4

Values (mean ± SD) of packed cell volume (PCV), total plasma protein (TPP), percent change in plasma volume (%PV), non-volatile weak acids (Atot), creatinine, anion gap (AG), and L-lactate measured in venous blood of neonatal calves with osmotic diarrhea and dehydration induced for 48 h and treated with commercial electrolyte concentrate diluted in milk (M), ingested at 0, 8, and 16 h, or diluted in water (W), administered orally, in a volume equivalent to 5% of body weight at 4 and 12 h. T = effect of time; D = effect of the dilution method; T × D = interaction between time and dilution

	-48	-24	0	8	16	24	48	P		
								T	D	T × D
PCV (%)										
M	31.41±4.56^{Abc}	35.00±4.80^{Aa}	36.16±4.91^{Aa}	31.91±4.64^{Ab}	29.25±5.34^{Acd}	28.75±4.67^{Ad}	29.08±4.73^{Ad}	<0.001	0.056	0.206
W	27.66±5.41^{Ab}	32.16±6.36^{Aa}	32.91±7.39^{Aa}	26.50±6.66^{Bbc}	24.33±5.64^{Bcd}	24.00±4.88^{Bd}	25.00±3.21^{Acd}			
TPP (g dL^{-1})										
M	7.15±0.90^{Ab}	8.08±1.16^{Aa}	8.31±1.16^{Aa}	7.28±0.81^{Ab}	6.86±0.79^{Abc}	6.51±0.69^{Ac}	6.66±0.72^{Ac}	<0.001	0.829	0.328
W	7.08±1.16^{Ab}	8.25±1.23^{Aa}	8.45±1.35^{Aa}	7.01±1.00^{Ab}	6.60±0.98^{Abc}	6.40±1.04^{Ac}	6.50±0.92^{Ac}			
%PV										
M	0±0^{Abc}	-11.07±5.14^{Ad}	-13.63±4.72^{Ad}	-1.62±7.74^{Bc}	4.26±5.78^{Babc}	9.72±5.47^{Aa}	7.63±10.96^{Aab}	<0.001	0.156	0.003
W	0±0^{Ac}	-13.68±6.28^{Ad}	-15.27±6.65^{Ad}	5.99±11.09^{Abc}	14.94±10.82^{Aa}	16.25±12.90^{Aa}	10.56±13.60^{Aab}			
Atot (mmol L^{-1})										
M	24.55±3.09^{Ab}	27.72±4.00^{Aa}	28.52±4.00^{Aa}	24.98±2.79^{Ab}	23.55±2.73^{Abc}	22.35±2.38^{Ac}	22.86±2.48^{Ac}	<0.001	0.829	0.328
W	24.29±3.98^{Ab}	28.29±4.25^{Aa}	28.98±4.66^{Aa}	24.06±3.45^{Abc}	22.63±3.37^{Ac}	21.95±3.58^{Ac}	22.29±3.18^{Ac}			
Creatinine (mg dL^{-1})										
M	1.03±0.16^{Ac}	1.99±0.66^{Ab}	3.05±1.59^{Aa}	2.39±1.82^{Aab}	2.12±1.88^{Aab}	1.86±1.85^{Abc}	1.46±1.30^{Abc}	<0.001	0.908	0.371
W	0.95±0,22^{Ab}	1.58±0.54^{Aab}	2.46±1.41^{Aa}	2.32±1.72^{Aa}	2.36±1.92^{Aa}	2.12±1.86^{Aa}	1.70±1.11^{Aab}			
AG (mmol L^{-1})										
M	12.61±1.31	13.24±1.36	13.75±1.07	13.14±1.08	13.62±2.08	13.51±1.28	12.72±1.31	0.057	0.218	0.51
W	13.44±0.96	13.39±1.42	13.22±1.92	14.50±1.90	14.90±2.82	13.64±2.34	13.80±2.19			
L-lactate (mmol L^{-1})										
M	1.58±0.65^{Aa}	1.79±0.77^{Aa}	1.39±0.88^{Aa}	1.33±0.72^{Aa}	1.58±1.01^{Aa}	1.29±0.79^{Aa}	1.21±0.90^{Aa}	0.004	0.810	0.657
W	1.61±0.98^{Aab}	1.96±0.95^{Aa}	1.37±0.63^{Aab}	1.36±0.73^{Aab}	1.24±0.53^{Aab}	0.92±0.49^{Ab}	1.37±0.79^{Aab}			

^{A,B} Different capital letters represent differences between treatments (P < 0.05)
^{a,b,c,d} Different lowercase letters represent differences between time points (P < 0.05).

The two treatments efficiently corrected water and acid-base imbalances; however, the electrolyte imbalance was only partially corrected. Diluting EC in milk was as efficient as traditional dilution in water for correcting dehydration. Recovery of BW; a decrease in the disease score, degree of dehydration, and PCV, TPP, A_{tot}, and creatinine values; and an increase in %PV were observed which proved the expansion of plasma volume and the reversal of hemoconcentration. The results obtained in the present study are consistent with those reported in healthy (Bachmann et al., 2009, 2012) or diarrheal calves (Miqueo et al., 2018; Wenge-Dangschat et al., 2020), in which the two methods of EC dilution had similar effects on plasma volume expansion.

Although the effects of the two EC dilution methods were similar, differences occurred in the PCV (8, 16, and 24 h) and %PV (8 and 16 h) values between the treatments. These differences can be explained by the volume of liquid that the GW calves received in the form of OES, corresponding to 5% BW at 4 and 12 h, which promoted faster plasma volume expansion. However, from 16 h onward, the two types of treatments maintained similar results. This agrees with previous observations in calves with naturally occurring diarrhea (Wenge-Dangschat et al., 2020). Conversely, the two rehydration methods did not differ in healthy calves when the EC was diluted in milk (Bachmann et al., 2012), or plasma volume expansion was greater with EC diluted in milk replacer than when administered as OES (Bachmann et al., 2009). When EC is diluted in a milk replacer, the final concentration of Na^+ is higher than that when EC is diluted in water or milk, as the milk replacer has a higher concentration of Na^+ (Byers et al., 2014). This may explain the differences in observations between the studies.

In the GM, the amount of water voluntarily ingested over time promotes the expansion of plasma volume. When EC is diluted in milk, it forms a hypertonic solution that increases the plasma concentration of Na^+, causing thirst. Therefore, for this treatment method to be efficient, the calves must have free access to water; otherwise, dehydration correction will not occur (Bachmann et al., 2012; Kirchner et al., 2014; Wenge et al., 2014; Wilms et al., 2020).

Over the course of the treatment day, no difference was observed in voluntary water intake between the calves in either treatment group. Nevertheless, GM calves ingested enough water to correct the hemoconcentration. The GM calves did not ingest a greater volume of water than the GW calves, probably because plasma Na+ concentrations remained low throughout the day of treatment, which may not have caused a sufficient stimulus for voluntary intake to be greater than that in the GW. In a previous study in which calves with diarrhea received OES or EC diluted in milk, no difference was observed in voluntary water intake between rehydration methods; however, the calves were monitored for only six hours, which makes the comparison difficult (Wenge-Dangschat et al., 2020). In other studies, the voluntary water intake was greater when EC was diluted in milk (Wenge et al., 2014) or milk replacer (Miqueo et al., 2018).

The EC used in this study was recently introduced into Brazilian commerce and no previous studies are available on its effects on correcting imbalances in calves with diarrhea.

In both treatment groups, moderate intensity metabolic acidosis was reversed and pH, pCO_2, HCO_3^-, and BE values were corrected after 8 h. The alkalizing potential can be attributed to the fact that this EC has a high effective SID3 (76 mmol L^{-1}). Similar effects were observed with another commercial EC containing 58 mmol L^{-1} effective SID_3, which was administered to diarrheal calves diluted in a milk replacer (Miqueo et al., 2018) or in the form of OES (Bregadioli et al., 2023) to correct electrolyte and acid-base imbalances. Distinctly, another commercial EC with an effective SID_3 of 49 mmol L^{-1}, when diluted in milk or administered as an OES, caused slight alkalinization in healthy calves (Bachmann et al., 2012), an effect that was not observed in diarrheal calves (Kirchner et al., 2014; Wenge-Dangschat et al., 2020). According to the strong ion theory, the effective SID3 of the administered electrolyte solution determines its impact on the acid-base balance and those with values greater than 40 mmol L^{-1} are alkalinizing. This effect intensifies as the effective SID3 value icreases (Constable, 2014; Constable et al., 2021).

The electrolyte imbalances were partially corrected. EC was able to correct hyponatremia in the GM; however, Na+ concentrations remained low throughout the day of treatment, which may have influenced voluntary water intake. At the end of the experimental monitoring period, the Na+ concentrations reached physiological values. In the GW, the hemodilution caused by OES accentuated hyponatremia and in the end, the values remained close to physiological limits (Carlson & Bruss, 2008). Regardless of the EC dilution method, Cl− concentrations decreased and did not return to baseline values. The results obtained demonstrate that, despite partial corrections of Na+ and Cl− concentrations, plasma SID3, which reflects the relationship between these two electrolytes, was corrected in both treatment groups, and had already returned to baseline values at 16 h. This result was only possible because of the high effective SID3 (76 mmol L-1) of the EC used, which promoted an increase in Na+ concentration and reversed relative hyperchloremia, thus increasing plasma SID3. This promoted alkalinization, according to the strong ion theory (Constable et al., 2005, 2021).

The results of the present study contradict the hypothesis raised by the authors that diluting EC in milk is not as effective as diluting it in water for correcting imbalances in calves with diarrhea. The ingestion of electrolyte-enriched milk is proved to be as effective as the administration of OES in correcting dehydration and strong ion metabolic acidosis of moderate intensities. Diluting EC in milk or milk replacers is more practical and facilitates the routine of the farm, which can increase treatment adherence, as the work involved in preparing the OES and additional administration to the calf is not necessary (Goodell et al., 2012). The success of this alternative treatment method depends on the free access of calves to water, as the reversal of dehydration occurs because of voluntary water intake. If a calf is deprived of water, a risk of hypernatremia will occur, which may be accompanied by neurological changes, in addition to the complete failure of this alternative rehydration method (Kirchner et al., 2014). The risk of hypernatremia increases when EC is diluted in a milk replacer instead of milk (Byers et al., 2014; Wilms et al., 2020).

Although promising, the results must be interpreted with caution, as they were obtained from calves with induced osmotic diarrhea and dehydration that remained alert with appetite and had an active sucking reflex. Calves affected by naturally occurring infectious diarrhea may be depressed and may not voluntarily drink enough water to correct dehydration. In these cases, the effectiveness of the alternative rehydration method tested would certainly be compromised and it would be prudent to opt for OES administration.

Conclusion

The administration of electrolyte-enriched milk is as effective as OES in reversing moderate dehydration and metabolic acidosis in non-depressed calves with induced osmotic diarrhea that have free access to water. Dehydration was reversed more rapidly with the OES treatment; however, both rehydration methods were equivalent at the end of the treatment day.

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