



Temporal changes of abomasal contents and volumes in calves fed milk diluted with oral rehydration salt solution

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ABSTRACT. Several manufacturers recommend to feed mixture comprising equal amounts of oral rehydration salt (ORS) solution and milk for diarrheic calves after milk withdrawal. Such a feeding method is expected to supply more nutrients and energy compared to feeding only the ORS solution. However, little is known about the effects of feeding milk diluted with ORS solution on calves' digestive process. This study examined the abomasal contents, volumes, and emptying rates in calves fed whole milk, milk diluted by 50% with ORS solution (50% ORS-milk), and ORS solution. Ultrasonography identified curds in the milk-fed calves, but not in the 50% ORS-milk-fed or the ORS-fed calves. The abomasal fluid of the 50% ORS-milk-fed calves contained not only β -lactoglobulin but also α -casein (CN), β -CN, and κ -CN, which were used for curd formation and undetectable in the milk-fed calves. Abomasal pH was relatively higher in the 50% ORS-milk-fed than that in the milk-fed calves. Abomasal emptying rates were significantly faster in the ORS-fed than in the 50% ORS-milk-fed and the milk-fed calves. These data indicate that the formation of abomasal curd is inhibited in the 50% ORS-milk-fed calves due to the resultant high abomasal pH and low κ -CN concentration. The 50% ORS-milk may not provide rehydration as quickly as the ORS solution. In conclusion, we do not recommend feeding 50% ORS-milk to calves.

KEY WORDS: abomasum, calf, curd formation, diarrhea, oral rehydration therapy

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Diarrhea is the most common cause of death in neonatal calves, thus causing economic problems for beef and dairy producers worldwide [27, 28]. When diarrhea occurs in calves, large amounts of body fluids and ions, such as bicarbonate and potassium, are lost due to frequent defecation of watery feces. In calves with severe dehydration, there is decrease of renal glomerular filtration of hydrogen ions and the accumulation of L-lactate, which results in metabolic acidosis [15, 26]. Force-feeding of milk to diarrheic calves may lead to dysfunction of the esophageal groove, which causes milk fermentation in the reticulorumen producing fatty acids that can further aggravate metabolic acidosis [14]. To prevent and/or decrease dehydration and metabolic acidosis in diarrheic calves, oral rehydration therapy by administration of an oral rehydration salt (ORS) solution is effective to replace lost fluids and restore acid-base balance [3, 24]. This therapy is inexpensive and non-invasive for calves with mild to moderate dehydration, and farmers can use it as a dietary supplement for calves without prescription by veterinarians.

There are numerous commercial ORS products for calves, which contain various types and amounts of electrolytes, sugars, and amino acids in addition to basic components such as sodium chloride, sodium citrate, potassium chloride, and glucose [29]. However, because the nutrients and energy contained in ORS are much lower than that of whole milk, which is rich in carbohydrates, proteins, and fat, the feeding of ORS solution only instead of whole milk causes loss of body weight (BW) in calves. Therefore, it has been recommended to feed both whole milk and ORS solution to diarrheic calves alternately for oral rehydration therapy [15]. Recent studies proposed that administration of milk, in which ORS powder or gel was directly dissolved, effectively provides both milk nutrients and electrolytes [1, 2, 4, 12, 13]. On the other hand, several manufacturers recommend dissolving ORS in water, but not in milk, and feeding milk diluted with ORS solution (ORS-milk) to diarrheic calves during the recovery period after milk withdrawal (Resorb, Zoetis US, Parsippany, NJ, U.S.A.; Lactade, Jurox, New South Wales, Australia; Lytafit, Albrecht GmbH, Aulendorf, Germany; Sacrolyte, Tulivin, County Cavan, Ireland; Lactade Plus, Liffey Mills, Roscrea, Ireland). Feeding ORS-milk is simple compared with feeding milk and ORS solution separately. However, there are no reports on

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whether calves digest ORS-milk in the same manner as milk alone.

The abomasum is the only functional stomach from birth until about two weeks in calves. During this period, milk bypasses the rumen via the esophageal groove and flows directly to the abomasum that secretes chymosin to coagulate milk. Milk contents are separated into curd and whey. Curd formation is important for digestion and absorption of milk nutrients and immune substances in calves [5, 8, 11, 16]. Previous studies showed that milk and milk replacers containing ORS had no effect on abomasal curd formation [2, 4, 13]. In contrast, the dilution of whole milk by 50% with ORS solution (50% ORS-milk) inhibited curd forming in an *in vitro* assay [19], suggesting that no abomasal curd is formed in calves fed this 50% ORS-milk without direct evidence. Thus, the specific aim of the present study was to examine the effects of milk dilution with ORS solution, which is recommended by several manufacturers, on abomasal curd formation and abomasal emptying rate in calves. We compared temporal changes in abomasal contents and volumes between calves fed whole milk, ORS solution, and 50% ORS-milk. Our results will contribute to improve our understanding of the digestive process of the current feeding method of diarrheic calves.

MATERIALS AND METHODS

Whole milk, ORS solution, and 50% ORS-milk

Whole milk was obtained from a bulk tank containing fresh cows' milk with 4.53% fat, 3.59% protein, and 8.85% solids-non-fat, which was certified quality of Ministerial Ordinance on Milk and Milk products Concerning Compositional Standards of Japan. The milk was stored at 4°C for up to 3 days before being fed to calves. The ORS solution used in the experiments was prepared by dissolving one Effydral tablet (48.71 g, Zoetis Japan, Tokyo, Japan) in 1 l of tap water according to the manufacturer's instruction. The tablet contained 2.34 g of sodium chloride, 1.12 g of potassium chloride, 6.72 g of sodium bicarbonate, 3.84 g of citric acid, 32.44 g of lactose, and 2.25 g of glycine, meaning that it contained bicarbonate at 80 mmol/l, citrate at 20 mmol/l, and a theoretical osmolality of 415 mOsm/kg. The 50% ORS-milk was prepared by mixing equal amount of the whole milk and the ORS solution according to the manufacturer's instruction. The pH of solutions used in the experiments was measured by a pH meter (TPX-90, Toko Chemical Laboratories Co., Ltd., Tokyo, Japan) before feeding.

In vitro rennet coagulation tests were carried out to examine the curd forming property of the whole milk, the 50% ORS-milk, and the milk diluted by 50% with water (50% water-milk), according to our previous study [16]. In brief, 10 ml of the whole milk, the 50% ORS-milk, and the 50% water-milk were incubated with rennet at 0.2–10 mg/ml for 2 hr at 38°C.

Animals and feeding

Three Holstein-Friesian calves with mean \pm standard deviation (SD) of BW at birth of 47.7 ± 1.7 kg were used. Each calf was kept at a calf hutch and fed the first-milking colostrum at 4% BW within 4.5 hr after birth, and then fed fresh cow's whole milk at 6% BW, using a bucket with a nipple, twice a day (at 8:00 and 20:00).

This study was conducted using a 3×3 Latin square design comprising the three calves between 6 and 16 day-old and following 3 solutions: whole milk, ORS solution, and 50% ORS-milk. Treatment order was different in each calf, with a minimum washout period of at least 12 hr between each treatment. Calves suckled each of the three solutions for 4 times at 12 hr intervals from the bucket with a nipple. Solutions were fed to calves at 38°C and 6% of BW which was determined immediately before each treatment. Ultrasonographic observation was carried out after the fourth feeding for evaluating abomasal contents, volumes, and emptying rates. Calves had no access to water or calf starter ration during the treatment. This study was approved by the Animal Research Committee and followed the guidelines for Animal Experiment of Iwate University.

Ultrasonography and estimation of abomasal volume and abomasal emptying rate

Ultrasonography was performed according to a previous method [17], using a HS-1500V Ultrasonic system equipped with a HLS-375 5.0 MHz 50-mm linear transducer (Honda Electronics Co., Ltd., Toyohashi, Japan) before (pre), immediately (0), 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hr after suckling started. Two or three sequential captured frames were combined into one picture to show abomasal contents in cross-sectional and longitudinal sectional images using Photoshop (Adobe Systems Inc., San Jose, CA, U.S.A.). Abomasal volume and emptying rate were calculated according to a previous report [30].

Analysis of abomasal fluid

The abomasal fluid was collected via abdominocentesis with an 18-gauge needle 0.5 and 2 hr after suckling started, under local anesthesia with 2% procaine hydrochloride. Ultrasonography was used to confirm the optimal location for abdominocentesis, i.e., where the lumen of abomasum was largest and adjacent to the abdominal wall without disturbance of other organs. The pH of abomasal fluid was measured using a pH meter (TPX-90, Toko Chemical Laboratories Co., Ltd., Tokyo, Japan). Protein concentrations were measured by the Bradford method, using bovine serum albumin as the standard.

SDS-PAGE

Protein compositions of 10 μ g aliquot from the three feeding solutions, the liquid phase of the rennet coagulation test, and abomasal fluid obtained 2 hr after feeding were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 10–20% gel) under non-reducing conditions, and the gel was stained with Coomassie Brilliant Blue R-250 (CBB). CBB stained bands of α -Casein (CN), β -CN, κ -CN, and β -lactoglobulin (LG) were determined based on the previous report [16, 31].

Table 1. *In vitro* rennet coagulation test

Concentration of rennet (mg/ml)	0.2	0.4	0.6	0.8	1	2	4	6	8	10
10 ml whole milk	+									
10 ml milk diluted by 50% with ORS solution	–	–	–	–	–	–	–	–	–	prec ^{a)}
10 ml milk diluted by 50% with water	–	–	±	±	±	+	+	+	+	+

+ Curd formed and no CNs remain in the liquid phase by SDS-PAGE. – Curd did not form and CNs remain in the liquid phase by SDS-PAGE. ± Soft curd formed but SDS-PAGE detected CNs in the liquid phase. a) White solid precipitated but SDS-PAGE detected CNs in the liquid phase.

Statistical analysis

All data are expressed as means ± SD. BW before and after the treatments were compared by paired *t*-tests. Abomasal volumes, pH, protein concentrations, and emptying rates were compared among groups at the same time point and time-dependent changes within each group using one-way analysis of variance (ANOVA) and repeated measure ANOVA, respectively, followed by multiple comparison using the Tukey–Kramer honestly significant difference test. All statistical analyzes were performed on JMP12 software (SAS Institute, Cary, NC, U.S.A.), considering $P < 0.05$ as significant.

RESULTS

Different coagulation activities between milk and 50% ORS-milk

In vitro rennet coagulation tests using rennet at 0.2–10 mg/ml showed that curd was not produced in the 50% ORS-milk, while the whole milk and the 50% water-milk were coagulated using rennet at 0.2 mg/ml and 2 mg/ml, respectively (Table 1). Protein concentrations in the whole milk, the 50% ORS-milk and the ORS solution were 40.2, 23.9, and 0 mg/ml, respectively. Figure 1 shows the protein compositions of the reaction mixtures before and after incubation of the 50% ORS-milk or the 50% water-milk. α -CN, β -CN, κ -CN, and β -LG were the major contents in both samples before incubation. Although α -CN, β -CN, and κ -CN, but not β -LG, were not present in the liquid layer (whey fraction) of the 50% water-milk after incubation, these proteins were detected in the 50% ORS-milk after incubation. These results indicated that curd was not formed from the 50% ORS-milk in *in vitro* assays.

Temporal changes of abomasal contents visualized by ultrasonography

No physical problems were detected in calves during the experiments, and all calves suckled the entire volumes of the whole milk, the 50% ORS-milk, and the ORS solution within 5 min. No significant differences in BW before and after treatments were found in milk-fed calves (48.9 ± 1.9 kg and 50.3 ± 2.3 kg, respectively), and 50% ORS-milk-fed calves (50.3 ± 2.0 kg and 50.7 ± 2.2 kg, respectively). There was a significant difference between BW before and after treatments (49.6 ± 2.4 kg and 48.5 ± 2.2 kg, respectively) in the ORS-fed calves.

Figure 2 shows representative ultrasonographic images of the abomasum before and after feeding. In all calves, the abomasum was visualized as an echogenic image in maximum volume immediately after feeding (0 hr). In the milk-fed calves, the echogenicity of abomasal contents increased at the bottom of abomasum 0.5 hr after feeding. Then, an echogenic image with clear outline, corresponding to a large curd, was observed between 1 and 2 hr after feeding; this image became indistinct at 4 hr. On the contrary, the ORS-fed calves showed only anechoic images corresponding to ORS solution from 0.5 to 2 hr, and the contents gained echogenicity with gathering abomasal folds indicating almost no contents at 4 hr. In the 50% ORS-milk-fed calves, there was no clear outlined image corresponding to curd. Echogenicity decreased until 1 hr after feeding, but increased again after 4 hr. Images of abomasal contents were similar for all calves from 6 hr after feeding to the end of the experiment.

Protein contents of abomasal fluid

The presence or absence of abomasal curd were also examined regarding the protein levels in the abomasal fluid samples obtained from calves 2 hr after feeding. There were significant differences in protein concentrations of abomasal fluid samples between the 50% ORS-milk-fed calves (10.8 ± 0.1 mg/ml) and the milk-fed calves (3.5 ± 0.1 mg/ml) or the ORS-fed calves (0.1 ± 0.1 mg/ml). In SDS-PAGE (Fig. 3), β -LG, but not α -CN, β -CN, and κ -CN, was detected as a major protein in the abomasal fluid of the milk-fed calves with curd formation. In contrast, α -CN, β -CN, and κ -CN as well as β -LG were detected in the abomasal fluid of the 50% ORS-milk-fed calves.

pH values of abomasal fluid

The pH value of ORS (7.2 ± 0.2) was significantly higher than that of the whole milk (6.7 ± 0.1) and the 50% ORS-milk (6.8 ± 0.2). The pH of abomasal fluid samples 0.5 hr after feeding were significantly lower in the milk-fed calves (5.6 ± 0.2) than in the 50% ORS-milk-fed calves (6.4 ± 0.1) or the ORS-fed calves (6.6 ± 0.2). The pH of samples 2 hr after feeding were significantly lower in the milk-fed calves (4.8 ± 0.1) than in the 50% ORS-milk-fed calves (6.1 ± 0.1) or the ORS-fed calves (6.3 ± 0.2).

Temporal changes of estimated abomasal volume

There was no significant difference on the feeding volumes of the 50% ORS-milk-fed calves ($3,012.0 \pm 120.0$ ml), the milk-fed

calves ($2,932.0 \pm 111.5$ ml), and ORS-fed calves ($2,976.0 \pm 141.5$ ml). Figure 4 shows the temporal changes in estimated abomasal volume in the three groups. Remarkable increases of abomasal volumes were observed in all calves immediately after feeding. The volume was significantly larger between 0 and 4 hr than before feeding in all calves. There was no significant difference in abomasal volume between the milk-fed and the 50% ORS-milk-fed calves. In contrast, the abomasal volume was significantly lower in the ORS-fed calves than in the milk-fed calves at 1, 1.5, and 2 hr, and the 50% ORS-milk-fed calves at 2 hr.

Abomasal emptying rate

Abomasal emptying rates measured by estimating abomasal emptying half time (T50) calculated from abomasal volume were 113.5 ± 14.6 min in the 50% ORS-milk-fed calves, 113.5 ± 11.5 min in the milk-fed calves, and 70.5 ± 12.2 min in ORS-fed calves.

DISCUSSION

This study demonstrated the effects of diluting milk with an ORS solution on the digestive process in calves by a comparison with that of milk and ORS solution. A major finding was the absence of curd in the 50% ORS-milk-fed calves. Although we did not directly evaluate secretion amounts and enzymatic activities of chymosin in each calf, ultrasonography showed that the same calves produced a large curd after feeding on the whole milk. Considering α -CN, β -CN, and κ -CN were used for curd formation and were not present in the abomasal fluid 2 hr after the ingestion, we strongly suggest that the 50% ORS-milk-fed calves did not form abomasal curd as the milk-fed calves did. These indirectly indicate that their abomasa had sufficient ability to form curd by chymosin during the experimental period. We suggest that the lack of curd formation in the 50% ORS-milk-fed calves was caused by dilution of the milk with the ORS solution and not by abomasal dysfunction, as reported previously [8, 18].

We speculate that high abomasal pH resulting from the 50% ORS-milk was the primary cause for the lack of curd formation in the calves. Abomasal curd formation begins with the hydrolysis of κ -CN by chymosin, which is secreted as an inactive proenzyme, prochymosin, that is activated upon exposure to acid [22]. The optimum pH for chymosin is 5.3–6.3 [7]. Calves fed the whole milk (pH 6.7) exhibited a remarkable decrease in abomasal pH, to 5.6 and 4.8, within 0.5 and 2 hr, respectively, after feeding, whereas calves fed the 50% ORS-milk (pH 6.8) varied slightly, with pH of 6.4 and 6.1 at 0.5 and 2 hr, respectively, after feeding. Previous studies reported that abomasal pH was higher in the ORS-milk-fed calves than in the milk-fed calves due to the presence of alkalizing agents, such as bicarbonate and citrate [4]. Thus, feeding milk diluted with ORS solution containing alkalizing agents increases abomasal pH in calves, and the high abomasal pH causes insufficient chymosin hydrolytic activity, resulting in no curd formation.

In contrast to our results, other studies have reported that calves fed milk containing ORS produced abomasal curd [4, 13]. We think that these conflicting results might be due to different ways of dissolving the ORS. The previous studies used milk in which ORS powder was directly dissolved without being diluted with water, whereas we diluted the milk with an ORS solution. The final concentrations of bicarbonate and citrate in the 50% ORS-milk used in this study were 40 and 10 mmol/l, respectively, similar to values reported in previous studies: 25 mmol/l bicarbonate and 12 mmol/l citrate [4], and 63 mmol/l bicarbonate or 93 mmol/l bicarbonate without citrate [13]. Therefore, the concentrations of alkalizing agents could not explain the cause for no curd formation in the 50% ORS-milk-fed calves. Here, we suggest that diluting milk with ORS solution is a secondary reason for the lack of curd formation in the calves. It has also been reported that κ -CN concentration in milk is a key factor in curd formation [9]. In general, kinetics explains that a low κ -CN concentration decreases the maximum rate of the chymosin reaction (V_{max}). This study showed that a 10-fold higher concentration of chymosin was necessary to coagulate the milk diluted to 50% with water rather than the whole milk. Related studies have reported that the time for curd to form was significantly prolonged in the 50% water-milk mixture than in the whole milk [19]. These results suggest that not only high abomasal pH but also a low κ -CN concentration caused by diluting the milk with ORS solution had a negative effect on abomasal curd formation in the 50% ORS-milk-fed calves.

Many calves are fed milk replacer instead of milk. There are both curd forming and non-curd forming milk replacers [10, 21], and abomasal curd formation is influenced by the curd forming properties of the milk replacer. Since this study focused on the effects of ORS solution used to dilute milk on abomasal curd formation, we primarily tested ORS-milk and not ORS-milk replacer. We believe that both the alkalizing agents contained in ORS and dilution of the milk replacer inhibit curd formation in calves fed

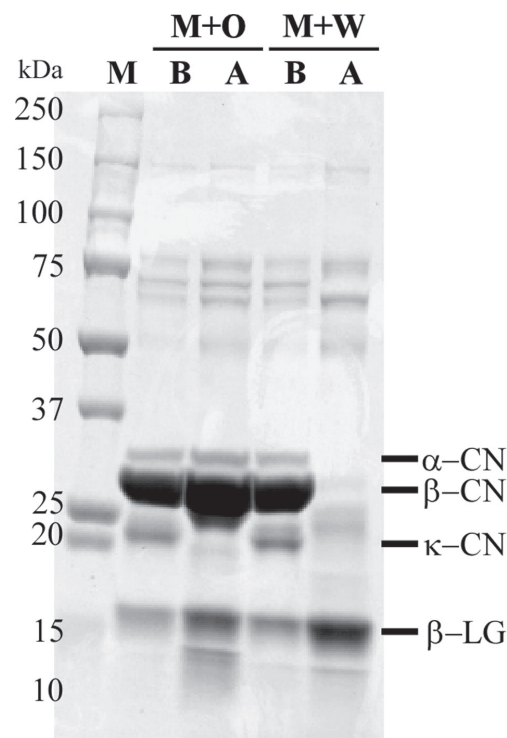


Fig. 1. Protein compositions of the milk diluted with oral rehydration salt (ORS) solution or water before and after incubation with rennet. SDS-PAGE (10–20% gradient gel) of proteins (10 μ g aliquot) from milk diluted by 50% with ORS solution or water before and after adding rennet. The gel was stained with Coomassie Brilliant Blue R-250. M+O: 50% ORS-milk, M+W: 50% water-milk. “B” and “A” mean before and after incubation with rennet. α -CN: α -Casein, β -CN: β -Casein, κ -CN: κ -Casein, and β -LG: β -lactoglobulin.

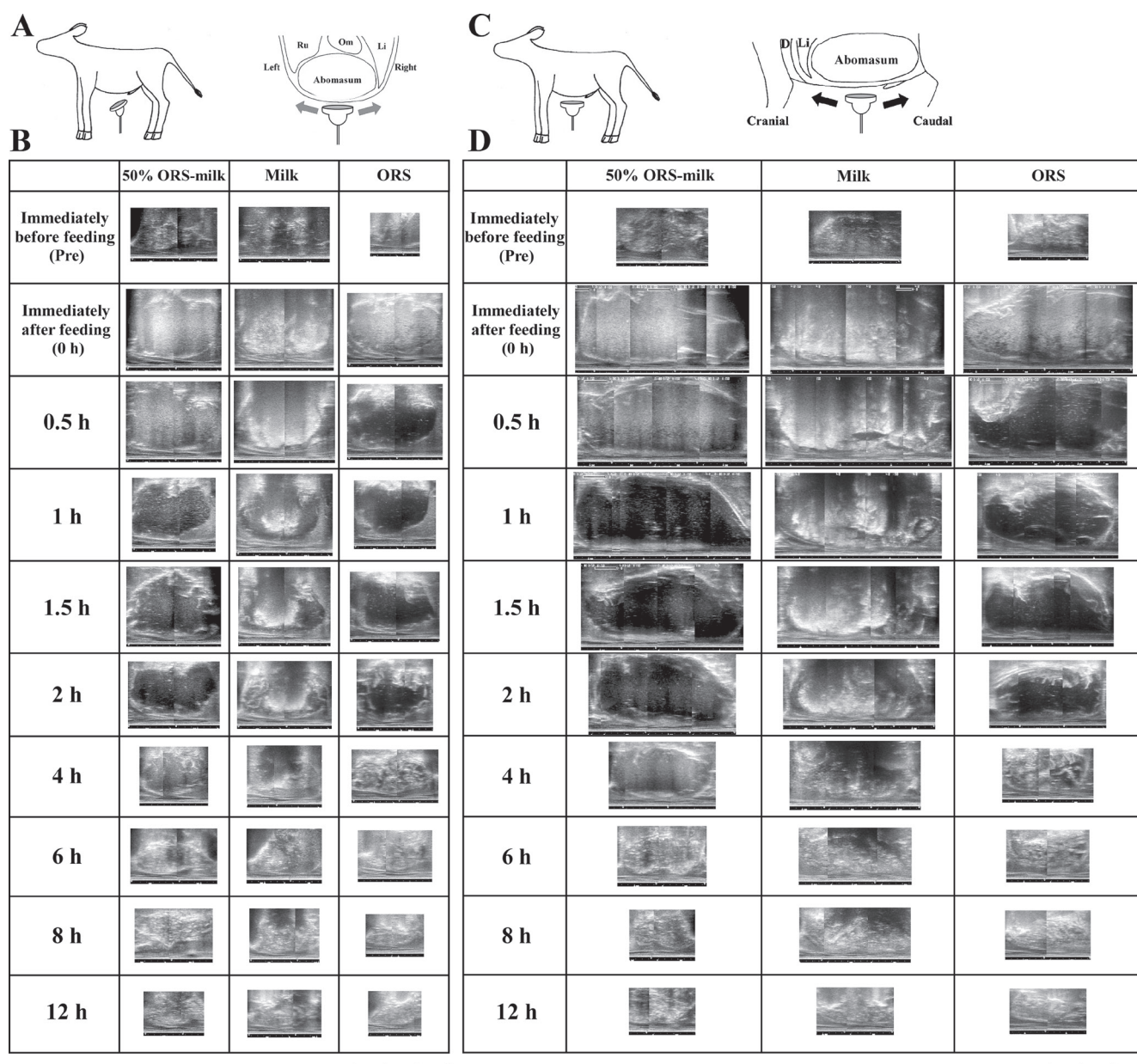


Fig. 2. Ultrasonographic images of the abomasal contents in a calf fed milk diluted by 50% with oral rehydration salt (ORS) solution (50% ORS-milk), whole milk (Milk), and ORS solution (ORS). A: Left schematic representation shows how to visualize cross-sectional images of the abomasum by the ultrasonography. The transducer was placed transversely across the ventral midline to determine transverse plane of the abomasum. Right schematic representation explains the cross-sectional ultrasonographic images. Rumen (Ru), omasum (Om), and liver (Li). B: The cross-sectional ultrasonographic images of a calf fed 50% ORS-milk (3.1 l), whole milk (3.0 l), and ORS solution (2.9 l), at 16-, 13-, and 11-days old, respectively. Upper, lower, right and left of images correspond to dorsal, ventral, right and left of the calf body, respectively. C: Left schematic representation shows how to visualize longitudinal-sectional images of the abomasum by the ultrasonography. The transducer was placed along the ventral midline to determine sagittal planes of the abomasum. Right schematic representation explains the longitudinal-sectional ultrasonographic images. Diaphragm (D) and liver (L). D: The longitudinal-sectional ultrasonographic images of the abomasal contents corresponding to Fig. 2B. A bar shows 15 cm.

ORS-milk replacers, as with ORS-milk.

Previous studies have reported that ultrasonography can measure abomasal volume [30] and evaluate abomasal curd formation [17] in calves. This study also showed that ultrasonography is useful for monitoring postprandial changes in abomasal contents and volumes, which determine the presence or absence of abomasal curd formation and abomasal emptying half time, respectively, in calves. In contrast to analyzes of abomasal fluid that was sampled via a cannula attached by surgery [2, 4] and a post-mortem visual inspection [5, 6, 23], ultrasonography is a non-invasive method that can be used to observe abomasal contents and volumes repeatedly in the same individual. We propose that ultrasonographic observations should be applied to clinical cases exhibiting

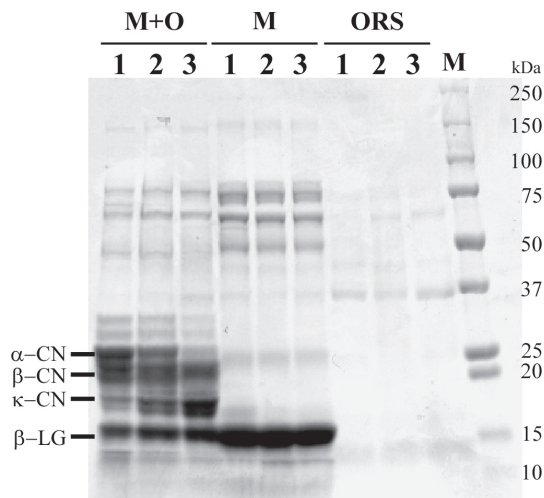


Fig. 3. Protein compositions of the abomasal fluid samples obtained from 50% oral rehydration salt (ORS)-milk-fed, whole milk-fed, and ORS-fed calves. SDS-PAGE (10–20% gradient gel) of abomasal fluid samples (10 μ g aliquot) obtained from calves 2 hr after feeding 50% ORS-milk, whole milk, and ORS solution. The gel was stained with Coomassie Brilliant Blue R-250. M+O: 50% ORS-milk, M: milk, ORS: ORS solution. Numbers mean identification numbers of calves. α -CN: α -Casein, β -CN: β -Casein, κ -CN: κ -Casein, and β -LG: β -lactoglobulin.

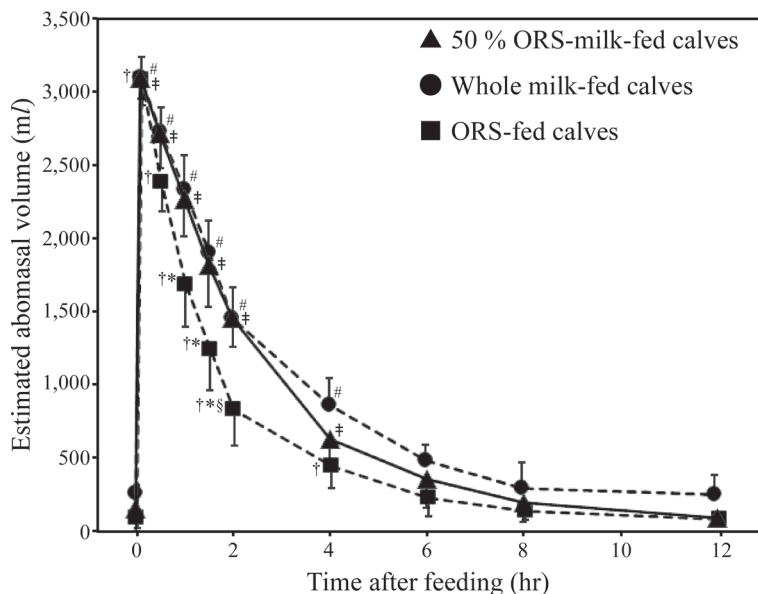


Fig. 4. Temporal changes of abomasal volume in 50% oral rehydration salt (ORS)-milk-fed (\blacktriangle), whole milk-fed (\bullet), and ORS-fed calves (\blacksquare). Abomasal volume of 50% ORS-milk-fed, whole milk-fed, and ORS-fed calves, which were estimated from ultrasonographic images. Data are expressed as mean \pm SD. $\S P < 0.05$ compared to the concentration of 50% ORS-milk-fed calves at the same time. $* P < 0.05$ compared to the concentration of whole milk-fed calves at the same time. $\# P < 0.05$, and $\dagger P < 0.05$ compared with before feeding (time=0) within each group.

anorexia or diarrhea for an evaluation of the treatment effects.

Based on a previous report that the lack of curd formation accelerates the abomasal emptying rate in calves [6, 23], we hypothesized that the abomasal emptying half time was shorter in the 50% ORS-milk-fed calves than in the milk-fed calves. However, our results show that the abomasal emptying half times were similar in the 50% ORS-milk-fed calves (113.5 ± 14.6 min) and the milk-fed calves (113.5 ± 11.5 min), both of which were within the normal range (104–160 min) for healthy euvolemic 5–13-day-old calves fed 2 l of cow's milk [4]. In contrast, different abomasal emptying half times were observed between the 50% ORS-milk-fed and ORS-fed calves (70.5 ± 12.2 min) that were similar to 65 and 75 min of the calves fed 2 l of isotonic glucose solution and hypertonic NaHCO_3 solution, respectively [25]. The results indicated that abomasal emptying rates were significantly faster in ORS-fed calves than in the 50% ORS-milk-fed calves and the milk-fed calves. It has also been reported that calves fed 2 l of ORS-milk containing electrolytes such as sodium, potassium, and chloride showed a remarkably slower abomasal emptying rate (260 min) compared to calves fed 2 l of milk (160 min) [4]. Based on these results, we reject our previous hypothesis and suggest that the 50% ORS-milk causes slow abomasal emptying in calves due to high electrolyte concentrations despite the lack of curd formation.

The formation of abomasal curd functions to digest milk in calves. Curd formation slows the passage of nutrients from the abomasum to the small intestine [6] and enhances feed efficiency, digestibility, and body weight gain [11]. Recently, we showed that curd formation enhances IgG absorption in neonatal calves [16]. Therefore, it is better to apply feeding methods that do not inhibit curd formation in calves. It has also been reported that faster abomasal emptying rates contribute to rehydration in healthy calves [20, 25]. Since abomasal emptying rates were slower in 50% ORS-milk-fed calves than ORS-fed calves, we speculate that the diluted milk remained in the abomasum longer than did the ORS solution, and it may not have caused rehydration as quickly as the ORS solution alone. Moreover, 50% ORS-milk is not suitable for treating dehydrated diarrheic calves; considering reports that abomasal emptying is prolonged in diarrheic calves compared with healthy calves [13], 50% ORS-milk may further delay the abomasal emptying in the former group. In conclusion, a feeding method using milk diluted 50% with ORS solution is not recommended for calves because the method impedes, rather than enhances, the functions of milk and ORS solution; ORS solution decreases the function of milk via the inhibition of curd formation while, conversely, a mixture of milk and ORS solution results in prolonged abomasal emptying of the ORS solution. Because the abomasal volume returns to the preprandial level at 6 hr after feeding (Fig. 4), one recommendation is to feed milk and an ORS solution alternately at least 6 hr apart. Feeding ORS powder dissolved in milk directly is also recommended, as reported previously [4, 13].

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