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Water intake and feeding with whole milk accentuate metabolic acidosis in calves with induced osmotic diarrhea

Ingestão de água e alimentação com leite integral acentuam a acidose metabólica em bezerros com diarreia osmótica induzida

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Highlights

Osmotic diarrhea was induced in calves fed with whole milk and not deprived of water.
Water intake during the inducing period caused less intense and hypotonic dehydration.
Hyponatremia occurred due to drinking water (hemodilution) and milk (no replacement).
Electrolyte and acid-base imbalances were similar to those of natural cases of diarrhea.

Abstract

Sucrose- and diuretics-based protocols are widely used to induce osmotic diarrhea and dehydration in calves, but they fail to cause metabolic acidosis. In previous studies, calves were fed milk replacers and deprived of water. In this study, we assessed the water, electrolyte, and acid-base imbalances in calves that were fed whole milk and were not completely deprived of water during the induction period. Healthy, male Holstein calves aged 10-12 days were assigned to two groups: free access to water (FWG; n=17) and water deprivation at night (DWG; n=21); and osmotic diarrhea was induced with sucrose added to milk, spironolactone (2mg kg^{-1}) and hydrochlorothiazide (2mg kg^{-1}) orally every 8h for 48h. pH, pCO_2, HCO_3^-, BE, Na^+, K^+, Cl^-, SID_3, TPP, AG, A_{tot}, glucose, L-lactate, D-lactate, SIG, and percentage change in plasma volume were measured in venous blood samples taken at 0, 24, and 48h. Data were analyzed using two-way repeated measures ANOVA. Calves showed diarrhea, mild (FWG) to moderate (DWG) dehydration, hyponatremia,

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and moderate (FWG) to severe (DWG) metabolic acidosis. AG and D-lactate levels were higher and SIG was lower in the DWG, and there was no hyper-L- or D-lactatemia. The magnitude of metabolic acidosis was similar to that observed in natural cases of diarrhea. The protocol for inducing osmotic diarrhea and dehydration should be applied to calves that are fed whole milk and are not completely deprived of water.
Key words: Electrolyte imbalances. Experimental induction protocol. Hyposmolar dehydration. Neonatal diarrhea. Strong ion acidosis.

Resumo

Protocolos baseados em sacarose e diuréticos são amplamente usados para induzir diarreia osmótica e desidratação em bezerros, porém não provocam acidose metabólica. Em estudos anteriores, os bezerros foram alimentados com substitutos de leite e privados de água. Neste estudo, avaliaram-se os desequilíbrios hídrico, eletrolítico e ácido base em bezerros que foram alimentados com leite integral e não foram completamente privados de água durante o período de indução. Bezerros HPB machos sadios com 10 a 12 dias de idade foram distribuídos por dois grupos: acesso livre à água (FWG; n=17) e privação de água durante a noite (DWG; n=21); e a diarreia osmótica foi induzida com sacarose adicionada ao leite, espironolactona (2mg kg^{-1}) e hidroclorotiazida (2mg kg^{-1}) por via oral a cada 8h durante 48h. pH, pCO_2, HCO_3^-, BE, Na^+, K^+, Cl^-, SID_3, PPT, AG, A_{tot}, glicose, lactato L, lactato D, SIG e alteração percentual no volume plasmático foram medidos em amostras de sangue venoso colhidas em 0, 24 e 48h. Os dados foram analisados usando ANOVA de medidas repetidas bifatorial. Os bezerros apresentaram diarreia, desidratação leve (FWG) a moderada (DWG), hiponatremia e acidose metabólica moderada (FWG) a grave (DWG). Os valores de AG e de lactato D foram maiores e o de SIG foi menor no DWG, e não houve hiperlactatemia L ou D. A magnitude da acidose metabólica foi semelhante à observada em casos naturais de diarreia. O protocolo para induzir diarreia osmótica e desidratação deve ser aplicado a bezerros alimentados com leite integral e não totalmente privados de água.
Palavras-chave: Acidose por íons fortes. Desequilíbrios eletrolíticos. Desidratação hipotônica. Diarreia neonatal. Indução experimental.

Introduction

Neonatal diarrhea is the main disorder that affects calves in the first month of life (Torsein et al., 2011; D. R. Smith, 2012; Windeyer et al., 2014). Viral, bacterial, and protozoan pathogens may be commonly involved in its etiology (Foster & Smith, 2009). Pathophysiological mechanisms can occur alone or in combination, including hypersecretion, malabsorption of nutrients, osmotic overload, and intestinal inflammation, which is occasionally accompanied by changes in intestinal motility (Heller & Chigerwe, 2017).

The loss of fluids and electrolytes leads to hypovolemia, hyponatremia, normochloremia or hyperchloremia, hyperkalemia, hyper-L-lactatemia, hyper-D-lactatemia, and, eventually, hypoglycemia (Trefz et al., 2013, 2015, 2017; Sayers et al., 2015; Gomez et al., 2017). Strong ion (metabolic) acidosis occurs due to loss of cations, mainly sodium (Na+), and increased concentration of anions, mainly chloride (Cl-). D-lactate, produced by microbiota in the large intestine, exacerbates metabolic acidosis when absorbed (Constable et al., 2005; Lorenz & Gentile, 2014).

Some experimental methods that do not rely on infectious agents have proven to be effective in inducing osmotic diarrhea and dehydration in newborn calves. Experimental induction protocols that mimic the imbalances present in natural cases of diarrhea are important because they allow testing and comparison of the effectiveness of treatments in a controlled manner. These methods include administration of sucrose and diuretic drugs, such as furosemide (Constable et al., 1996), furosemide in combination with hydrochlorothiazide (Kirchner et al., 2014), furosemide in combination with spironolactone and hydrochlorothiazide (Walker et al., 1998a,b; Constable et al., 2001; Doré et al., 2019), and spironolactone in combination with hydrochlorothiazide (Leal et al., 2008, 2012), for 48h. These protocols are effective in causing moderate (Constable et al., 1996, 2001; Taylor et al., 2017) and severe dehydration (Walker et al., 1998a,b; Leal et al., 2008, 2012; Kirchner et al., 2014; Doré et al., 2019). However, they are generally ineffective in causing electrolyte imbalance and metabolic acidosis.

In previous studies, mild metabolic acidosis was induced by increasing the amount of sucrose administered, doubling the doses of diuretics, eliminating the use of furosemide (Leal et al., 2008, 2012), or extending the induction period to 96h (Doré et al., 2019). One important distinction between the studies by Leal et al. (2008, 2012) and other studies (Walker et al., 1998a, b; Constable et al., 1996, 2001; Kirchner et al., 2014; Taylor et al., 2017; Doré et al., 2019) was that they fed calves whole milk instead of milk replacers, which could explain the occurrence of the observed acid-base imbalance since milk replacers have a higher sodium concentration than milk (Gaucheron, 2005; Ollivett & McGuirk, 2013; Doré et al., 2019).

Only two studies have explicitly stated that the calves were deprived of water during the induction period (Constable et al., 2001; Taylor et al., 2017). In the other studies, although not stated, it is logical to assume that the calves were also deprived of water. We hypothesized that calves subjected to the protocol for inducing osmotic diarrhea may present more marked electrolyte and acid-base imbalances when fed milk instead of milk replacers and that access to water for voluntary intake can alleviate the degree of dehydration. This study aimed to compare water, electrolyte, and acid-base imbalances caused by induced osmotic diarrhea in neonatal calves fed natural whole milk with free access to water or water deprivation at night for 12h.

Materials and Methods

This is a randomized controlled trial with a repeated measures design previously approved by the Ethics Committee on the Use of Animals at the Universidade Estadual de Londrina (CEUA/UEL; protocol 9847.2017.10). Thirty-eight healthy, male Holstein calves aged 10-12 days with a weight of 42.89 ± 3.70kg were used. During the adaptation period (7-8 day), the calves were kept in individual pens covered with sawdust and fed natural whole milk from a bottle in a volume corresponding to 12% of their body weight (BW), divided into two feedings per day. Water, a commercial pre-starter feed, and coast-cross grass hay (*Cynodon dactylon*) were available ad libitum.

In all calves, osmotic diarrhea and dehydration were induced using a standardized protocol for 48h (Leal et al., 2008, 2012) - ingestion of whole milk (16.5mL kg^{-1} BW) plus 4g kg^{-1} BW of sucrose diluted to 20% in warm water every 8h and oral administration of spironolactone (Espironolactona 50mg; Eurofarma Laboratórios SA, Itapevi, SP, Brazil) and hydrochlorothiazide (Hidroclorotiazida 50mg; EMS S/A., Hortolândia, SP, Brazil) both at a dose of 2mg kg^{-1} BW every 8h immediately after feeding. Calves were randomly assigned to two groups according to access to water during the 2 days of diarrhea induction - free water access group (FWG; n=17) and water deprivation at night for 12h group (DWG; n=21).

In all calves, physical examinations were performed every 8h by a single practitioner who was blind of the group allocation. The color and moisture of the mucous membranes, state of hydration, degree of enophthalmia, skin turgor, capillary refill time, appetite, vigor of milk sucking, fecal consistency, posture, and behavior were evaluated. We defined scores to assess the consistency of the feces, the degree of dehydration, and behavioral characteristics, posture, and sucking reflex, according to the proposed classification (Walker et al., 1998a; G. W. Smith, 2009) with some modifications (Table 1). The general disease score was determined according to the sum of the defined scores, with a score of 0 indicating a healthy status and a score of 10 indicating a worse disease status.

The calves were weighed and the blood samples were collected at the following three predefined times before the first meal of the day: 0h (start of the induction protocol), 24h (one day of diarrhea), and 48h (end of the induction protocol). The jugular vein was catheterized with an 18-G catheter, which remained sealed with a closing luer connector. Blood samples were collected directly from the catheter using a needle and syringe in vacuum flasks containing EDTA anticoagulant with and without sodium fluoride. Fluoridated plasma was obtained by centrifugation (1,500 × *g* for 10min) up to a maximum of 10min after collection and preserved by freezing (−20°C) until processing. For blood gas analysis, blood samples were collected using heparinized plastic syringes. The analyses were performed shortly after sample collection.

Table 1
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	Letargic, 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

Total plasma protein (TPP) concentrations were measured by refractometry using a portable manual refractometer after centrifugation in a microhematocrit centrifuge. D-lactate levels in the fluoridated plasma were measured using a D-Lactate colorimetric assay kit (BioVision Inc., Milpitas, CA, USA) and read using a plate reader (iMark; Bio-Rad Laboratories, Inc., Tokyo, Japan). Blood pH, pCO_2, HCO_3^-, BE, Na^+, K^+, Cl^-, L-lactate, and glucose levels were measured using a blood gas analyzer (RAPIDpoint 500 System; Siemens Healthcare Diagnostics Inc., Deerfield, Illinois, USA).

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 level before induction and TPP_2 is the TPP level at subsequent timepoints (Carlson & Bruss, 2008).

The volume of water voluntarily ingested throughout each day was measured at 24 and 48h. After sample collection at 48h, the calves were rehydrated orally (n=33) with

oral electrolyte solutions or intravenously (n=5) with infusion of lactated Ringer's solution (LRS) in calves with more severe imbalances. In these calves, metabolic acidosis was corrected using a 6% sodium bicarbonate solution in a volume calculated according to the BE value or by enriching LRS with 50% sodium lactate to increase the effective SID of the solution (SIDeffective=84mEq/L).

The 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 (0 and 48h for D-lactate and 0, 24, and 48h for the other variables), effect of water deprivation factor (FWG × DWG), and the interaction between them. Tukey's test was used for multiple comparisons. An error probability of 5% was considered. Values are presented as means and standard deviations. The SigmaPlot 13.0 package for Windows (Systat Software Inc., San Jose, CA, USA) was used for statistical analysis.

Results and Discussion

The protocol used effectively induced osmotic diarrhea and dehydration. At 8h, most calves had semi-liquid (12/38) or liquid (23/38) stools, and at 48h, all calves had liquid stools. The feces had a yellowish color and a slightly foul odor. Up to 16h, dehydration was absent (n=31) or mild (n=7). At 24h, dehydration was absent (n=7), mild (n=27), moderate (n=3), or severe (n=1). At 48h, all calves had dehydration. In the FWG, dehydration was mild (n=8) and moderate (n=9), and in the DWG, dehydration was mild (n=3), moderate (n=17), and severe (n=1). Regarding behavior, posture, and sucking reflex, all calves remained alert, in standing position, and with vigorous sucking reflex until 24h. At 48h, 25 calves maintained these characteristics, seven calves were dull, five calves were obtunded, and one calf was in a comatose state and lateral recumbency and had no sucking reflex. Ten of these 13 calves belonged to the DWG. The disease score increased up to 48h in both groups ($P<0.001$), but it was higher in the DWG than in the FWG ($P=0.031$) at the end of the experimental period (Table 2). The BW gradually decreased in both groups ($P<0.001$), but the reduction was greater in the DWG than in the FWG ($P=0.002$; Table 2). The average percentage loss of BW was 5.2% in the FWG and 9.3% in the DWG.

Table 2
Values (mean ± SD) of BW, difference in BW, disease score, and daily water intake measured in neonatal calves subjected to induction of osmotic diarrhea for 48h with free access to water (n=17) or with deprivation of water at night for 12h (n=21)

Group	0h	24h	48h
FWG	43.25 ± 4.35Aa	41.01 ± 4.03Ab	40.96 ± 3.74Ab
DWG	42.61 ± 3.18Aa	39.59 ± 3.22Ab	38.60 ± 3.23Ac
FWG	0Aa	-2.24 ± 1.19Bb	-2.28 ± 1.44Bb
DWG	0Aa	-3.02 ± 1.19Ab	-4.00 ± 1.23Ac
FWG	2.53 ± 1.01Aa	4.71 ± 0.69Ab	5.82 ± 0.88Bc
DWG	2.62 ± 0.59Aa	4.90 ± 0.54Ab	6.38 ± 0.92Ac
FWG		4.15 ± 1.06Aa	4.36 ± 1.34Aa
DWG		2.54 ± 0.99Bb	3.96 ± 0.77Aa

[A,B] Superscript capital letters represent differences between the groups (P<0.05).
[a,b,c] Superscript lowercase letters represent differences between time points (P<0.05).
BW, body weight; SD, standard deviation; DWG, deprivation of water at night for 12h group; FWG, free access to water group.

In both groups, the induced diarrhea and diuresis resulted in a continuous decrease in pH, HCO_3^-, BE, Na^+, SID_3 (Figure 1), %PV, and SIG values (Figure 2) and a continuous increase in TPP, AG, A_{tot}, D-lactate, and glucose values (Figure 2). The concentrations of K^+ and L-lactate increased only in the DWG and those of Cl- and pCO_2 decreased only in the FWG (Figure 1 and 2).

The differences between groups were observed at 24 and 48h. At 48h, the DWG calves showed lower values of pH, HCO_3^-, and BE and higher values of K^+ and D-lactate than the FWG calves. After 24h, the values of Na^+, Cl^-, and AG were higher in the DWG than in the FWG, and the values of %PV and SIG were higher in the FWG than in the DWG. The values of pCO_2, SID_3, TPP, A_{tot}, L-lactate, and glucose did not differ between the groups (Figure 1 and 2).

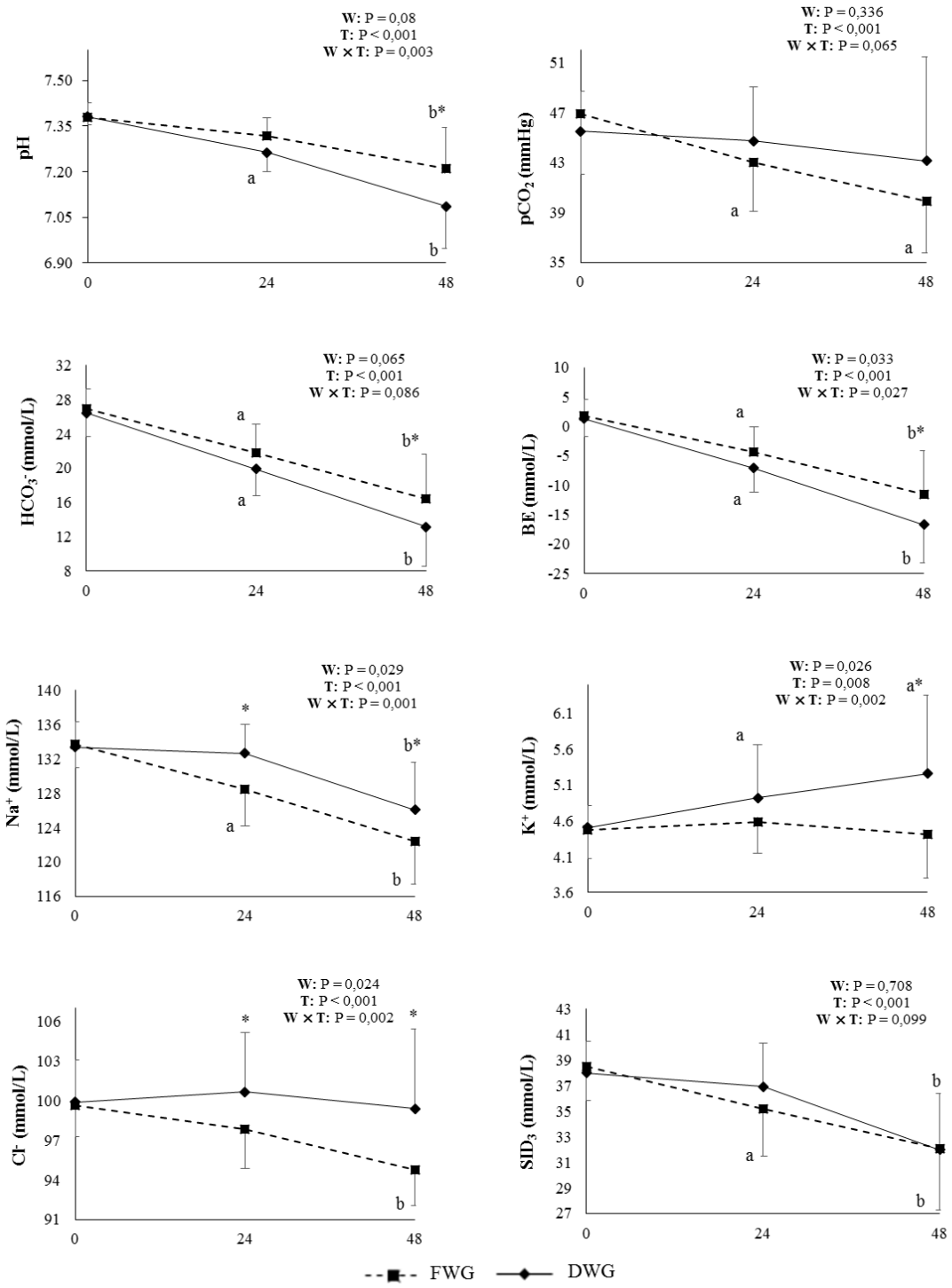

Figure 1. Values (mean and SD) of pH, pCO_2, HCO_3^-, BE, Na^+, K^+, Cl^-, and SID_3 measured in venous blood over time (hours) in neonatal calves subjected to induction of osmotic diarrhea for 48h with free access to water (FWG; n=17) or deprivation of water at night for 12h (DWG; n=21). Effects of factors: water intake (W) and time (T) and interaction between them (W × T). a means differences with 0h; b means difference with 0 and 24h; * means difference between the groups.

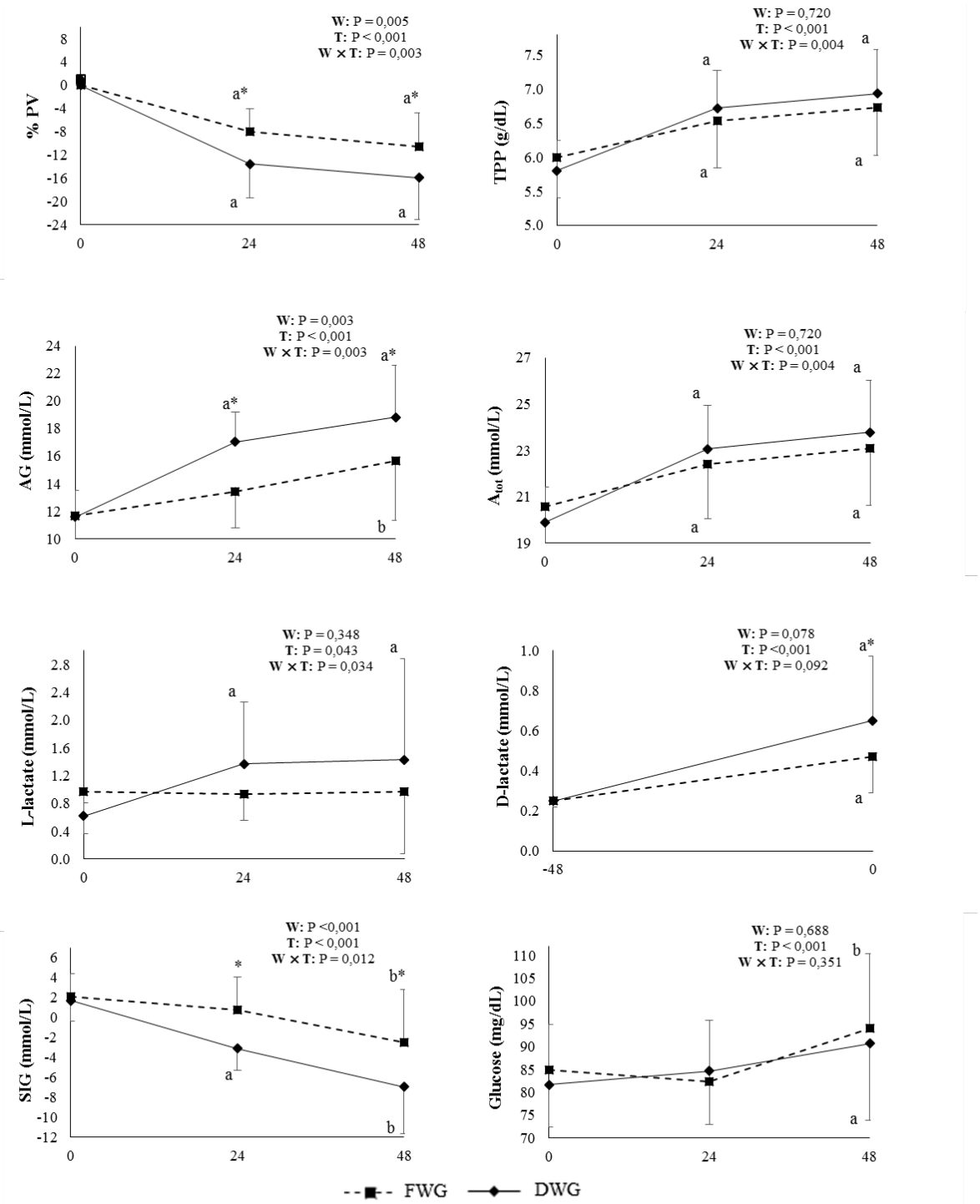

Figure 2. Values (mean and SD) of percentage change in plasma volume (%PV), TPP, AG, Atot, L-lactate, D-lactate, SIG, and glucose measured over time (hours) in neonatal calves subjected to induction of osmotic diarrhea for 48h with free access to water (FWG; n=17) or deprivation of water at night for 12h (DWG; n=21). Effects of factors: water intake (W) and time (T) and interaction between them (W × T). [a] means differences with 0h; [b] means difference with 0 and 24h; * means difference between the groups.

All calves recovered after treatment at the end of the experiment.

Therefore the experimental induction protocol applied to calves fed whole milk with free access to water or with nightly restriction of water caused osmotic diarrhea, mild-to-moderate dehydration, electrolyte imbalances characterized by hyponatremia and relative hyperchloremia, and moderate-to-severe metabolic acidosis after 48h. K+ and glucose levels were maintained at physiological values, and despite high AG values and low SIG values, none of the claves developed hyper-L-lactatemia or hyper-D-lactatemia. In general, these imbalances were more accentuated in DWG calves.

Considering the reduction percentage in BW, dehydration was mild (average reduction of 5.2%) in FWG calves and moderate (average reduction of 9.3%) in DWG calves. The percentage reduction in plasma volume showed a similar trend (average reduction of 10% and 16% in the FWG and DWG, respectively). Therefore, the magnitude of water imbalance observed in our study was lower than that observed in previous studies, in which the degree of dehydration varied from moderate to severe, with reductions in BW of 8% (Constable et al., 1996; Taylor et al., 2017), 8%-10% (Constable et al., 2001), 12% (Kirchner et al., 2014), 13% (Leal et al., 2008, 2012), 14% (Walker et al., 1998a,b), and 15% (Doré et al., 2019). In these studies, the percentage reduction in plasma volume was also more pronounced than that observed in our study, varying between 20% and 26% (Constable et al., 1996; Walker et al., 1998a,b; Leal et al., 2008, 2012; Kirchner et al., 2014; Doré et al., 2019).

The voluntary intake of water alleviated the induced dehydration. Voluntary water intake was higher in the FWG ($P<0.001$) than in the DWG during the first day (24h), but it did not differ between the groups ($P=0.243$) on the second day (48h; Table 2). FWG calves ingested a daily volume of water equivalent to approximately 10% of their BW. However, DWG calves ingested a daily volume equivalent to approximately 5% of their BW on the first day and increased their intake on the second day (Table 2). In addition to the volume of milk and sucrose solution ingested, FWG calves ingested a total of fluid volume equivalent to 21% of their BW per day. However, DWG calves ingested a total of fluid volume equivalent to 16% of the BW on the first day and 21% of their BW on the second day. Fluid lost due to osmotic diarrhea and increased diuresis was partially replaced by drinking water.

The dehydration induced in the studied calves can be considered hyposmolar based on the reduced Na^+ concentration. This finding is inconsistent with that reported in previous studies, in which isosmolar dehydration was observed because the plasma concentrations of Na^+, K^+, and Cl^- (Constable et al., 1996, 2001; Walker et al., 1998a,b; Doré et al., 2019), and blood serum osmolality (Constable et al., 1996; Kirchner et al., 2014) were unchanged. The induction protocols used in these studies were unable to induce electrolyte imbalances, contrary to our study findings. There are two explanations for the presence of hyponatremia in the calves in this study - feeding with natural milk instead of milk replacers and voluntary water intake.

Whole milk has low concentrations of Na^+, ranging from 16 to 30mmol L^{-1} (Gaucheron, 2005; Ollivett & McGuirk, 2013; Foroutan et al., 2019). In contrast, milk replacers may have higher Na^+ concentrations, varying between 30 and 88mmol L^{-1} (Angelos et al., 1999; Ollivett & McGuirk, 2013; Kirchner et al., 2014; Doré et al., 2019). Therefore, the induced loss of Na^+ in the feces and urine can be compensated when milk replacers are used to feed the calves, and the use of whole milk would be more appropriate to guarantee the occurrence of hyponatremia. However, Leal et al. (2008, 2012) also used whole milk, but they were unable to induce electrolyte imbalances, contrary to the above hypothesis.

Therefore, voluntary intake of water during the experimental induction period may have contributed to the occurrence of hyponatremia and relative hyperchloremia, which was confirmed by the reduction in plasma SID3 values, due to the hemodilution effect. The partial correction of plasma volume without the concomitant replacement of electrolytes may have promoted dilution of the plasma components, leading to hyposmolar-type dehydration, a differential feature of the present study. This was reinforced by the fact that hyponatremia was more intense in FWG calves (Figure 1), which had lesser reduction in plasma volume (Figure 2) than DWG calves. At 24h, hypovolemia was pronounced and Na^+ concentrations remained unchanged in DWG calves compared to those in FWG calves. At the end of induction, FWG calves showed lower intensity of hemoconcentration and lower concentrations of Na^+, K^+, and Cl^- than DWG calves.

In the protocol used, the loss of electrolytes occurred mainly via elimination in the urine, although they are also eliminated in the feces, and it is assumed that both hydrochlorothiazide and spironolactone have equivalent natriuretic and chloruretic effects (Walker et al., 1998a). However, the observed reduction in plasma SID_3 values indicates that the loss of Na+ was more pronounced than the loss of Cl^-, since SID_3 expresses the balance or imbalance between the concentrations of Na^+ and Cl^- in the plasma (Constable, 2014). In the previous studies, plasma SID_3 values did not change (Constable et al., 1996; Leal et al., 2008, 2012; Kirchner et al., 2014) or increased (Walker et al., 1998a,b; Constable et al., 2001). It is important to consider that furosemide was used as a diuretic in most of these studies, which may have led to a different pattern of urinary electrolyte excretion. Hence, the results of previous studies are inconsistent with those of our study.

Acidemia occurred in both groups, and the average decreases in the values of HCO_3^- (10mmol L^{-1} in the FWG and 13mmol L^{-1} in the DWG) and BE (13mmol L^{-1} in the FWG and 18mmol L^{-1} in the DWG) indicated that FWG calves developed moderate metabolic acidosis and DWG calves developed severe metabolic acidosis. The induced metabolic acidosis, as well as its magnitude, markedly distinguishes the present results from those obtained in previous studies. This acid-base imbalance was not induced in other studies, except in studies by Leal et al. (2008, 2012) and Doré et al. (2019), who observed a mild degree of metabolic acidosis. The direct comparison between our study results and those reported by Leal et al. (2008, 2012) is relevant since the induction protocols

used were identical, except voluntary intake of water. In the studies by Leal et al. (2008, 2012), the average reduction in the values of HCO_3^- and BE at the end of induction were 6mmol/L and 7mmol/L, respectively, which characterizes a less intense imbalance.

The reduced plasma SID_3 values indicated that there was strong ion acidosis resulting from hyponatremia and relative hyperchloremia. In other words, the voluntary intake of water caused electrolyte imbalances, which secondarily determined the occurrence of hyperchloremic acidosis. However, as SID_3 values did not differ between the groups, this was not the only mechanism that caused metabolic acidosis. DWG calves showed higher AG and lower SIG values than FWG calves (Figure 2). Further, AG values were slightly above the upper limits and SIG values were below the lower limits considered physiological for neonatal calves (Trefz et al., 2017). This means that acidosis was accentuated by a certain accumulation of organic acids in the DWG. L-lactate concentrations increased slightly in the DWG, but remained within the range of physiological variation (Trefz et al., 2017). D-lactate concentrations increased in both groups, but were higher in the DWG than in the FWG (Figure 2). However, considering the physiological upper limit of 3.9mmol/L for neonatal calves (Trefz et al., 2017), it is possible that the studied calves did not have hyper-D-lactatemia. Although hyper-D-lactatemia is considered one of the most important causes of metabolic acidosis in diarrheal calves (Lorenz & Gentile, 2014), based on previous observations (Kirchner et al., 2014), it can be said that the protocol for inducing osmotic diarrhea with sucrose is probably unable to cause this type of change consistently. Therefore, other unmeasured anions must be involved in the genesis of the most pronounced acidosis in DWG calves.

Finally, it can be stated that the water, electrolyte, and acid-base imbalances caused by the protocol used in our study are representative of those observed in natural cases of diarrhea. Surveys with a large number of calves with natural diarrhea have demonstrated that the changes are commonly characterized by non-compensated metabolic acidosis; hyponatremia; hyperchloremia; hyperkalemia; decreased SID3 and SIG values; increased AG, TPP, and Atot values; hypoglycemia; and hyper-L-lactatemia and/or hyper-D-lactatemia (Trefz et al., 2013, 2015, 2017; Sayers et al., 2015; Gomez et al., 2017). Except hyperkalemia, hypoglycemia, and hyperlactatemia, all the aforementioned changes occurred in the studied calves.

The limitations of the present study are the absence of a group in which the calves received milk replacers instead of whole milk and the absence of a group in which the calves were completely deprived of water. In the first case, the hypothesis that Na+ depletion is more intense when whole milk is used could be proven since the intake of milk replacers may contribute to the replacement of this electrolyte, mitigating or correcting the imbalance induced by the protocol. In the second case, the hypothesis that hemodilution contributes to the occurrence of hyponatremia, relative hyperchloremia, and hyperchloremic acidosis could be more consistently proven. Unfortunately, the cost involved in forming one or two more experimental groups was the decisive impeding factor.

Conclusion

Based on the observed results, it can be concluded that the protocol for inducing osmotic diarrhea and dehydration, which consists of the oral administration of sucrose, hydrochlorothiazide, and spironolactone, should be applied to calves fed whole milk that are not completely deprived of water. Under these circumstances, the induced dehydration is not very intense, but hyponatremia, relative hyperchloremia, and metabolic acidosis are present, more realistically mimicking the imbalances that occur in natural cases of neonatal diarrhea in calves.

Conflict of interest statement

There are no conflicts of interest.

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