Received: 18 April 2019 | Revised: 24 November 2019 | Accepted: 14 December 2019

DOI: 10.1111/jpn.13306

ORIGINAL ARTICLE

Journal of Animal Physiology and Animal Nutrition | WILEY

Abomasal emptying rate of diarrhoeic and healthy suckling calves fed with oral rehydration solutions

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Funding information
This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Abstract

The aim of the study was to determine the abomasal emptying rate (AER) of calves suffering from naturally occurring diarrhoea compared with that of healthy calves. Furthermore, the effects of an oral rehydration solution (ORS) mixed into milk replacer on the AER were determined. Acetaminophen absorption test (APAT) was performed to estimate the AER. Sixty Holstein–Frisian calves (age < 14 days) were included in the study and divided into groups as follows: healthy calves (H; n = 16), healthy calves fed with ORS (HORS; n = 14), diarrhoeic calves (D; n = 15) and diarrhoeic calves fed with ORS (DORS; n = 15). For the APAT, the calves were fed 2 L of milk replacer containing 50 mg acetaminophen (AP)/kg body weight. Venous blood samples were collected before and after milk replacer and AP intake in 30–60 min intervals for 12 hr. During the APAT, no significant differences in median maximum acetaminophen concentration (C_{max}) were observed among all groups. Time to reach maximum acetaminophen concentration (T_{max}) in DORS (median 390 min, 25/75 quartiles: 300/480 min) was significantly higher compared with that in H (median: 270 min 25/75 quartiles: 210/315 min) and HORS (median: 300 min (25/75 quartiles: 240/360 min). Non-linear regression revealed that the calculated abomasal half-life (AP $t_{1/2}$) tended to be delayed in DORS (median: 652 min, 25/75 quartiles: 445/795 min, p = .10). The area under the AP curve values (AUC) from 0 to 120 min and 0 to 240 min of the observation period were significantly higher in H than D and DORS. In conclusion, significant differences in the AER indices reflected delayed abomasal emptying in diarrhoeic calves. Furthermore, the hypertonic ORS tended to have an additive delaying impact on the AER, which needs attention for the feeding management of diarrhoeic calves.

KEYWORDS

abomasum, acetaminophen, calves, diarrhoea, oral rehydration solution

Abbreviations: ABE, anion base excess; AER, abomasal emptying rate; AP, acetaminophen; APAT, acetaminophen absorption test; AUC, area under the concentration curve; Cmax, maximum acetaminophen concentration; D, Diarrhoea group fed with milk replacer only; DORS, Diarrhoea group fed with milk replacer and ORS; H, Healthy group fed with milk replacer only; controls; HORS, Healthy group fed with milk replacer and ORS; ORS, Oral rehydration solution; T_{max}, Time to reach maximum acetaminophen concentration.

1 | INTRODUCTION

Neonatal diarrhoea is the most important risk factor for mortality in dairy calves and can lead to considerable economic losses (Windeyer et al., 2014). During an episode of diarrhoea, calves lose large amounts of water and electrolytes, which may lead to the inability to suckle, lethargy, metabolic acidosis and circulatory disorders. Kirchner et al. (2015) recently described delayed abomasal emptying during diarrhoea in suckling calves. According to these findings, a delay in abomasal emptying and changes in intraluminal conditions, such as temperature, were confirmed by using an acetaminophen absorption test (APAT) and a telemetric device in diarrhoeic calves (Hildebrandt et al., 2017). Impaired abomasal motility might be a risk factor for bacterial mal-fermentation processes facilitating the production of short-chain fatty acids (SCFA). SCFA are discussed as predisposing factors for damaging gastric mucosa cells by interrupting the sodium transport system under in vitro conditions, for example in horses (Nadeau et al., 2003), but data in calves are lacking. Furthermore, an increase in gas-producing bacteria such as *Clostridium perfringens*, *Sarcina ventriculi* or *Lactobacillus spp* is considered to be a predisposing factor of abomasal tympany (Marshall, 2009).

Oral rehydration solutions (ORS) represent the most common treatment for diarrhoeic calves under the assumption of a sufficient suckle reflex (Wenge, Steinhöfel, Heinrich, Coenen, & Bachmann, 2014). The absorption of bicarbonate and electrolytes is essential to compensate for metabolic acidosis and the replacement of electrolytes in diarrhoeic calves. Bachmann, Schmidt, Rauwolf, Wenge, and Coenen (2012) recommended hypertonic ORS or ORS mixed with milk replacer as the most effective treatment to correct the fluid homeostasis in diarrhoeic calves. In that context, the abomasal emptying rate is dependent on various parameters such as volume, caloric content, type of proteins or osmolarity (Hunt & Knox, 1968; Hunt, Smith, & Jiang, 1985; Nouri & Constable, 2006; Sen, Constable, & Marshall, 2006; Siegel et al., 1988).

To investigate the abomasal emptying rate (AER), various methods of assessment, such as the D-xylose test, ultrasonographic measurement, luminal pH return and APAT, have been validated in healthy calves and heifers (Ahmed, Constable, & Misk, 2002; Burgstaller, Wittek, & Smith, 2017; Marshall, Constable, Crochik, & Wittek, 2005; Wittek, Constable, Marshall, & Crochik, 2005; Wittek, Schreiber, Fürll, & Constable, 2005).

APAT is known as a reliable and accurate method to assess abomasal emptying as previously described by Marshall et al. (2005). The pharmacokinetic model according to Siegel's equation of the biphasic nature of gastric emptying (Siegel et al., 1988) leads to the most accurate AER values (Marshall et al., 2005). However, APAT is challenging in diarrhoeic calves, possibly due to the altered absorption capacity of the damaged mucosa cells in the small intestine.

Therefore, the aim of this study was to compare AER by APAT in healthy and diarrhoeic calves fed with and without ORS. We hypothesized that the abomasal emptying and absorption rate in the small intestine of diarrhoeic calves will be delayed in comparison with those of healthy calves. As the treatment with ORS is the treatment method of choice for diarrhoeic calves, the impact of ORS mixed in the milk replacer on AER was compared between healthy and diarrhoeic calves. It was speculated that mixing ORS in the milk replacer will have an impact on abomasal emptying namely by changes in osmolality and energy intake.

2 | MATERIALS AND METHODS

2.1 | Animals

The study was performed with 60 suckling Holstein–Frisian calves of (female; 3–14 days old, mean age ± *SD*: 8.0 ± 2.7 days; mean body weight (BW) ± *SD*: 42.1 ± 5.3 kg) housed in single stalls (length × width ×height; 1.90 m × 1.14 m × 1.35 m) and bedded with straw. Excluding the examination day, the animals were fed twice daily with 4 L of combined skimmed milk and whey protein milk replacer containing ingredients as labelled: crude protein 20.0%; crude fat 17.0%; crude ash 7.2%; phosphorus 0.7%; calcium 0.8%; and sodium 0.6% (FOK TOP, Alpuro Breeding). A daily milk replacer intake of 8 L is necessary to meet the energy and nutrient requirements in 3- to 14-day-old calves. The animals had free access to tap water.

2.2 | Experimental design

Calves were arranged in subsequent order into four groups: healthy calves (H, n = 16) as controls, healthy calves additionally fed with oral rehydration solution (HORS, n = 14), calves with naturally occurring diarrhoea (D, n = 15) and diarrhoeic calves additionally fed with ORS (DORS, n = 15). The inclusion criterion for the diarrhoea groups was a faecal score ≥ 2 on a scale of 0–3 according to Walker et al. (1998). A score of 0 to 1 reflected well-formed faeces up to abnormal faeces that tended to be pasty. A score ≥ 2 described pasty faeces, mainly liquid with solids up to liquid faeces. Diarrhoea had to have occurred for at least 24 hr before the experimental day. The animals suffering from other diseases, such as pneumonia or omphalitis (see clinical examination), were excluded from the study. After the experimental day, all diarrhoeic calves were treated with ORS, and diarrhoea improved without any further medical intervention.

2.3 | Clinical examination

In the morning before blood sampling, all calves were weighted on an electronic scale. Heart rate and respiratory rate were measured by auscultation and counting. Rectal temperature was determined with an electronic thermometer. Calves were manually checked for omphalitis.

2.4 | Test ration

After a fasting period of 12 hr, calves were fed 2 L of a milk replacer via a bucket with a teat. Fifty milligrams of acetaminophen (AP)/kg BW (Pracetam, Animedica) was mixed into the milk replacer. HORS and DORS were provided 75 g of an ORS (Lytafit, Albrecht) added into milk replacer containing the following ingredients as labelled: crude protein 10.0%; crude ash 15.0%; calcium 0.13%; sodium 5.0%; potassium 1.3%; magnesium 0.05%; and chloride 4.4%. Further ingredients included lactose and glycine. The product was a HCO_3-containing ORS that has a similar composition to the WHO recommended ORS (Na^+ 81 mmol/L, K^+ 13 mmol/L, Cl^- 45 mmol/L, 361 mOsmol/kg). During observation period water intake was withheld.

2.5 | Faecal collection and analysis

Rectal faecal samples were collected once before feeding. The samples were analysed for *Rota virus*, *Corona virus*, *Escherichia coli* and *Cryptosporidium parvum* by using a commercial enzyme-linked immunosorbent assay (Fassisi BoDia, Fassisi).

2.6 | Blood collection

After shaving and skin disinfection, an indwelling catheter (14 G, 2.5 inches) was inserted into the right or left jugular vein and flushed with physiological saline after every blood sampling. The blood samples were collected 30 min before feeding, eight hours postprandial in 30 min intervals and the following 4 hr in 60 min intervals. The blood was collected in tubes containing lithium heparin (10 ml Monovette, Sarstedt). After centrifugation of the lithium heparin tubes at 1,000 × g for 10 min, the plasma was harvested and stored at −20°C until analysis. Additionally, venous blood samples from a representative number of calves in each group (n = 5) were taken 30 min before feeding with a 3 ml syringe (PICO 50, Radiometer) containing heparin for oxymetric analysis.

2.7 | Blood analysis

The measurements of the plasma samples were performed in the Institute of Pharmacology of the University Medicine of Greifswald.

AP concentrations were determined with a validated liquid chromatography–mass spectrometry method (LC-MS/MS) after liquid-liquid extraction and internal standard method estimation.

The first derivative of Siegel's modified power exponential equation was used to generate an AP-time curve according to the protocol of Marshall et al. (2005):

$$C(t) = mk\beta e^{-kt}\left(1e^{-kt}\right)^{(\beta-1)} \quad (1)$$

C was the calculated acetaminophen concentration (µg/mL) depending on the time t (time from the start of suckling in minutes). The constant m was the total cumulative recovery of acetaminophen when the time was infinite, the constant k was the estimated rate of abomasal emptying (min^{-1}), and the constant β was an estimate of the duration of the lag phase before the exponential emptying phase was reached. Non-linear regression analysis was used to determine the constants. The acetaminophen abomasal half-life was calculated by applying the generated constants of the following equation:

$$APt_{\frac{1}{2}} = \left(-\frac{1}{k}\right)\ln\left(1-2^{\left(-\frac{1}{\beta}\right)}\right) \quad (2)$$

This pharmacokinetic model was validated and recommended by Marshall et al. (2005), providing the most accurate abomasal emptying indices for calves.

The area under the curve (AUC) was calculated by the trapezoidal method from the AP-time plot from 0 to 60 min, 0 to 120 min, 0 to 240 min and 0 to 720 min using the following equation:

$$AUC_{(0-t)} = \sum_{i=0}^{n-1}\left(t_{i+1}-t_i\right)\left(\frac{C_i+C_{i+1}}{2}\right)$$

where C_i was the acetaminophen concentration corresponding to the post-suckling time t_i

After centrifugation, each plasma sample was analysed for total plasma protein (TPP) by using a refractometer. The change in plasma volume (ΔP, expressed in percent) was calculated using following equation (van Beaumont, Greenleaf, & Juhos, 1972):

$$\Delta P_{(t=x)} = \left(P_{(t=0)} - P_{(t=x)}\right) * \frac{100}{P_{(t=x)}}$$

where $P_{(t=0)}$ was the blood plasma concentration before feeding and $P_{(t=x)}$ was the blood plasma concentration at the time x after suckling the test meal.

The venous blood samples were analysed oxymetrically by a blood gas analyser (corrected to rectal temperature, ABL80 Flex, Radiometer). The following parameters were measured: pH, partial pressure of carbon dioxide (pCO_2) and oxygen (pO_2), sodium, chloride, potassium, calcium, haematocrit (Hct), haemoglobin (Hb) and hydrogen carbonate (HCO_3^-). Anion base excess (ABE) and anion gap were calculated (ABE) by a blood gas analysing device.

2.8 | Statistical analysis

Data analysis was performed by a statistical software program (Statistica 7.1, StatSoft). Power analysis was performed by using abomasal emptying rates in healthy and diarrhoeic calves using ultrasonographic imaging as described by Kirchner et al. (2015). A minimum number of 12 calves had been calculated to detect significant

differences. In case of animal losses, we decided to include a total of 16 calves in each group. Due to an inadequate milk replacer intake, animal numbers were reduced in HORS: n = 14, D: n = 15 and DORS: n = 15. Data were analysed for normal distribution by the Shapiro–Wilks test. Outlier tests were performed, and the data were excluded when they differed from the mean by more than the double standard deviation.

The normally distributed data such as vital and blood gas parameters were subjected to unpaired t tests. In case of non-normally distributed data such as AP concentrations, data were subjected to Mann–Whitney U test with Bonferroni correction to compare the different groups. Time-related differences during APAT were tested by Friedman ANOVA. To calculate the AP half-time, the acetaminophen concentration data were analysed by non-linear regression. Normally distributed data are presented as means ± *SD*. Non-normally distributed data are presented as medians and 25/75 quartiles. Statistical significance was accepted at $p < .05$. A trend was postulated at $p < .1$.

3 | RESULTS

3.1 | Faeces and vital parameters

All calves included in D (n = 15) and DORS (n = 15) were tested positive for diarrhoea-causing pathogens. *Cryptosporidium parvum* was found in all calves in D and 12/15 calves in DORS. *Rota virus* was found in 13/15 calves in D and 14/15 calves in DORS. Seven calves belonging to H (n = 16) and 6 calves included in HORS (n = 14) tested also positive for diarrhoea-causing pathogens (Table 1). The mean (± *SD*) rectal temperature in D and DORS was 39.1 ± 0.47°C and 39.1 ± 0.42°C respectively. In H and HORS, the mean (±*SD*) rectal temperature was 38.8 ± 0.45°C and 38.9 ± 0.27°C respectively (p = .24).

The mean (±*SD*) heart rate and mean (±*SD*) respiratory rate were similar for diarrhoeic and healthy calves (heart rate p = .67; respiratory rate p = .59).

The vital parameter findings were not significantly different between healthy or diarrhoeic calves (Table 1).

3.2 | Blood gas analysis

The blood haematocrit, electrolyte and anion gap levels were not significantly different among the calves (Table 2). Blood pH in D (7.34 ± 0.04) was significantly lower compared with that in healthy calves (pH (H) = 7.4 ± 0.03 and pH (HORS) = 7.39 ± 0.03; p = .02). pH in DORS (7.37 ± 0.04) was not significantly different. Higher bicarbonate concentrations were observed in HORS (33.3 ± 4.1 mmol/L) compared with those in diarrhoeic calves (HCO_3: D = 27.4 ± 3.3 mmol/L and DORS = 28.9 ± 4.6; p = .05). Furthermore, ABE was lower in D (2.2 ± 3.4 mmol/L) than in H and HORS (H = 6.5 ± 2.2 mmol/L and HORS = 7.93 ± 3.6; p = .04). ABE in DORS was numerically lower (3.95 ± 4.40) mmol/L but did not lead to significance.

3.3 | TPP and changes in plasma volume

Overall, D had significantly lower TPP levels than H. Significant time-related changes were found after milk replacer intake with or without ORS, but the interactions between time and health status were not significantly different among the four groups (p = .12; data not shown). In H, the calculated changes in plasma volume (P) significantly increased from baseline at t = 210 min (5.92 ± 4.44%). In HORS ΔP (4.42 ± 5.32%) was significant different to $P_{(t=0)}$ at t = 180 min. The changes in plasma volume in D (4.93 ± 5.90%) and DORS (5.71 ± 6.38%) increased significantly from baseline levels at t = 300 min.

3.4 | APAT

Median maximum AP concentrations (C_{max}) revealed no significant differences among the groups (p = .67). The median C_{max} of D and DORS was 49.8 µg/mL (25/75 quartiles: 41.9/55.5 µg/mL) and 55.8 µg/mL (25/75 quartiles: 47.3/79.6µg/mL), and the median C_{max} of H and HORS was 60.6 µg/mL (25/75 quartiles: 48.9/71.2 µg/mL) and 56.2 µg/mL (25/75 quartiles: 44.9/62.1 µg/mL) respectively (Table 3).

Significant differences were found in the median time to reach the maximum AP concentration (T_{max}) as follows: T_{max} (DORS) = 390 min (25/75 quartiles: 300/480 min was significantly higher than that in H and HORS (T_{max} (H) = 270 min (25/75 quartiles: 210/315 min), T_{max} (HORS) = 300 min (25/75 quartiles: 240/360 min; p = .02) and tended to be higher than in D (T_{max} (D) = 300 min (25/75 quartiles: 240/390 min) (Table 3). The calculated median AP abomasal half-life (AP $t_{1/2}$) was significantly longer in DORS = 652 min (25/75 quartiles: 445/795 min) than in H = 493 min (25/75 quartiles: 374/626 min) (p = .05) and HORS = 465 min (25/75 quartiles: 422/554min) (p = .03) (Table 3). The calculated median AP abomasal half-life of HORS tended to be longer than in H (p = .08). Two and four hours after milk replacer intake, the median AUC of H was significantly higher (AUC_{120} (H) = 3,314 µg*min/mL (25/75 quartiles: 2,410/3,976 µg*min/mL), AUC_{240} (H) = 9,100 µg*min/mL (25/75 quartiles: 6,691/10,759 µg*min/mL) than in D and DORS. A trend (p = .06) for a lower AP AUC_{720} was found for D in comparison with H (Table 3). Within 720 min of the sampling period, the median AP concentrations did not reach the basal values measured before the experimental meal intake within all groups (Table 4).

4 | DISCUSSION

In the present study, we used calves with naturally occurring diarrhoea, which was reflected by diarrhoea-causing pathogens. All calves suffering from diarrhoea were infected by either *Rota virus* or *Cryptosporidium parvum*. Although some healthy calves also tested positive for diarrhoea-causing pathogens, the infection did not lead to profound diarrhoea. During the examination period, all healthy

TABLE 1 Vital parameter findings (data are expressed as the means ± *SD*) and the distribution of diarrhoeic pathogens from the faecal samples of each group (data are expressed as numbers)

Item	D (*n* = 15)	DORS (*n* = 15)	H (*n* = 16)	HORS (*n* = 14)	P
Vital parameters					
Rectal temperature [°C]	39.2 ± 0.51	38.9 ± 0.54	38.9 ± 0.54	38.9 ± 0.26	.74
Heart rate [bpm]	113 ± 15.6	111 ± 13.6	117 ± 12.4	107 ± 11.3	.21
Respiratory rate [bpm]	46.4 ± 15.0	50.9 ± 10.9	45.9 ± 11.1	47.6 ± 12.7	.68
Diarrhoeic pathogens					
Rota virus	13	14	5	5	
Corona virus	3	3	0	2	
Escherichia coli	2	4	1	0	
Cryptosporidium parvum	15	12	4	2	

TABLE 2 Parameters of the blood gas analysis of select calves from each group (*n* = 5) before test meal intake (data are expressed as the means ± *SD*)

Item	D	DORS	H	HORS	*P*
pH (T)	7.34 ± 0.04^{a}	7.37 ± 0.04^{ab}	7.40 ± 0.03^{b}	7.39 ± 0.28^{b}	.02
Hct [%]	30.8 ± 3.87	27.5 ± 2.69	26.0 ± 2.28	32.8 ± 9.99	.17
Na^+ [mmol/L]	136 ± 1.03	134 ± 6.77	137 ± 1.63	139 ± 2.61	.22
K^+ [mmol/L]	4.61 ± 0.40	4.63 ± 0.32	4.71 ± 0.23	4.72 ± 0.44	.92
Ca^{2+} [mmol/L]	1.38 ± 0.04	1.31 ± 0.07	1.36 ± 0.02	1.35 ± 0.03	.12
Cl^- [mmol/L]	101 ± 2.58	96.7 ± 5.96	100 ± 2.77	98.0 ± 3.74	.22
Hb [g/L]	100 ± 12.3	89.0 ± 8.25	83.3 ± 7.34	93.8 ± 13.3	.07
HCO_3^- [mmol/L]	27.4 ± 3.25^{a}	28.9 ± 4.64^{a}	31.1 ± 2.51^{ab}	33.3 ± 4.05^{b}	.05
ABE [mmol/L]	2.20 ± 3.42^{a}	3.95 ± 4.40^{ab}	6.55 ± 2.11^{b}	7.93 ± 3.59^{b}	.04
Anion Gap (K^+) [mmol/L]	12.4 ± 2.36	13.6 ± 3.79	11.2 ± 1.80	12.4 ± 3.79	.60

Note: Means in the same row with unlike superscripts are different with $p < .05$.

TABLE 3 Indices from APAT in H (*n* = 16), HORS (*n* = 14), D (*n* = 15) and DORS (*n* = 15) calves after test meal intake; data are expressed as medians and 25/75 quartiles (in brackets)

Item	D (*n* = 15)	DORS (*n* = 15)	H (*n* = 16)	HORS (*n* = 14)
C_{max} [µg/mL]	49.8 (41.9/55.5)^{a}	55.8 (47.3/79.6)^{a}	60.6 (48.9/71.2)^{a}	56.2 (44.9/62.1)^{a}
T_{max} [min]	300 (240/390)^{ab}	390 (300/480)^{b}	270 (210/315)^{a}	300 (240/360)^{a}
$t_{1/2}$(AP) [min]	591 (452/680)^{ab}	652 (445/795)^{b}	482 (374/626)^{a}	465 (422/554)^{a}
AUC_{60} [(µg*min)/mL]	777 (599/864)^{a}	642 (449/869)^{a}	976 (620/1147)^{a}	811 (563/1002)^{a}
AUC_{120} [(µg*min)/mL]	2,336 (2,079/2,727)^{ab}	2,154 (1,577/2,537)^{a}	3,314 (2,410/3,976)^{cb}	2,700 (1,623/3,197)^{b}
AUC_{240} [(µg*min)/mL]	6,518 (5,789/6,915)^{ab}	6,957 (4,235/7,713)^{a}	9,100 (6,691/10,759)^{b}	7,500 (5,012/9,222)^{ab}
AUC_{720} [(µg*min)/mL]	21,771 (18,932/26,130)^{a,*}	25,038 (19,460/32,286)^{ab}	26,544 (23,198/34,204)^{b,*}	26,224 (19,928/29,471)^{ab}

Note: Medians in the same row with unlike small superscripts are different with $p < .05$.
$^{a}p = .06$.

calves fulfilled the inclusion criteria (faecal score ≤ 1). However, it was not in the scope of the present study to determine whether the calves suffered from diarrhoea after finishing the observation period. In addition to including calves in D and DORS with a faecal score ≥ 2, we had to ensure that all calves voluntarily consumed 2 L of the test meal. To ensure this premise, the calves that suffered from profound diarrhoea leading to a severe state of lethargy and inappetence were not included in the study.

In this study, diarrhoea led to significant changes in the blood buffering system, such as in HCO_3^- and ABE, which were lower in D and DORS than in the other groups. Blood pH was significantly lower in D than H and HORS, whereas blood pH in DORS was similar to the different groups. These results confirmed an impaired acid-base balance at least in D but to a lesser extent in DORS. The APAT model was used to determine the AER indices that have already been described in previous studies with healthy ruminants and healthy

TABLE 4 Acetaminophen concentrations [μg/mL] depending on time after suckling test meal [min]; data are expressed as medians and 25/75 quartiles (in brackets)

Time	D (*n* = 15)	DORS (*n* = 15)	H (*n* = 16)	HORS (*n* = 14)
30	14.5 (12.0/17.9)^{a*}	11.6 (7.6/16.7)^{a*}	16.9 (10.9/23.6)^{a*}	15.3 (9.8/21.0)^{a*}
60	20.2 (16.0/25.8)$^{a\#}$	18.7 (14.5/26.9)$^{a\#}$	29.2 (17.7/33.5)$^{a\#}$	23.9 (14.7/33.6)$^{a\#}$
90	29.1 (25.8/32.4)$^{a\#}$	23.2 (20.4/33.5)$^{a\#}$	46.4 (30.3/53.5)$^{b\#}$	30.4 (20.3/42.1)$^{a\#}$
120	26.2 (19.0/29.3)$^{a\#}$	24.5 (16.5/31.3)$^{ab\#}$	37.1 (29.4/49.1)$^{b\#}$	30.1 (24.0/44.3)$^{b\#}$
150	30.0 (21.9/34.2)$^{ab\#}$	30.9 (20.1/36.4)$^{a\#}$	40.4 (35.4/52.5)$^{b\#}$	36.4 (28.0/46.2)$^{ab\#}$
180	31.7 (24.4/39.6)$^{ab\#}$	34.8 (22.1/42.4)$^{ab\#}$	48.5 (35.2/60.1)$^{ab\#}$	40.0 (25.9/46.2)$^{ab\#}$
210	36.5 (26.5/40.5)$^{a\#}$	41.8 (23.8/45.6)$^{a\#}$	52.5 (39.6/61.3)$^{b\#}$	44.8 (31.5/52.7)$^{ab\#}$
240	39.7 (25.9/46.6)$^{ab\#}$	37.4 (26.3/45.8)$^{ab\#}$	48.4 (37.9/64.8)$^{ab\#}$	45.5 (31.7/56.2)$^{ab\#}$
270	41.5 (31.8/47.7)$^{a\#}$	40.6 (23.2/57.5)$^{a\#}$	49.9 (41.6/60.7)$^{a\#}$	49.2 (30.9/56.9)$^{a\#}$
300	39.0 (25.2/46.3)$^{a\#}$	45.1 (23.3/54.8)$^{a\#}$	45.4 (40.6/61.3)$^{a\#}$	45.8 (39.3/53.9)$^{a\#}$
330	42.2(33.0/49.8)$^{a\#}$	35.3 (29.0/48.2)$^{a\#}$	49.6 (40.3/57.4)$^{a\#}$	44.9 (34.3/53.4)$^{a\#}$
360	36.0 (33.0/40.5)$^{a\#}$	45.6 (31.8/51.3)$^{a\#}$	45.7 (40.0/55.3)$^{a\#}$	41.0 (34.9/52.5)$^{a\#}$
390	37.0 (30.2/45.0)$^{a\#}$	46.7 (30.9/55.8)$^{a\#}$	45.6 (39.4/56.3)$^{a\#}$	40.3 (35.4/46.5)$^{a\#}$
420	35.5 (29.5/45.3)$^{a\#}$	43.8 (30.8/52.0)$^{a\#}$	39.2 (36.9/50.0)$^{a\#}$	38.4 (31.3/46.7)$^{a\#}$
450	31.5 (27.2/41.8)$^{a\#}$	40.8 (33.2/47.9)$^{a\#}$	37.6 (32.3/46.2)$^{a\#}$	34.2 (31.6/43.3)$^{a\#}$
480	31.0 (24.7/36.0)$^{a\#}$	35.1 (29.3/45.0)$^{a\#}$	34.7 (30.7/44.1)$^{a\#}$	36.0 (29.7/42.9)$^{a\#}$
540	23.0 (21.0/37.5)$^{a\#}$	37.3 (29.9/47.4)$^{a\#}$	31.0 (24.2/46.3)$^{a\#}$	29.0 (25.8/33.8)$^{a\#}$
600	22.9 (18.0/31.3)$^{a\#}$	30.4 (20.5/39.0)$^{a\#}$	29.0 (22.2/36.5)$^{a\#}$	26.7 (21.3/38.0)$^{a\#}$
660	28.3 (23.4/37.1)$^{a\#}$	38.3 (26.2/57.9)$^{a\#}$	34.1 (26.2/44.3)$^{a\#}$	31.2 (25.6/50.8)$^{a\#}$
720	27.5 (20.9/33.5)$^{a\#}$	30.9 (24.7/47.3)$^{a\#}$	29.7 (24.8/38.4)$^{a\#}$	27.1 (19.5/48.4)$^{a\#}$

Note: Medians in the same row with unlike small superscripts are different with $p < .05$.
Medians in the same column with unlike symbols are different with $p < .05$ in comparison with time 30.

calves (Ehsani-Kheradgerdi, Sharifi, & Mohri, 2011; Marshall et al., 2005; Nouri & Constable, 2006). Calves are functional monogastric individuals with a biphasic gastric emptying which is best described by the equation of Siegel (Marshall et al., 2005). A delayed AP uptake leads to higher AP $t_{1/2}$ under the assumption of undisturbed liver metabolism. In the present study, differences in the abomasal AP $t_{1/2}$ and AUC were observed in diarrhoeic calves compared with healthy calves, which might be explained by a reduction in abomasal motility. In particular, initial AUC values from 0 to 120 min and 0 to 240 min post-suckling were significantly lower in D and DORS compared with those in H. Interestingly, considering the entire observation period, basal AP levels were not reached at t720 min in all groups. However, a trend for a lower AP AUC was found for D in comparison with H. It remained open whether a delay in abomasal emptying or/and a reduced absorption capacity are reflected by these findings. A delay in abomasal emptying was supported by the similar C_{max} values and different T_{max} in all groups, as the AP peaks reached their maximum later in DORS than in H and HORS. Marshall et al. (2005) postulated that the time to reach maximum serum AP levels was the most accurate index to describe AER. Further, lower abomasal pressure data and higher abomasal temperature data were described in diarrhoeic calves than in healthy calves (Hildebrandt et al., 2017).

After fluid meal intake changes in TPP concentrations and the calculation of the plasma volume are also used as a tool to describe the abomasal emptying Bachmann et al. (2012) described changes in plasma volume dependent on test meals with different osmolarities. In our study, we determined the changes in plasma volume dependent on health status in combination with test meals with and without ORS. Interestingly, the changes in plasma volume were faster in H and HORS compared with diarrhoeic calves which may also reflect an impaired AER. In summary, based on the AER indices, the abomasum in diarrhoeic calves showed a delayed emptying rate compared with that in healthy calves. In our previous study, we postulated that the higher intraluminal temperatures in diarrhoeic calves fed with 2 L of milk replacer were the result of bacterial fermentation due to a delay in abomasal emptying. Consequently, fermentation products such as SCFA might impair abomasal motility by damaging the abomasal mucosa. Other mechanisms such as interference with the enteric nerve cells by metabolites are possible explanations; however, data are missing. It remains unclear whether larger test meals or more severe diarrhoea may have more significant effects on abomasal emptying. However, AP absorption might be challenged in diarrhoeic calves because of an impaired AP absorption in the proximal small intestine due to mucosa damage (Prescott, 1980) induced by infection such as by *Rota virus* or *Cryptosporidium parvum* (García et al., 2000; Zu, Fang, Fayer, & Guerrant, 1992). But present data evidenced that APAT data reflect a delay in abomasal emptying rather than an impaired absorption as indicated by similar median Cmax and median AUCs of AP between 0 and 720 min postprandial. Profound diarrhoea leads to significant losses of electrolytes, which results in

metabolic acidosis. The supplementation of ORS is considered the most reliable treatment to compensate for electrolyte losses and to balance the acid-base status (Bachmann et al., 2012; Kirchner et al., 2015; Tsukano, Ajito, Abe, & Sarashina, 2017). Currently, different feeding protocols are published for the supplementation of ORS in diarrhoeic calves and equivocal results have been obtained. Rademacher, Lorenz, & Klee, (2002) recommended alternating the administration of milk replacer and ORS in at least 2-hr intervals to avoid milk clotting imbalances. In accordance, Miyazaki, Okada, Yamashita, and Miyazaki (2019) found an impaired formation of abomasal curd and a delay in rehydration in healthy calves fed a 50% ORS-milk.

In contrast, the administration of ORS mixed in milk replacer did not impair milk clotting (Bachmann et al., 2009, 2012; Kirchner et al., 2015).

Sen et al. (2006) described changes in AER when comparing different ORS mixed into tap water and all-protein milk replacer in healthy calves. Hypertonic bicarbonate solutions or milk replacers delayed emptying compared with isotonic bicarbonate solutions, which highlights that osmolality has a delaying effect on AER. Likewise, the impact of osmolality on AER has been discussed by Kirchner et al. (2015), who found changes in abomasal diameter after feeding different ORS protocols to calves with naturally occurring diarrhoea. In addition, by the ORS supplementation a 10% higher energy intake had been added to the diet. The impact of caloric content on AER has been discussed in humans (Hunt et al., 1985). In calves, Bell and Webber (1979) used an infusion model with different monosaccharides into the duodenum, and it was concluded that the energy content was not a primary determinant of AER. In conclusion, a 10% higher energy intake by ORS on AER remained open in our study. In the present study, ORS did not delay AER in healthy calves (H vs. HORS), as no significant differences were observed in the abomasal emptying indices. However, with naturally occurring diarrhoea, the AP $t_{1/2}$ of DORS was significantly prolonged compared to H and HORS and tended to be prolonged compared to D (p = .05). Therefore, our data support the hypothesis that a higher osmolality induced by mixing the hypertonic ORS with milk replacer impairs AER, at least in diarrhoeic calves. As abomasal emptying was delayed in diarrhoeic calves, small meal sizes are recommended to avoid abomasal congestion. In diarrhoeic calves, the provision of ORS is highly recommended to replace electrolyte and fluid losses, but as high osmolality had an impact on AER, the effect of different concentrations of ORS on AER needs further elucidation.

ACKNOWLEDGEMENTS

The authors thank Neue Salower Milchviehbetriebs GmbH & Co KG for providing the calves.

CONFLICT OF INTEREST

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

ANIMAL WELFARE STATEMENT

This study was approved by the State Department of Agriculture, Food Safety and Fisheries of Mecklenburg-Western Pomerania, Germany (AZ 7221.3–1-018/15) and followed the guidelines for Animal Experiments of University Leipzig.

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REFERENCES

Ahmed, A. F., Constable, P. D., & Misk, N. A. (2002). Effect of feeding frequency and route of administration on abomasal luminal pH in dairy calves fed milk replacer. *Journal of Dairy Science*, *85*(6), 1502–1508. https://doi.org/10.3168/jds.S0022-0302(02)74219-7

Bachmann, L., Homeier, T., Arlt, S., Brueckner, M., Rawel, H., Deiner, C., & Hartmann, H. (2009). Influence of different oral rehydration solutions on abomasal conditions and the acid-base status of suckling calves. *Journal of Dairy Science*, *92*(4), 1649–1659. https://doi.org/10.3168/jds.2008-1487

Bachmann, L., Schmidt, B., Rauwolf, U., Wenge, J., & Coenen, M. (2012). Change of plasma volume, osmolality, and acid–base status in healthy calves after feeding of milk and water- and milk-based oral rehydration solutions. *Journal of Dairy Science*, *95*(10), 6006–6014. https://doi.org/10.3168/jds.2012-5562

Bell, F. R., & Webber, D. E. (1979). A comparison of duodenal osmolality and energy content as controlling factors of gastric emptying in the calf. *The Journal of Physiology*, *297*(1), 379–385. https://doi.org/10.1113/jphysiol.1979.sp013046

Burgstaller, J., Wittek, T., & Smith, G. W. (2017). Invited review: Abomasal emptying in calves and its potential influence on gastrointestinal disease. *Journal of Dairy Science*, *100*(1), 17–35. https://doi.org/10.3168/jds.2016-10949

Ehsani-Kheradgerdi, A., Sharifi, K., Mohri, M., & Grünberg, W. (2011). Evaluation of a modified acetaminophen absorption test to estimate the abomasal emptying rate in Holstein-Friesian heifers. *American Journal of Veterinary Research*, *72*(12), 1600–1606. https://doi.org/10.2460/ajvr.72.12.1600

García, A., Ruiz-Santa-Quiteria, J. A., Orden, J. A., Cid, D., Sanz, R., Gómez-Bautista, M., & De La Fuente, R. (2000). Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. *Comparative Immunology, Microbiology and Infectious Diseases*, *23*(3), 175–183. https://doi.org/10.1016/S0147-9571(99)00071-5

Hildebrandt, T., Scheuch, E., Weitschies, W., Grimm, M., Schneider, F., Bachmann, L., & Vervuert, I. (2017). Measurement of abomasal pH, luminal pressure and temperature in healthy and diarrheic dairy calves using a wireless ambulatory capsule. *Livestock Science*, *203*, 41–47.

Hunt, J. N., & Knox, M. T. (1968). A relation between the chain length of fatty acids and the slowing of gastric emptying. *The Journal of Physiology*, *194*(2), 327–336. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5639357. https://doi.org/10.1113/jphysiol.1968.sp008411

Hunt, J. N., Smith, J. L., & Jiang, C. L. (1985). Effect of meal volume and energy density on the gastric emptying of carbohydrates. *Gastroenterology*, *89*(6), 1326–1330. https://doi.org/10.1016/0016-5085(85)90650-X

Kirchner, D., Schwedhelm, L., Wenge, J., Steinhöfel, I., Heinrich, C., Coenen, M., & Bachmann, L. (2015). Ultrasonographic imaging of abomasal milk clotting and abomasal diameter in healthy and diarrheic calves. *Animal Science Journal*, *86*(11), 929–936. https://doi.org/10.1111/asj.12382

Marshall, T. S. (2009). Abomasal Ulceration and Tympany of Calves. *Veterinary Clinics of North America: Food Animal Practice*, *25*(1), 209–220. https://doi.org/10.1016/j.cvfa.2008.10.010

Marshall, T. S., Constable, P. D., Crochik, S. S., & Wittek, T. (2005). Determination of abomasal emptying rate in suckling calves by use of nuclear scintigraphy and acetaminophen absorption. *American Journal of Veterinary Research*, *66*(3), 364–374. https://doi.org/10.2460/ajvr.2005.66.364

Miyazaki, T., Okada, K., Yamashita, T., & Miyazaki, M. (2019). Temporal changes of abomasal contents and volumes in calves fed milk diluted with oral rehydration solution. *Journal of Veterinary Medical Science*, *81*(2), 256–262.

Nadeau, J. A., Andrews, F. M., Patton, C. S., Argenzio, R. A., Mathew, A. G., & Saxton, A. M. (2003). Effects of hydrochloric, valeric, and other volatile fatty acids on pathogenesis of ulcers in the nonglandular portion of the stomach of horses. *American Journal of Veterinary Research*, *64*(4), 413–417. https://doi.org/10.2460/ajvr.2003.64.413

Nouri, M., & Constable, P. D. (2006). Comparison of two oral electrolyte solutions and route of administration on the abomasal emptying rate of Holstein-Friesian calves. *Journal of Veterinary Internal Medicine/American College of Veterinary Internal Medicine*, *20*(3), 620–626. https://doi.org/10.1111/j.1939-1676.2006.tb02906.x

Prescott, L. F. (1980). Kinetics and metabolism of paracetamol and phenacetin. *British Journal of Clinical Pharmacology*, *10*, 291S–298S. https://doi.org/10.1111/j.1365-2125.1980.tb01812.x

Sen, I., Constable, P. D., & Marshall, T. S. (2006). Effect of sucklign isotonic or hypertonic solutions of sodium bicarbonate or glucose on abomasal emptying rate in calves. *Journal of Chemical Information and Modeling*, *53*, 1689–1699. https://doi.org/10.1017/CBO9781107415324.004

Siegel, J. A., Urbain, J. L., Adler, L. P., Charkes, N. D., Maurer, A. H., Krevsky, B., … Malmud, L. S. (1988). Biphasic nature of gastric emptying. *Gut*, *29*(1), 85–89. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artxml:id=1433265&tool=pmcentrez&rendertype=abstract. https://doi.org/10.1136/gut.29.1.85

Rademacher, G., Lorenz, I., & Klee, W. (2002). Tränkung und Behandlung von Kälbern mit Neugeborenendurchfall. *Tierärztliche Umschau*, *57*, 177–189.

Tsukano, K., Ajito, T., Abe, I., Sarashina, S., & Suzuki, K. (2017). Rehydration and catabolic preventive effects depend on the composition of oral electrolyte solutions for diarrheic calves. *Journal of Veterinary Medical Science*, *79*(11), 1776–1779. https://doi.org/10.1292/jvms.17-0398

Van Beaumont, W., Greenleaf, J. E., & Juhos, L. (1972). Disproportional changes in hematocrit, plasma volume, and proteins during exercise and bed rest. *Journal of Applied Physiology*, *33*(1), 55–61. https://doi.org/10.1152/jappl.1972.33.1.55

Walker, P. G., Constable, P. D., Morin, D. E., Drackley, J. K., Foreman, J. H., & Thurmon, J. C. (1998). A reliable, practical, and economical protocol for inducing diarrhea and severe dehydration in the neonatal calf. *Canadian Journal of Veterinary Research = Revue Canadienne De Recherche Vétérinaire*, *62*(3), 205–213. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artxml:id=1189477&tool=pmcentrez&rendertype=abstract

Wenge, J., Steinhöfel, I., Heinrich, C., Coenen, M., & Bachmann, L. (2014). Water and concentrate intake, weight gain and duration of diarrhea in young suckling calves on different diets. *Livestock Science*, *159*, 133–140. https://doi.org/10.1016/j.livsci.2013.11.004

Windeyer, M. C., Leslie, K. E., Godden, S. M., Hodgins, D. C., Lissemore, K. D., & LeBlanc, S. J. (2014). Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Preventive Veterinary Medicine*, *113*(2), 231–240. https://doi.org/10.1016/j.prevetmed.2013.10.019

Wittek, T., Constable, P. D., Marshall, T. S., & Crochik, S. S. (2005). Ultrasonographic measurement of abomasal volume, location, and emptying rate in calves. *American Journal of Veterinary Research*, *66*(3), 537–544. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15822600. https://doi.org/10.2460/ajvr.2005.66.537

Wittek, T., Schreiber, K., Fürll, M., & Constable, P. D. (2005). Use of the D-xylose absorption test to measure abomasal emptying rate in healthy lactating Holstein-Friesian cows and in cows with left displaced abomasum or abomasal volvulus. *Journal of Veterinary Internal Medicine / American College of Veterinary Internal Medicine*, *19*(6), 905–913. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16355689. https://doi.org/10.1111/j.1939-1676.2005.tb02786.x

Zu, S. X., Fang, G. D., Fayer, R., & Guerrant, R. L. (1992). Cryptosporidiosis - Pathogenesis and Immunology. *Parasitology Today*, *8*(1), 24–27. https://doi.org/10.1016/0169-4758(92)90307-N

How to cite this article: Hildebrandt T, Scheuch E, Weitschies W, et al. Abomasal emptying rate of diarrhoeic and healthy suckling calves fed with oral rehydration solutions. *J Anim Physiol Anim Nutr*. 2020;104:462–469. https://doi.org/10.1111/jpn.13306