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Short communication: Neonatal calves coagulate first-milking colostrum and produce a large curd for efficient absorption of immunoglobulins after first ingestion

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ABSTRACT

Calves are fed milk and milk replacer for their growth until approximately 2 mo after birth. During this period, their abomasa produce curd and whey from milk. It has been thought that curd formation is important for digestion and absorption of milk nutrients and immune substances in calves. However, no study has been done observing abomasal contents in neonatal calves after first ingestion of first milking colostrum. Here we report curd formation in neonatal calves and its physiological function with a focus on immunoglobulin absorption. We first examined curd formation by ultrasonography in 3 neonatal calves after first ingestion of first-milking colostrum. Between 0.5 and 8 h after colostrum ingestion, a curd was visualized as a large echogenic image with a clear outline, which was surrounded by an anechoic image corresponding to whey. We next compared serum IgG and IgA concentrations in 10 calves fed the pooled colostrum and 7 calves fed the whey solution that did not coagulate into curds. Serum from 1 calf in the pooled colostrum sample set was excluded due to incomplete curd formation in that the whole colostrum did not coagulate into a large mass of curd and a portion of the colostrum remained as its residue caseins detectable from the abomasal fluid. Serum IgG and IgA concentrations were significantly higher in the 9 calves fed the colostrum than the 7 calves fed the whey solution. One calf exhibiting incomplete curd formation showed low levels of serum IgG and IgA after ingestion, similar to the calves fed the whey solution. These results indicate that curd formation is associated with IgG and IgA absorption in neonatal calves after first ingestion of colostrum.

Key words: neonatal calf, curd, immunoglobulin G, colostrum

Short Communication

Colostrum is a rich source of nutrients and immune substances for neonatal calves (Tsioulpas et al., 2007; Elizondo-Salazar and Heinrichs, 2009). Neonatal calves can absorb colostral IgG by intestinal pinocytosis within first 24 h after birth (Jochims et al., 1994; Kaup et al., 1996). Therefore, a common management technique is to feed colostrum to neonatal calves during this period (Quigley and Drewry, 1998; Godden, 2008). Quality and quantity of colostrum and age of first feeding are mainly focused as important factors for efficient IgG absorption from colostrum in neonatal calves (Stott and Fellah, 1983; Furman-Fratczak et al., 2011; Osaka et al., 2014; Yang et al., 2015; Halleran et al., 2017). It has been also suggested that abomasal curd formation is involved in the absorption of colostral IgG (Cruywagen, 1990; Longenbach and Heinrichs, 1998; Gregory, 2003).

Through curd formation, milk components are separated into curd and whey in the abomasum of calves. Milk caseins and lipids are major contents of the curd, whereas whey contains other milk proteins such as immunoglobulins and lactoglobulins, lactose, and minerals. A study showed that plasma IgG levels are higher in neonatal calves fed unadjusted colostrum than in neonatal calves fed colostrum with added oxalic acid to inhibit milk coagulation (Cruywagen, 1990). Additionally, one report showed that over one-third of abomasal fluid samples obtained from neonatal calves lacked enzyme activities for curd formation, and serum levels of γ-glutamyl transferase (**GGT**), which is used as an indicator of IgG absorption, were higher in calves fed colostrum with rennet than in calves fed only colostrum (Gregory, 2003). We recently found that some neonatal calves exhibited low levels of IgG and oligosaccharides including lactose, candidates absorbed by intestinal pinocytosis, until 12 h after first ingestion of first-milking colostrum (Miyazaki et al., 2017). Considering also our previous finding that 28% (95% CI, 15–46%) calves (2 d old) exhibited no curd formation after feeding a clot-

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ting milk replacer (Miyazaki et al., 2010), we hypothesized that there is individual variation in abomasal conditions for curd formation among neonatal calves and the variations may cause differences in colostral IgG and oligosaccharide absorptions.

In our previous studies, ultrasonography showed abomasal curd formation only in calves older than 2 d after feeding fresh milk and some milk replacers (Miyazaki et al., 2009; Okada et al., 2009). However, no studies have focused on abomasal curd formation of neonatal calves after first ingestion of first-milking colostrum. We proposed that it is necessary to examine abomasal curd formation in individual neonatal calves using ultrasonography. Such study will also help to discuss why there was a wide variation in apparent efficiency of absorption (**AEA**) of IgG in calves (Stott and Menefee, 1978), and why there were conflicting results regarding the IgG absorption in neonatal calves after feeding sodium bicarbonate-containing colostrum replacers; such replacers increased serum IgG concentrations in neonatal calves after ingestion (Morrill et al., 2010; Cabral et al., 2012), or not (Cabral et al., 2011, 2014).

The purpose of this study is to examine whether a large mass of curd is produced in individual neonatal calves after first ingestion of first-milking colostrum, as well as calves older than 2 d. We also examined the existence of calves that exhibited incomplete curd formation, such as small curds and no curd. Furthermore, we compared serum IgG and IgA levels between neonatal calves with and without curd formation under evidence that each neonatal calf produced curd or not. Finally we discussed whether abomasal curd formation is involved in AEA of IgG in neonatal calves after first ingestion of first-milking colostrum.

This study was approved by the Animal Research Committee and followed the guidelines for Animal Experiments at Iwate University. First-milking colostrum (approximately 100 L) was collected from 37 Holstein cows at 5 private dairy farms. Then, the pooled colostrum (aliquot 2 L) was divided into plastic bags; 20 of the 50 bags were immediately stored at −20°C until later feeding. In the remaining 30 bags, 40 mL of 100 mg/mL of rennet (MP Biomedical, Santa Ana, CA) solution in 0.1 *M* sodium phosphate buffer (pH 6.3) was added to each bag and incubated at 38°C for 2 h until a large clot of curd and yellowish transparent whey were observed. The whey was then separated from curds using a strainer and pooled in a plastic bucket. The whey (aliquot 2 L) was divided into plastic bags containing 260 g (1,350 kcal) of human milk formula (Sukoyaka, Bean Stalk Snow Co., Ltd., Tokyo, Japan) as supplements, whose amounts were decided based on the reported dairy cow colostrum contents of protein, fat, and carbohydrates (Tsioulpas et al., 2007; Elizondo-Salazar and Heinrichs, 2009). The pooled whey with milk formula (whey solution) was stored at −20°C until later feeding.

Curd formation of the pooled colostrum was examined in in vitro rennet coagulation tests according to a modified method of our previous study (Miyazaki et al., 2009). Briefly, 10 mL of the colostrum was mixed with 200 μL of 100 mg/mL rennet solution, and then the mixture was incubated at 38°C for 2 h. Coagulation was evaluated every 30 min by filtering the mixture through a 1.4-mm testing sieve (Tokyo Screen Co., Tokyo, Japan). The curd on the sieve was weighed. The IgG concentrations in the filtrate were measured using a bovine IgG ELISA quantitation set (Bethyl Laboratories Inc., Montgomery, TX). Curd formation of the pooled colostrum in the presence of amniotic fluid was also examined in in vitro rennet coagulation tests. Five milliliters of the colostrum was mixed with 5 mL of amniotic fluid that was obtained from 3 Holstein dams during cesarean section, and the mixture was incubated with 200 μL of 100 mg/mL rennet solution as described above.

In the primary experiment, 3 male Holstein-Friesian calves, with a mean BW at birth of 48.5 ± 2.2 kg (mean ± SD), were used for the temporal observation of abomasal curd formation after first ingestion of first-milking colostrum. Their physical conditions were evaluated based on appearance, vitality, and blood cell counts. Calves were fed the colostrum once at a volume of 4% of BW at 38°C using a rubber nipple attached to a bucket within 4.1 ± 0.4 h (mean ± SD) after birth. Ultrasonographic images of the abomasal contents were captured using a HS-1500V ultrasonic system equipped with a HLS-375 5.0-MHz 50-mm linear transducer (Honda Electronics Co., Ltd., Aichi, Japan) immediately before (0) and 0.5, 1, 2, and 8 h after first ingestion of the pooled colostrum. Two or 3 sequential captured frames were combined into 1 picture to show abomasal contents in cross-sectional and longitudinal sectional images using Photoshop software (Adobe Systems Inc., San Jose, CA).

In the secondary experiment, 17 Holstein calves (12 male and 5 female) from 6 private dairy farms in Hokkaido, Japan, were separated from their dams before suckling occurred, and housed individually in calf hutches. They were divided into 2 feeding groups. Ten of the 17 calves were fed 2 L of pooled colostrum and the remaining 7 calves were fed 2 L of pooled whey solution, at 38°C within 3 h after birth. A bias was present regarding the numbers of calves between the 2 groups. This was due to the following reason. Considering our previous report that approximately 28% (95% CI, 15–46%) of calves may exhibit no abomasal curd