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No abomasal curd formation in pre-ruminant calves after ingestion of a clotting milk replacer

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ABSTRACT

The aim of this study was to ultrasonographically evaluate curd formation in 29 pre-ruminant calves that were fed a clotting milk replacer. Abomasal curd was absent in 8/29 calves at 2 h after feeding. In these eight calves, abomasal contents were observed as an anechoic image with small echogenic spots (five calves), or as an echogenic image with an unclear outline (three calves), but there was no echogenic image with a clear outline corresponding to curd that was visualised in the other 21 calves. The curd was not observed until 7 h after feeding in the eight calves. Our analysis also indicated that the absence of curd formation in the pre-ruminant calves did not have a significant impact on the appearance, appetite and vigour of pre-ruminant calves or on their blood parameters, including serum triglyceride, blood urea nitrogen and glucose concentrations. The study provided the first evidence that the abomasum of some calves does not form curd despite ingestion of a clotting milk replacer.

Introduction

Abomasal curd formation is a unique feature of the digestive system that occurs in milk-fed pre-ruminant calves. In this process, milk is coagulated under the action of chymosin (EC 3.4.23.4) and casein, and the lipids are trapped in the curd, whereas other milk proteins, lactose and minerals are separated in the whey (Hill et al., 1970; Longenbach and Heinrichs, 1998; Tomkins and Jaster, 1991). Several reports have suggested that curd formation is important for the efficient digestion and absorption of nutrients (Jenkins and Emmon, 1982; Petit et al., 1987a; Cruywagen et al., 1990). Cruywagen (1990) reported that curd formation is involved in the effective absorption of immunoglobulin G from the colostrum. In contrast, other studies have reported that curd formation does not provide physiological advantages or protective effects against diseases in calves (Petit et al., 1988, 1989; Constable et al., 2005; Cruywagen and Horn-Quass, 1991). Thus, a controversy exists as to the exact function of curd formation. Importantly, previous studies did not use real-time techniques to confirm whether curd was formed in the abomasum of calves that were fed milk replacer, while clotting or non-clotting properties were determined by in vitro assays.

Recently, we established a simple and practical technique for real-time visualisation of abomasal curd in pre-ruminant calves by ultrasonography (Miyazaki et al., 2009). Ultrasonographic images of the abomasum show curd and whey as an echogenic image with a clear outline and as an anechoic image, respectively. We also confirmed that ultrasonography is a useful technique to distinguish the presence and absence of curd in the abomasum of calves by using milk replacers with clotting and non-clotting properties. Temporal analysis of abomasal contents indicated that the best time for evaluating curd formation is between 1–2 h after feeding milk replacer, when a large curd mass could be imaged, whereas the curd image becomes indistinct 6 h after feeding. Based on these results, we proposed that ultrasonography provides direct evidence to show that curd is formed in the abomasum of pre-ruminant calves.

During the previous ultrasonographic investigation of abomasal curd formation in pre-ruminant calves, we unexpectedly found no abomasal curd in one of six calves at 2 h after feeding the clotting milk replacer (Miyazaki et al., 2009). This finding led us to hypothesise that in some calves, the abomasum does not form curd despite being fed clotting milk or milk replacer. To test this hypothesis, the current study was designed to investigate abomasal curd formation in 29 pre-ruminant calves by ultrasonography after feeding clotting milk replacer. We also analysed the physical condition and blood parameters of the calves, to investigate any negative effects of non-curd-formation.

Materials and methods

Animals and feeding

Twenty Holstein Friesian (HOL) calves (9 males, 11 females; mean birth bodyweight [BW] 43 ± 5 kg) and nine F1 hybrids of Holstein Friesian and Japanese black (F1) calves (4 males, 5 females; mean birth BW 37 ± 3 kg) were used. The calves

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were born and kept with a large dairy cattle breeding herd and housed individually in hutches. They were fed milk replacers at 10% BW daily at 0800 and 1700 h, and had access to fresh water at all times, but were not fed a calf starter ration. The experiment was performed at the same farm. Twenty-eight (19 HOL and 9 F1) calves were examined for a single day, while one HOL calf was examined for 2 days. At the time of the ultrasonographic examination, the calves ranged from 4 to 14 days old. The physical condition of each calf was determined from its appearance, appetite, and vigour on the day of study.

The study was approved by the Animal Research Committee of the Faculty of Agriculture, Iwate University, Japan. Experiments were conducted according to the Iwate University Guidelines for Animal Experimentation.

Milk replacer

A commercial milk replacer (Neo Calf Milk Tsuyoshikun, Snow Brand Seed) was used in this experiment. This milk replacer had clotting properties that were determined by an in vitro rennet coagulation test, as described in a previous report (Miyazaki et al., 2009). The milk replacer contained approximately 24% crude protein, 16% crude fat, 1% crude fibre, 0.5% calcium and 0.3% phosphorus. The percentage of milk proteins, including skim milk and whey proteins, was 66%. Since this was a commercial product, more detailed information on its composition was not available. Milk replacer powder was dissolved at 125 g/L in water at 38 °C.

Ultrasonography

To evaluate the presence or absence of abomasal curd formation in pre-ruminant calves, ultrasonography was performed 2, 4 and 7 h after the morning feeding, using a Super Eye SSD-500 (Aloka) with a 5.0 MHz linear transducer, as described previously (Miyazaki et al., 2009). Briefly, the transducer was placed transversely across the ventral midline and moved along the ventral midline from the xiphoid process to the penis or the corresponding area in female calves. Then, at the point where maximal abomasal area was observed, both paramedian regions were examined by moving the transducer laterally, and still frames were recorded several times using a Video Graphic Printer. Still frames were combined using Photoshop software (Adobe Systems). All examinations by ultrasonography were performed by the same investigator (TM).

Biochemical analysis

Blood samples were obtained just before the morning feeding (0 h), and 1, 2, 4 and 7 h after the feeding. Samples were collected from the jugular vein of each calf. Serum triglyceride (TG), blood urea nitrogen (BUN), and glucose were measured using a MAT Super kit (MC Medical).

Statistical analysis

Means and standard errors (SE) were calculated for serum TG, BUN and glucose concentrations of each curd-forming calves and non-curd-forming calves at 0, 1, 2, 4 and 7 h after feeding. A two-way analysis of variance (ANOVA) with repeated measures of split-plot design was used to detect the effect of curd formation and the time-dependent changes. When the factor was significant according to the Mauchly sphericity test and/or the Greenhouse-Geisser Epsilon adjustment, time-dependent change of each group was analysed by Tukey's honest significant difference test as a multiple comparison. The statistical model included serum concentrations as dependent variable, time after feeding as fixed factor, and calves as random factor. Results were considered significant at $P < 0.05$. Data were analysed using SPSS for Windows version 11.0.1.

Results

Curd formation in the abomasum of pre-ruminant calves

Ultrasonography was used to visualise the abomasal contents of 29 calves (20 HOL and 9 F1, 8.7 ± 3.3 days old) 2 h after feeding with the clotting milk replacer. In 21 calves (14 HOL and 7 F1), a large curd mass was visualised as an echogenic image with a clear outline (Figs. 1A and B). In 20 of the calves, more than two still frames (6 cm/frame) were needed to visualise the maximum cross-sectional width of the curd, with a representative image shown in Fig. 1A. In the one HOL calf (Fig. 1B), the maximum cross-sectional width of the curd was about 6 cm, and it could be visualised in one still frame. The curd (asterisk) was surrounded by the abomasal outline (arrows) and occupied a large space in the abomasum. An anechoic image corresponding to whey (W) was evident in a residual space of the abomasum. In the remaining eight calves (six HOL and two F1), no echogenic image with a clear outline corresponding to curd was observed (Fig. 1C and D). The abomasal outline was visualised as an echogenic line (arrows), and its contents were visualised as images containing an anechoic image with small echogenic spots (C) in five calves (one F1 and four HOL) and an echogenic image with an unclear outline (D) in three calves (one F1 and two HOL).

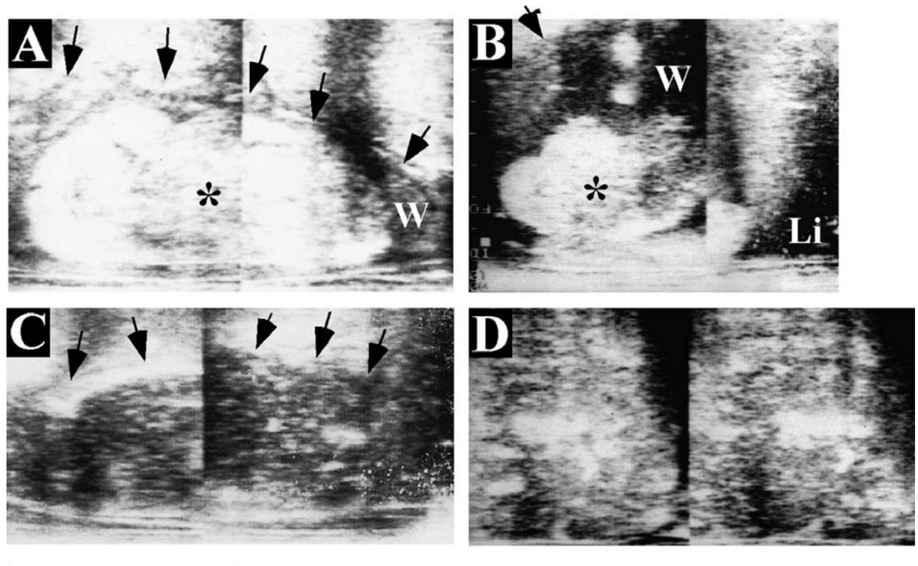

Fig. 1. Ultrasonographic images of abomasal contents in calves 2 h after feeding clotting milk replacer. Panels show the representative images of calves with curd (A, B) and without curd (C, D). (A) An image of abomasal contents in a 13 day old HOL calf with an outlined echogenic image corresponding to a large clot of curd. (B) An image of abomasal contents in an 8 day old HOL calf with an outlined echogenic image corresponding to a clot of curd whose size was markedly smaller than that of other curd-forming calves. (C) An image of abomasal contents in a 9 day old F1 calf with an anechoic image with small echogenic spots. (D) An image of a 6 day old HOL calf showing an echogenic image with an unclear outline. Top, bottom, right, and left of each panel correspond to dorsal, ventral, right, and left of the calf body, respectively. Asterisk: Curd; W: Whey; Li: Liver; arrows: abomasal wall. Bar = 6 cm.