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Measurement of abomasal conditions (pH, pressure and temperature) in healthy and diarrheic dairy calves using a wireless ambulatory capsule

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ABSTRACT

This study investigated abomasal luminal parameters in healthy and diarrheic calves by using a wireless ambulatory capsule (WAC). The acetaminophen absorption test (APAT) was used to determine abomasal emptying rate. Four healthy and five diarrheic female Holstein-Friesian calves (age < 14 days) were included in the study. For APAT, calves were fed 2 L of milk replacer containing 50 mg acetaminophen/kg body weight, and blood samples were taken during a 12-h period afterward. Concomitantly, a WAC in the abomasum continuously measured luminal pH, pressure, and temperature. Five hours post suckling, intraluminal temperature was significantly higher in diarrheic calves than in healthy calves. Abomasal pH and pressure were not significantly different, but intraluminal pressure was always numerically lower in diarrheic calves. During APAT no significant differences in maximum acetaminophen concentrations (C_{max}) and time to reach maximum acetaminophen concentration (T_{max}) were observed. Nonlinear regression findings revealed a longer acetaminophen half-time (AAP $t_{1/2}$) in diarrheic calves compared to healthy calves [564 ± 96 min vs. 393 ± 84 min, respectively; $P = 0.04$] and lower area under the concentration curve values (e.g., 60 min postprandial AUC_{60} 681 ± 244 (μg·min)/mL vs. 1064 ± 23 (μg·min)/mL, respectively; $P = 0.04$). In conclusion, abomasal luminal conditions were different between diarrheic and healthy calves. Significant differences in APAT reflected a delay in abomasal emptying in diarrheic calves. Impaired abomasal movement may induce enhanced bacterial fermentation processes as indicated by a higher abomasal temperature in diarrheic calves, which should be considered in management of their feeding.

1. Introduction

Neonatal diarrhea is the main cause of death during the first 2 weeks of life in calves (Torsein et al., 2014). Furthermore, diarrhea results in poor growth performance and increases the susceptibility to other infections (Windeyer et al., 2014). Kirchner et al. (2015) recently reported that neonatal diarrhea affects abomasal motility, which consequently delays the abomasal emptying rate (AER) after milk intake. Impaired abomasal motility may increase bacterial fermentation and the production of short-chain fatty acids (SCFAs) (Nouri and Constable, 2006), and luminal temperature may rise as a result of bacterial fermentation. The production of SCFAs contributes to the inhibition of abomasal motility by affecting chemoreceptors in the abomasal epithelium (Leek, 1977; Crichlow and Leek, 1986; Crichlow, 1988). In addition, growth of gas-producing bacteria such as *Clostridium perfringens*, *Sarcina ventriculi*, and *Lactobacillus* spp. is considered to be a predisposing factor for abomasal tympany (Marshall, 2009). SCFAs have been found to impair the sodium transport system of the gastric mucosa of equines under in vitro conditions (Nadeau et al., 2003), leading to an osmotic influx of water into the cells and swelling and degradation of the mucosa (Argenzio, 1999; Carney et al., 1981). Abomasal conditions can be characterized by several intraluminal

Abbreviations: AAP, Acetaminophen; AER, Abomasal emptying rate; APAT, Acetaminophen absorption test; AUC, Area under the concentration curve; SD, Standard deviation; SCFA, Short-chain fatty acids; WAC, Wireless ambulatory capsule

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parameters such as pH, temperature, and abomasal pressure. As previously described, intraluminal pH is determined by invasive methods such as cannulation or through post-mortem study (Bachmann et al., 2009; Constable et al., 2009). A noninvasive wireless ambulatory capsule (WAC) for intraluminal pH, pressure, and temperature measurements has been already described in ponies (Stokes et al., 2012), dogs (Boillat et al., 2010), and humans (Koziolek et al., 2015). To our knowledge, noninvasive WAC measurements have not been described in calves. To investigate the AER, a variety of methods have been used, such as the acetaminophen absorption test (APAT, Constable et al., 2009), D-xylose absorption test (Wittek et al., 2005a, 2005b), scintigraphic evaluation (Marshall et al., 2005), and ultrasonographic measurements (Sen et al., 2006; Wittek et al., 2005a). In particular, the APAT has been evaluated as an appropriate nonradioactive method in cows (Wittek et al., 2009), heifers (Ehsani-Kheradgerdi et al., 2011), and healthy calves (Constable et al., 2009; Marshall et al., 2005; Sen et al., 2006). Acetaminophen (AAP) is absorbed in the proximal small intestine (Prescott, 1980), and it serves as an accurate parameter of gastric (Snyder et al., 2014) or abomasal emptying in healthy animals (Marshall et al., 2005; Nouri and Constable, 2006; Sen et al., 2006). However, to our knowledge data about diarrheic calves and the AER are lacking.

The aim of the study was to investigate abomasal conditions (abomasal pH, luminal pressure temperature and emptying rate) in healthy and diarrheic calves using WAC tool and APAT. We hypothesized that abomasal emptying is delayed in diarrheic calves compared to healthy calves. In consequence abomasal conditions differ with respect to abomasal pH, temperature, and luminal pressure.

2. Materials and methods

2.1. Animals

Nine Holstein-Friesian suckling calves [female; 4–14 days old, mean age (± SD): 7.7 ± 3.5 d; mean body weight (BW ± SD): 43.7 ± 6.3 kg] from one farm were used in this study; four were healthy, and five were diarrheic. The calves were housed in individual stalls from birth until 14 days postpartum (length × width × height; 1.90 m × 1.14 m × 1.35 m) and bedded on straw. Except for the experimental day the animals were fed twice daily with 4 L of combined skimmed milk and whey protein milk replacer containing ingredients as labeled: crude protein, 20.0%; crude fat, 17.0%; crude ash, 7.2%; phosphorus, 0.7%; calcium, 0.8%; sodium, 0.6% (FOK TOP, Alpuro Breeding). The animals had free access to water except for the day of the measurements.

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.

2.2. Experimental design

The study was performed in subsequent order including healthy calves as controls and calves with naturally occurring diarrhea. Inclusion criteria for the healthy group were a fecal score ≤ 1 on a scale of 0–3 according to Walker et al. (1998) A score of 0–1 indicated well-formed feces up to abnormal feces that tend to be pasty. Inclusion criteria for the diarrhea group were a fecal score ≥ 2 on a scale of 0–3. A score of ≥2 describes pasty feces, mainly liquid with solids up to liquid feces. Calves had diarrhea for 24 h before the experimental day. Animals suffering from other diseases such as pneumonia or omphalitis (see clinical examination) were excluded from the study. After the experimental day diarrheic calves were treated with oral rehydration solutions, state of diarrhea improved without any medical intervention.

Calves were equipped with a WAC to determine abomasal pH, pressure, and temperature. The APAT was performed in each animal to analyze AER.

2.3. Clinical assessment

In the morning before sampling all calves were weighed on an electronic scale. Clinical examinations included the measurement of heart rate and lungs (auscultation), respiratory rate (counting) and rectal temperature (electronic thermometer). Calves were manually checked for omphalitis.

2.4. Acetaminophen absorption test

After a fasting period of 12 h calves were fed 2 L of a milk replacer by a bucket with a teat according to the protocol previously published by Marshall et al. (2005). Healthy calves were fed with 49.1 ± 4,4 mL/kg BW, and diarrheic calves were fed with 44.6 ± 4.4 mL/kg BW (amount of feeding per kg BW: $p = 0.31$). Fifty milligrams acetaminophen (AAP)/kg BW (Pracetam, Animedica) was mixed into the milk replacer. The mean (± SD) milk replacer temperature was 12.7 ± 2.9 °C when provided to the animals.

2.5. Feces collection

Rectal fecal samples were collected once during APAT.

2.6. Blood collection

Before the animals were fed, an indwelling catheter [14 G, 6.4 cm (2.5 in.)] was inserted into the right or left jugular vein after the skin was shaved and disinfected. The catheter was flushed with physiological saline after each blood sampling. Blood samples were collected 30 min before the test meal was fed to the calves and then at 30-min intervals for 8 h and at 60-min intervals for another 4 h. Blood was collected into tubes containing lithium heparin (10 mL Monovette, Sarstedt). Immediately after blood collection, lithium heparin tubes were centrifuged at 3000*g* for 10 min. Plasma was removed and stored at −20 °C until analysis. Additionally one venous blood sample was taken 30 min preprandially into a 3-mL syringe (PICO 50, Radiometer) containing heparin for oxymetric analysis.

2.7. Administration of WAC

Before administration of a WAC, the ventral abdominal region of each calf was shaved. All capsules (13 mm × 26 mm) were calibrated with a buffer solution containing citric acid (pH 6.0) and activated by the appropriate software tool (MotiliGI, SmartPill Corp). The pH, temperature and pressure sensors in the WAC

send data at 434 MHz and, according to the manufacturer's information, the WAC measures pH from 0.5 to 9.0 pH units with an accuracy of ± 0.5 units; pressure from 0 to 350 mmHg with an accuracy of ± 5 mmHg; and temperature from 25 to 49 °C with accuracy of ± 1 °C. In the case of pressure, the software provided a baseline correction. In our study we used the temperature compensated data as these data considered real values measured in the animal (Koziolek et al., 2015). The capsule was administered to the calves with a modified pill applicator before feeding them the milk replacer. A data receiver was fixed on the left side of each calf with an elastic bandage (CoFlex Vet, Andover). The successful placement of the capsule into the abomasum was verified by ultrasonographic imaging using a 3.5-Hz sector probe (A6Vet Sonoscape, Sonoring, see Supplementary file 1) 30 min after WAC application and afterwards at 60-min intervals for at least 12 h after milk intake. Approximately every 30 s the integrated antenna of the WAC sent pH, temperature, and pressure sensor information to the receiver. According to the manufacturer's information, the pH sensor took measurements every 5 s in a range of 0.5–9 units with an accuracy of ± 0.5 units. The temperature was measured every 20 s in a range from 25 to 49 °C, with an accuracy of ± 1 °C. Pressure data were taken at a frequency of 2 Hz in a range from 0 to 350 mm Hg

(± 5 mm Hg). The pressure data are expressed in kilopascals. Artificial pressure peaks due to manipulations of the examiner were marked by pressing the 'Event' button on the data receiver. These data were excluded from the statistics. 90% of the collected data were measured during a lying position of the calf.

2.8. Feces analysis

Feces were analyzed for *Rota virus*, *Corona virus*, *Escherichia coli*, and *Cryptosporidium parvum* by a commercial enzyme-linked immunosorbent assay (Fassisi BoDia, Fassisi).

2.9. Blood analysis

Blood plasma was analyzed by liquid chromatography–mass spectrometry analysis to determine AAP concentration.

The maximum AAP concentration (C_{max}) and time to maximum AAP concentration (T_{max}) after oral AAP application are primarily dependent on the AER correlating with a faster rate of absorption compared with the rate of elimination. An AAP time curve was generated by using the first derivative of Siegel's modified power exponential equation as follows:

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

where C is the calculated AAP concentration (µg/mL) depending on the time t (time from the start of suckling in minutes). The constant m is the total cumulative recovery of AAP when the time is infinite; the constant k is the estimated rate of abomasal emptying (min^{-1}), and constant β is an estimate of duration of the lag phase before the exponential emptying phase is reached. All constants were determined through nonlinear regression analysis. Acetaminophen abomasal half-time was calculated by applying the generated constants into following equation:

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

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

Area under the curve (AUC) data were determined from the AAP concentration–time plot from 0 to 60 min, 0–120 min, 0–240 min, and 0–720 min calculated by the trapezoidal method using the following equation as previously described (Marshall et al., 2005):

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

where C_i is the AAP concentration at the postsuckling time t_i.

Before calves were fed, venous blood samples were analyzed oxymetrically by a blood gas analyzer (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, and hematocrit (Hct) Bicarbonate (HCO_3^-) was calculated according to the equation of Hasselbalch. Anion base excess and anion gap were calculated by a blood gas analyzing device.

Total plasma protein (TPP) was analyzed by refractometry (Euromax).

2.10. Statistical analysis

Data analysis was performed with a statistical software program (Statistica 7.1, StatSoft). Data were assessed for normal distribution by the Kolmogorov–Smirnov test. Outlier tests were performed, and data were excluded when they were assumed to be an error of assessment.

Normally distributed data were subjected to unpaired *t*-test. In case of nonnormal distribution of data, the data were subjected to Mann–Whitney *U* test. To calculate AAP half-time, AAP concentration data were analyzed by nonlinear regression. Data are expressed either as mean ± standard deviation (SD) considering normal distribution or as median, 25 and 75 percentiles in case of nonnormal distribution. Statistical significance was accepted at $P < 0.05$. A trend was postulated at $P < 0.10$.

3. Results

3.1. Feces and vital parameters

All healthy calves were tested negative for diarrhea-causing pathogens. Calves with diarrhea were tested positive for *Rota virus* and *Cryptosporidium parvum*. Three of the five diarrheic calves were infected with *Corona virus*, and *Escherichia coli* was found in two of five fecal samples (see Supplementary file 2).

Vital parameter findings were not significantly different between healthy and diarrheic calves. Mean (± SD) rectal temperature 39.0 ± 0.26 °C and 38.8 ± 0.53 °C for diarrheic and healthy calves, respectively. Mean (± SD) heart rate and mean (± SD) respiratory rate were similar for diarrheic and healthy calves: 105 ± 16 bpm vs. 125 ± 9 bpm, and 45 ± 20 bpm vs. 41 ± 4 bpm.

3.2. APAT

The mean (± SD) maximum AAP concentration for diarrheic calves was 45 ± 12 µg/mL (range, 29–51.5 µg/mL), which was not significantly different from that for healthy calves (59.5 ± 8 µg/mL; range, 50.7–66.4 µg/mL, $P = 0.13$; Fig. 1). Times to reach maximum AAP concentration (T_{max}) were similar in both groups (Table 1). Within the 720 min of the observation period, AAP concentrations did not reach basal values measured before milk replacer intake. The calculated AAP abomasal half-time (AAP $t_{1/2}$) was longer in diarrheic calves than in health calves (245 ± 42 min vs. 171 ± 36 min, respectively; $P = 0.04$). The AUCs were significantly higher in healthy calves than in diarrheic calves (Table 1).

3.3. Blood gas analysis

Blood pH, hematocrit, electrolytes, and anion gap were not significantly different between the two groups of calves (Table 2). Lower bicarbonate concentrations were observed in diarrheic calves (27.7 ± 3.4 mmol/L) compared to healthy calves (32.7 ± 2.6 mmol/L; $P = 0.04$). Furthermore, the anion base excess was lower in diarrheic calves (2.7 ± 3.8 mmol/L) than in healthy calves (7.8 ± 1.9 mmol/L; *P*

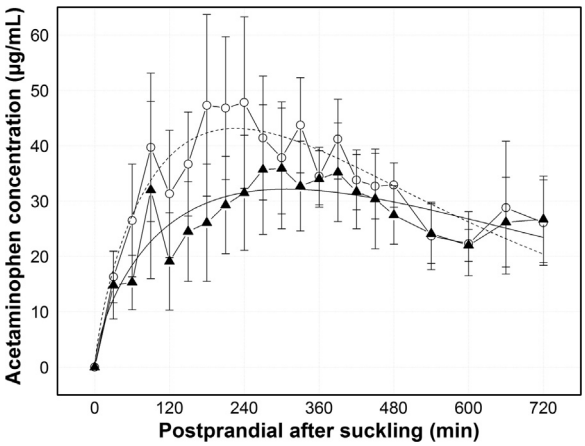

Fig. 1. Acetaminophen concentrations(µg/mL); O – healthy calves (H); Δ – diarrheic calves (D); dashed line – nonlinear regression curve of H; straight line – nonlinear regression curve of D, time $P < 0.001$, diagnosis $P = 0.208$, time*diagnosis $P = 0.127$ (data are expressed as means ± SD).

Table 1
Indices of abomasal emptying rate in healthy (n = 3) and diarrheic (n = 5) calves after test meal intake (data are expressed as means ± SD).

Item	Healthy	Diarrheic	*P*-value
Acetaminophen absorption			
C_{max} [μg/mL]	59.5 ± 8.03	44.7 ± 12.4	0.13
T_{max} [min]	210 ± 30.0	312 ± 132	0.25
$t_{1/2}$(AAP) [min]	393 ± 84	564 ± 96	0.04
AUC_{60} [(μg min)/mL]	1064 ± 23	681 ± 244	0.04
AUC_{120} [(μg min)/mL]	3416 ± 261	1929 ± 558	0.01
AUC_{240} [(μg min)/mL]	9245 ± 732	5089 ± 1586	0.01
AUC_{720} [(μg min)/mL]	24,986 ± 2752	18,989 ± 4203	0.07

Table 2
Parameters of blood gas analysis of healthy (n = 4) and diarrheic (n = 5) calves before test meal intake (data are expressed as means ± SD).

Item	Healthy	Diarrheic	*P*-value
Rectal temperature [°C]	38.8 ± 0.57	39.0 ± 0.26	0.60
pH (T)	7.41 ± 0.04	7.36 ± 0.05	0.18
Hct [%]	29.3 ± 1.50	28.6 ± 2.88	0.70
Na^+ [mmol/L]	138 ± 1.26	136 ± 1.14	0.14
K^+ [mmol/L]	4.74 ± 0.47	4.50 ± 0.15	0.73
Ca^{2+} [mmol/L]	1.34 ± 0.03	1.37 ± 0.05	0.40
Cl^- [mmol/L]	100 ± 3.30	100 ± 2.30	0.66
Hb [g/L]	94 ± 4.76	93 ± 9.33	0.85
HCO_3^- [mmol/L]	32.7 ± 2.59	27.7 ± 3.38	0.04
Anion base excess [mmol/L]	7.83 ± 1.87	2.74 ± 3.75	0.04
Anion gap (K^+) [mmol/L]	9.90 ± 2.03	12.6 ± 2.35	0.11

= 0.04).

3.3.1. TPP

Postprandial, TPP showed time related variations (P < 0.02), but TPP was not significantly different between the two groups of calves (data not shown).

3.4. WAC data reception

The ultrasonographic findings revealed that the WAC remained in the abomasum of each calf for the duration of the experiment (720 min postprandial). The mean reception rate (ratio between the collected data and calculated maximum of data within 720 min) of all WAC sensors was 74.8 ± 11.7% and ranged from 48.5% to 89.2%.

3.5. Abomasal pH

Immediately after feeding (t = 60 s post-suckling) mean (± SD) abomasal pH averaged 5.64 ± 1.01 with a minimum of 4.58 and a maximum of 6.60 in healthy calves and 6.11 ± 0.14 in diarrheic calves, with a minimum of 5.14 and a maximum of 6.26. Post prandial, the intraluminal pH decreased until reaching < 2 in both groups (Fig. 2) The mean time (± SD) to reach pH < 2 was similar in both groups after feeding (342 ± 50 min vs. 362 ± 61 min in diarrheic and healthy calves, respectively; *P* = 0.4). Six hours after feeding, pH averaged 1.95 ± 0.77 in healthy calves and 1.89 ± 0.63 in diarrheic calves. In three calves (one healthy calf, two diarrheic calves) pH values were below 1. The minimum pH of all WAC data was pH 0.61 detected at t = 620 min in a diarrheic calf.

3.6. Abomasal temperature

In the first hour after milk intake mean (± SD) minimum abomasal temperatures of 35.2 ± 2.1 °C and 35.7 ± 0.3 °C were detected in healthy and diarrheic calves, respectively. No significant difference was observed between the two groups. The maximum temperature was 40.13 °C at 545 min in a diarrheic calf. After feeding mean (± SD) abomasal temperature increased continuously until 39.0 ± 0.3 °C at 240 min in healthy calves and 39.6 ± 0.4 °C at 540 min in diarrheic calves. Abomasal temperatures of healthy and diarrheic calves were similar in the first 420 min postprandial. Within the last 5 h of examination (420–720 min postprandial) significantly higher temperatures were measured in diarrheic calves (Fig. 3).

3.7. Abomasal pressure

The median abomasal pressure was −4.1 kPa (−5.6/−3.1; 25th and 75th percentile) and ranged from −11.5 kPa at 658 min to 13.1 kPa at 530 min. During the whole observation period (0–720 min postprandial) abomasal pressure data were not significantly different between the two groups. One hour postsuckling, the median pressure was −2.8 kPa (−3.5/−1.7) in healthy calves and −3.1 kPa (−5.3/−2.7) in diarrheic calves. The median pressure continuously decreased until reaching −4.7 kPa (−6.1/−2.9) at 600 min in healthy calves and −6.0 kPa (−10.0/−4.0) in diarrheic calves (Fig. 4).

3.7.1. WAC excretion

Within 14–24 days after WAC application, the capsules were still found in the abomasum as confirmed by ultrasonic imaging. However, further monitoring was not in the target of the study.

4. Discussion

In the present study we used calves that appeared to be in an early phase of neonatal diarrhea as reflected by similar results for hematocrit and electrolytes compared to healthy calves but with significant lower bicarbonate concentrations and a lower base excess. Diarrheic calves were positively evaluated by clinical signs (fecal score) and diarrhea-causing pathogens. Only calves with voluntary feed intake were included to ensure an adequate milk intake of 2 L in all calves for APAT.

All calves tolerated the administration of the WAC by a pill applicator well. The WAC was inserted after calves suckled a small amount of the test meal, which provoked the reticular groove reflex. Fasting values of abomasal pH, temperature, and pressure were therefore not measured.

The appearance of the WAC in the abomasum of each calf was confirmed by ultrasonography at intervals of 60 min. Interestingly, based on ultrasonography, the WAC was not transported from the abomasum into the small intestine within 24 days after application, in contrast to humans (Koziolek et al., 2015) and ponies (Stokes et al., 2012).

In all calves, the median abomasal pressure values were generally described as subatmospheric, with a minimum abomasal pressure of −11.5 kPa. In ponies a mean minimum gastric pressure of 0.0 kPa has been reported (Stokes et al., 2012). In calves, a maximum pressure of 13.1 kPa (98.3 mm Hg) was similar to the mean maximum gastric pressure reported in ponies (126 ± 36 mm Hg; Stokes et al., 2012). Maximum pressure peaks up to 50 kPa were identified as the capsule passed from the stomach into the duodenum in dogs (Boillat et al., 2010), ponies (Stokes et al., 2012), and humans (Koziolek et al., 2015). In our study we did not observe similar maximum pressure peaks because the capsule was not passed from the abomasum into the small intestine either during the observation period of 720 min or at more than 24 days after WAC application (Fig. 5). However, the median intraluminal pressure tended to be lower in diarrheic calves than in healthy calves; however, the difference was not significant, probably because of the low number of subjects.

Maximum pH was obtained immediately after feeding and steadily decreased until 12 h postprandial. The pH values measured by WAC were similar to postprandial pH values obtained in other studies using calves with an abomasal cannula that were fed either milk or milk replacer (Constable et al., 2005; Sen et al., 2006) or oral rehydration

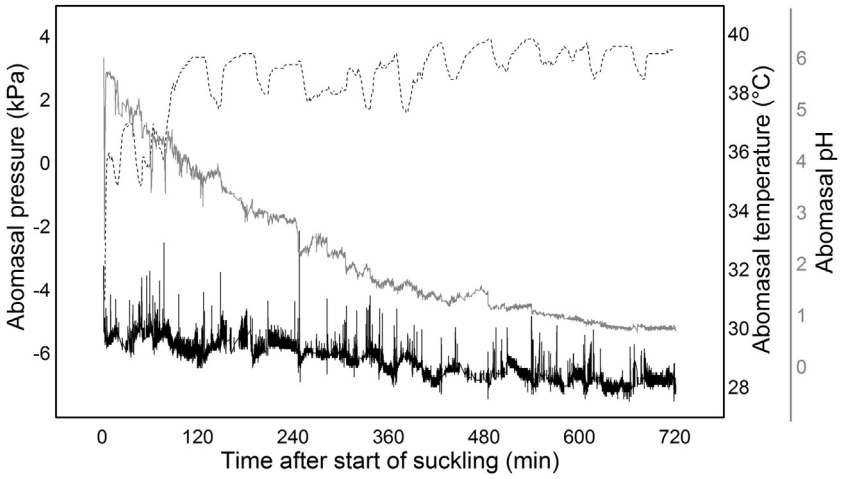

Fig. 2. Representative WAC data from a diarrheic calf, abomasal temperature (dashed line), pH (straight gray line), and pressure (straight black line) against time.

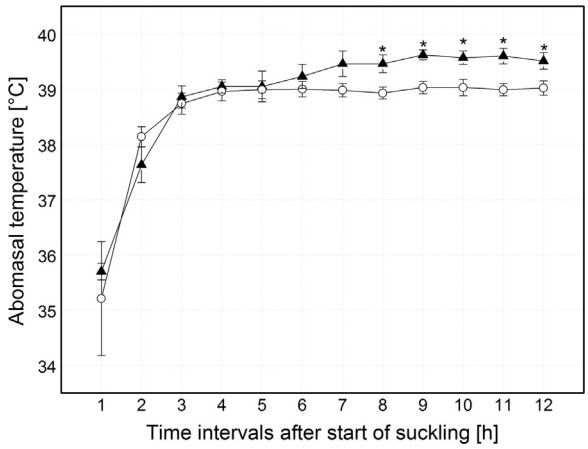

Fig. 3. Abomasal temperature data against time; O – healthy calves; Δ – diarrheic calves; * data were significantly different ($P < 0.05$), (data are expressed as means ± SD).

solutions (Sen et al., 2006; Bachmann et al., 2009). The time to pH < 2 was not significantly different in healthy or diarrheic calves. However, times to pH < 2 have been confirmed by other studies in calves fed milk replacer (Sen et al., 2006). It must be emphasized that pH variations depend on the location of the WAC in the abomasum. WACs that are close to the abomasal wall are primarily located in whey and probably experience lower pH values than WACs located in the middle of the chyme, particularly in the curd formation. Furthermore, McLauchlan et al. (1989) found pH differences in the gastric body and antrum in humans due to varied buffering effects of the chyme. The WAC presence in the abomasum was always confirmed by ultrasonography, but the exact location of the WAC in each term was not documented in our study. However, the WAC can be speculated to have been close to the abomasal mucosa in the xiphoidal region given the similar pH trends during the observation period in both groups. Related to the location of the WAC, pH measurements may reflect continuous hydrochloric acid production by the parietal cells of the mucosa and the acidification by the whey. In that context, Constable et al. (2005) reported lower pH values after the intake of cow's milk, which was either a result of the clotting process by the extrusion of low whey pH or by a fast AER, among other possibilities. In the present study, AER was delayed in diarrheic calves, and therefore the location of the WAC in the acidifying whey near the abomasal mucosa might be a possible explanation.

The low temperatures at the beginning of the observation period were probably related to the low temperature of the milk fed to the calves, but the abomasal temperature increased within the first 3 h after milk intake. Afterward, the mean abomasal temperature was higher than the mean rectal temperature in both groups. However, the abomasal temperature of diarrheic calves steadily increased until a mean maximum temperature of 39.6 °C was reached 9 h postprandial. Higher temperatures were found in diarrheic calves than in healthy calves 7 h after milk replacer intake. One explanation for the higher intraluminal temperatures might be related to fermentation processes in the

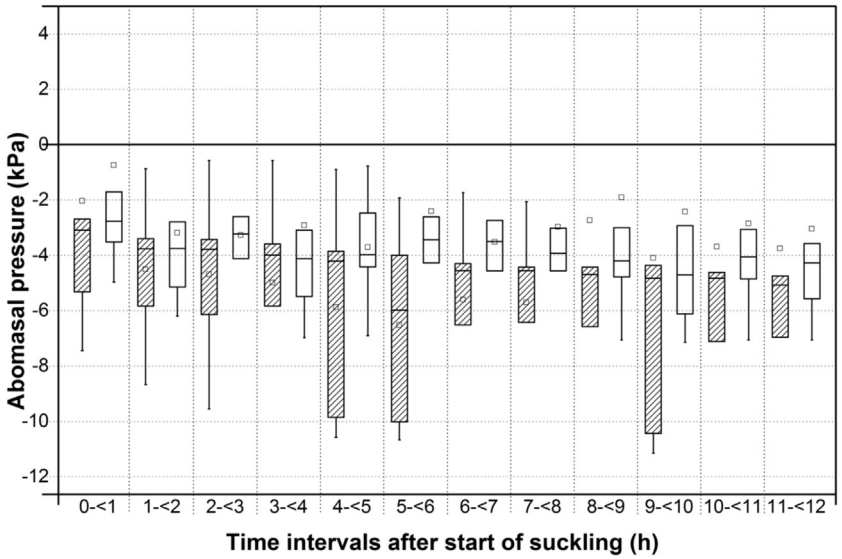

Fig. 4. Box plots of abomasal pressure data against time at each hour after the test meal; clear boxes – healthy calves; banded boxes – diarrheic calves (bottom and top of the box correspond to 25th and 75th percentiles).

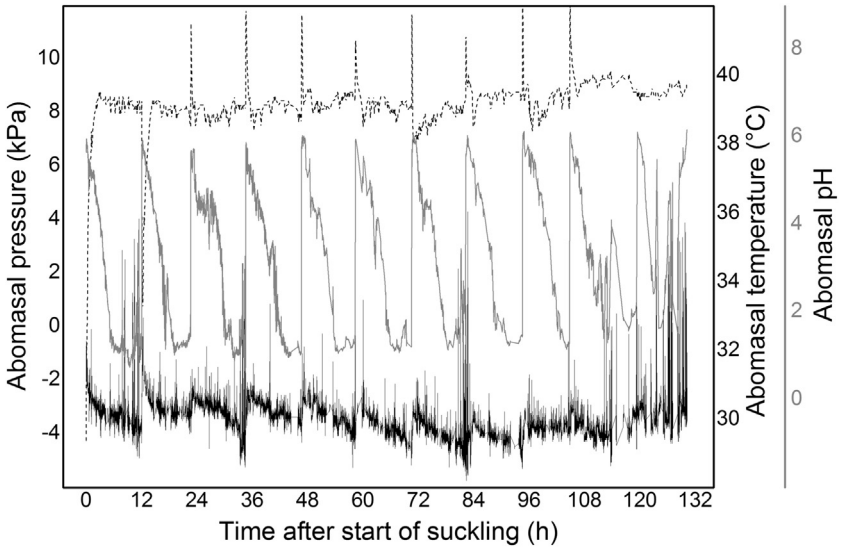

Fig. 5. Representative WAC data from a healthy calf that was observed for 130 h post suckling; alternating pH curves indicate feeding process, abomasal temperature (dashed line), pH (straight gray line), and pressure (straight black line) against time.

abomasum. In that context pH-tolerant mucosa-associated *Lactobacilli* ssp. were recently found by Hund et al. (2014) in the abomasum in calves. One consequence of bacterial fermentation is the production of SCFAs. SCFAs were reported to cause cell edema by interrupting the sodium transport under in vitro conditions in horse gastric tissue (Nadeau et al., 2003). A similar impairment of the sodium transport might also occur in the abomasum; however, data are lacking in the calf. If cell edema impairs the functionality of the abomasal movement, it may lead to delayed abomasal emptying. In addition, we detected pathogens such as *Cryptosporidium parvum*, *Rota virus*, and *Corona virus* in all diarrheic calves, and these pathogens are known for their mucosa-injuring potential (Tzipori, 1983; Tzipori et al., 1983).

Another likely explanation for the higher intraluminal temperatures in the abomasum might be related to dehydration in diarrheic calves. With respect to dehydration a decreased heat loss has been described by Walker et al. (1998) due to impaired peripheral perfusion in experimentally induced diarrhea in neonatal calves. As TPP and other parameters such as electrolytes and hematocrit were similar between healthy and diarrheic calves, it can be speculated that calves did not suffer under severe dehydration. However, from our study it remained open whether fermentation processes or a decreased heat loss impaired intraluminal temperature in the abomasum.

APAT data confirm the results of Kirchner et al. (2015) who reported delayed abomasal emptying measured by ultrasonography in diarrheic calves. The AAP half-time was nearly 3 h faster in healthy calves (393 ± 84 min) than in diarrheic calves (564 ± 96 min). However, no significant differences in C_{max} and T_{max} were observed between the two groups of calves. The AUC values of diarrheic calves were about 25% lower compared to those of healthy calves, which could also be explained by malabsorption processes in the small intestine. More severely injured small intestine might alter AAP indices more noticeably. A validation of APAT with other methods of assessment (scintigraphy, ultrasonography) is necessary.

Interestingly, serum AAP curve showed an initial increase until 90 min postprandial, followed by a decrease 120 min postprandial, and a second rise in serum AAP in the following in both groups. In recent research it has been discussed that the abomasal emptying might be influenced by initial insulin and glucagon-like peptide-1 response in the early stage of glucose absorption (Stahel et al., 2016, MacPherson et al., 2016) which may explain the early postprandial fluctuations.

Considering WAC and APAT data, we confirmed our hypothesis that abomasal conditions differ between healthy and diarrheic calves. However, we emphasize that we used calves with mild symptoms of diarrhea without any impairment of suckle reflex and hydration or electrolyte status. Changes in abomasal luminal conditions and abomasal emptying could be more substantial in calves with severe diarrhea.

5. Conclusion

WAC is a tool to assess intraluminal pH, temperature, and pressure data. A slower AER and bacterial fermentation processes should be considered in the feeding management of diarrheic calves. In particular, smaller meal sizes might decrease the risk for fermentation processes in the abomasum. Based on the second finding that WAC was not transported from the abomasum into the small intestine within 24 days after application, the capsule might offer a new therapy tool in applying drugs with a constant flow of agents (e.g. buffering substances or trace elements such as selenium for calves on pasture) over several days or weeks.

Conflict of interest statement

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

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Appendix A. Supporting information

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