



Changes in the abomasal contents of pre ruminant calves fed probiotics and kitchen herbs fortified in whole milk

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ABSTRACT

The aim of the study was to determine curd formation in abomasum of the pre-ruminant calves when fed whole milk fortified with probiotics and kitchen herbs (carom seeds, cinnamon and turmeric) as prophylaxis against calf diarrhoea. Ultrasonographic imaging reveals that these products enhanced clotting process and abomasal emptying. It can be suggested that addition of probiotics and herb mix in whole milk accelerated the process of gastric emptying less than 3 hours (hrs) of feeding.

Keywords: Abomasal Curd, Kitchen herbs, Pre-ruminant Calves, Probiotics, Ultrasonography

The pathogenic *E. coli* carrying virulence activation factor (VAF) is widely distributed and a common cause of neonatal calf diarrhoea (Lorenz *et al.* 2011). These are mainly enterotoxigenic (ETEC), enteropathogenic *E. coli* (EPEC) and enterohemorrhagic (EHEC)/Shiga toxin producer *E. coli* (STEC) (Menge *et al.* 1999, Mainil and Fairbrother 2014) often causing mortality and morbidity in young animals. The virulent form of *E. coli* adheres to the epithelial cells forming biofilms during the early stage of bacterial infection (Kaper *et al.* 2004). Many antibiotics have been used to control infections successfully (Constable 2004) but problem of resistance is a growing concern even in well established dairy farms (Rai *et al.* 2020). Alternatively, herbs and spices such turmeric (*Curcuma Longa*), cinnamon (*Cinnamomum* sp.) and carom seeds (*Trachyspermum ammi*) besides flavouring agents have continued to gain popularity due to its strong antioxidant, anti inflammatory and antimicrobial properties (Yadav *et al.* 2013, Jagetia *et al.* 2003, Bentely and Trimmen 1999, Shahidi and Ambigaipalan 2015). Besides, little is also known of herbal medicines possessing microbial resistance (Singh 2013). Many workers have suggested the potential use of probiotics in regulating gut microbiota and proven to reduce diarrhoea among neonates (Liu *et al.* 2022, Pascher *et al.* 2008).

Many interventions in calf feeding systems such as milk replacers, probiotics, milk acidifiers etc. (Godden *et al.* 2005, Burgstaller *et al.* 2017) and nutraceuticals have been

introduced for betterment in overall health of calves but, little is known of the temporal changes in the abomasum after feeding probiotics and kitchen herbs as prophylaxis against diarrhoea. In pre-ruminant calves the milk fed bypasses the rumen via the esophageal groove and flows directly to the abomasum which coagulates as a result of hydrolysis of kappa casein by chymosin separating the curd and whey respectively. The formation of curd is important for digestion, absorption of milk nutrients and immunoglobulins by the calf (Miyazaki *et al.* 2017). Delay in abomasal emptying of milk increases the chance of gastrointestinal disease such as abomasal bloat and diarrhoea (Glenn Songer and Miskimins 2005).

Therefore, the study is conducted to visualize the Abomasal curd formation and its gastric emptying in the pre-ruminant calves after feeding probiotics and kitchen herbs by ultrasonography method (USG).

MATERIALS AND METHODS

Animal feeding: The study was approved by the Institute Research Council of ICAR- National Dairy Research Institute, Eastern Regional Station, Kalyani, India. Fifteen Jersey crossbred calves aged within 3 weeks of age having the mean body weight of 24.40±0.42kgs, were assigned to three groups of five calves each; control, T1 and T2, respectively. Animals in the control group was fed whole milk, T1 group were offered probiotics fortified whole milk while, T2 group was fed probiotics and kitchen herbs mixed in whole milk. The probiotics offered was *Lactobacillus fermentum* NCDC605 and *Lactobacillus rhamnosus* NCDC610 @1010 cfu/mL (source: National Dairy Research Institute, Karnal, India) and kitchen herbs was powdered turmeric, cinnamon and carom seeds (@3%

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per litre of milk). Milk fed to all the calves through bottle @ 10% body weight at 8 AM and 4 PM daily. The fortified mix was supplied until the crucial period of 3 weeks where incidence of diarrhoea is prominent (Cho and Yoon 2014). All the calves had access to clean drinking water at all times but grains were not fed to them when USG was imaged. The time between milk intake and appearance in the abomasums was noted.

In vitro rennet coagulation test: Before the feeding trial, the clotting properties of whole milk, whole milk fortified with probiotics and whole milk probiotics kitchen herb mix were assessed by in vitro rennet coagulation test (Miyazaki *et al.* 2009) where milk clot was caught in the sieve (Fig. 1).

Examination of the abomasum: The abomasum is the largest organ in the pre ruminant calves and its appearance via ultrasonography depends upon milk ingestion. The curd formation in the abomasum was assessed using Ultrasonography (Digi 1100 CD-E Vet, SS Medical Systems (I), Pvt. Ltd, Uttarakhand, India) with a 5.0 MHz linear transducer of high resolution. Hair was clipped and area cleaned with alcohol, contact gel was applied on the transducer and placed in the region specified. The calf was in standing position and restrained well so as to allow continuous recording of all images by the ultrasound machine. The site of the abomasum was scanned between 5th to 12th intercostal space starting from the ventral midline, progressing laterally and dorsally with the transducer held parallel to the ribs (Braun *et al.* 1997). Five calves in each group were analysed at least thrice.

RESULT AND DISCUSSION

Coagulation properties of milk: The fat, SNF, protein and lactose content in the whole milk to be fed to the calves were 4.48 ± 0.34 , 8.91 ± 0.17 , 3.25 ± 0.05 and $4.87 \pm 0.09\%$ respectively. The *in vitro* rennet coagulation test performed in whole milk; whole milk fortified with probiotics and whole milk probiotics kitchen herb mix clotted within 0.30 min as curd was retained on the sieve. The pH (6.0-6.5)



Fig. 1. *In vitro* rennet coagulation test

remained unchanged in all the groups. Softer clots formed in the whole milk as compared to probiotics herb mix.

Ultrasonographic images of the abomasum in empty stomach: The ultrasonographic image of empty calf abomasum is shown in Fig. 2. It is visible between 5th to 12th intercostals space in the ventral right flank region. The abomasal walls appears as distinct fine echoic lines with a circular pylorus having characteristic spoke wheel appearance (Jung 2002). Fine abomasal fold is clearly visible in empty stomach with slow contractions. Next to the abomasum, omasum is detected caudal to the 8th intercostals space with distinct shape and omasal leaves but no detectable motility was visualized.

Ultrasonographic image at 15 mins of milk ingestion: The transducer was placed from the dorsal to ventral region between 6th to 12th intercostal spaces after 15 mins of milk ingestion (Fig. 3). The influx of whole milk, whole milk fortified with probiotics and whole milk probiotics kitchen herb mix appeared as a cloudlike homogenous hyperechoic mass entering to entire abomasum area. There was no difference in these components while visualizing through USG. Abomasal folds were clearly visible as clotting was not initiated.

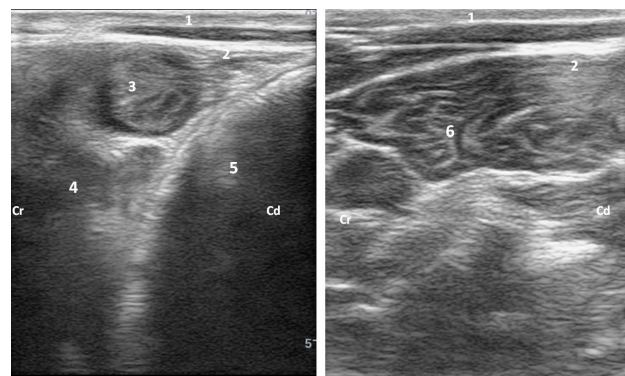


Fig. 2. Empty calf abomasums before feeding milk: 1. Ventral abdominal wall, 2. Abomasal wall, 3. Pylorus, 4. Abomasum, 5. Omasum (hyperechoic rim with anechoic structure), 6. Abomasal folds, Cr: Cranial, Cd: Caudal

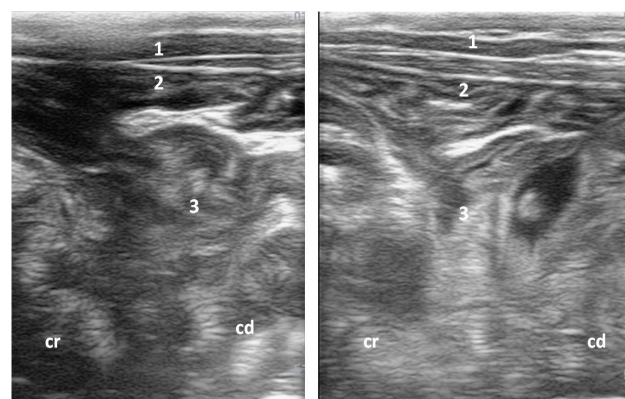


Fig. 3. Abomasum after 15 mins ingestion of whole milk or with fortification (probiotics or kitchen herbs): Unclotted milk, 1. Ventral abdominal wall, 2. Abomasal wall, 3. Abomasal contents, Cr: Cranial, Cd: Caudal

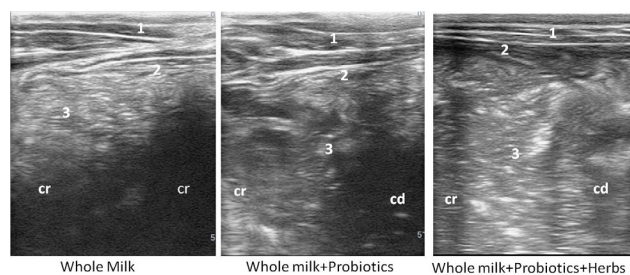


Fig. 4. Abomasum after 30 mins ingestion of whole milk or with fortification (probiotics or kitchen herbs): 1. Ventral abdominal wall, 2. Abomasal wall, 3. tiny clots, Cr: Cranial, Cd: Caudal

Ultrasonographic image at 30 mins of milk ingestion: After 30 mins ingestion of whole milk or with fortification (probiotics or kitchen herbs), milk coagulation started with tiny hyperechoic clots. The contents appear heterogenous with presence of gas formation; abomasal folds were slowly masked by these tiny clots. However, flow of the milk contents was easily visible with strong contractions of the abomasum. But, intensity of the contraction was not measured in the present study. Among all three feedings, presence of gas was more prominent when whole milk and whole milk + probiotics were fed, while whole milk fortified with probiotics and herbs were visualized with sparse gas formation. It could be true that some kitchen herbs possess beneficial effect on digestion, reducing problems of bloating, flatulence, abominal pain and gas formation (Boskabady *et al.* 2014).

Ultrasonographic image at 60 mins of milk ingestion: After 60 min ingestion of whole milk and with fortification (probiotics, herbs), there is formation of larger hyperechoic clumps where the milk has separated into curd (*) and whey. It had been noted that the clot formation looks firmer when whole milk is fortified with probiotics and herbs (Fig. 5). Here also the abomasal folds cannot be visualized by USG. The USG probe was placed slightly left to the ventral midline to assess abomasal clot.

Ultrasonographic image at 2 hrs of milk ingestion: The whole milk with or without fortification (probiotics and herbs) have been represented in Fig. 6. during 2 hrs of milk ingestion. Clear demarcation of hyperechoic abomasal

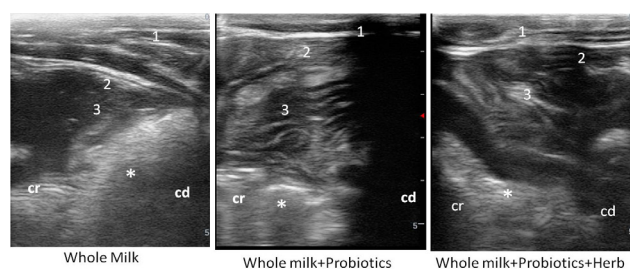


Fig. 6. Abomasum after 1 hr ingestion of whole milk or with fortification (probiotics or kitchen herbs): 1. Ventral abdominal wall, 2. Abomasal wall, 3. Abomasal folds, 4. Clot *, Cr: Cranial, Cd: Caudal

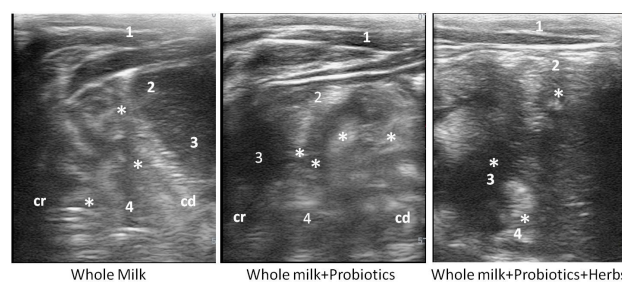


Fig. 5. Abomasum after 60 mins ingestion of whole milk or with fortification (probiotics or kitchen herbs): 1. Ventral abdominal wall, 2. Abomasal wall, 3. Whey, 4. Curd *, Cr: Cranial, Cd: Caudal

folds have been visualized in abomasums of the calves fed whole milk with fortification. However, the echoic clot (*) formation seems larger in calves fed only whole milk.

Ultrasonographic image during 3-4 hrs after milk ingestion: At 3 to 4 hrs of whole milk with or without fortification is represented in Fig. 7. The abomasums present at the dependent part of the abdomen on the ventral midline post xiphoid region clearly shows the milk clots. Larger clot is still evident at 3 to 4 hrs of milk ingestion. The movement of whey is clearly visible with stronger contractions. While the whole milk with probiotics and herbs are holding comparatively smaller milk clots with presence of abomasal folds.

Although many investigators have studied the role of probiotics and herbs as nutraceuticals in calf diets, there is lack of *in vivo* evidence of curd formation in the abomasum in a non-invasive way. In pre-ruminant calves, the most functional part of the stomach, the abomasum, located in the dependent part of the abdomen allows its contents to be readily radiographed by USG. After feeding whole milk, with fortification with probiotics and herbs, the curd and clot formation was clearly visualized by echogenic image with clear outline. The size of the USG linear probe was small (6 cm) which hindered in taking measurements of the clot. But the status of the abomasum remained clearly visible (clot formation, whey, contractions etc.). Abomasal volume, location and emptying rate was studied by many methods (radioactive agents, magnetic resonance imaging) Nappert and Lattimer (2001) but, the non-invasive method

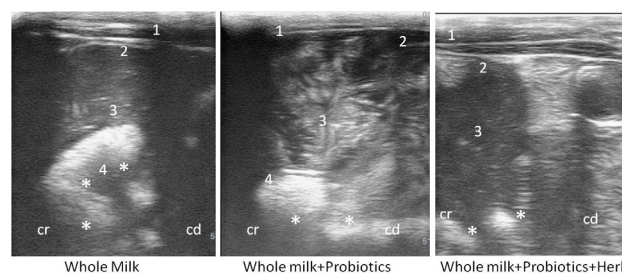


Fig. 7. Abomasum after 1 hr ingestion of whole milk or with fortification (probiotics or kitchen herbs): 1. Ventral abdominal wall, 2. Abomasal wall, 3. Abomasal folds, 4. Clot *, Cr: Cranial, Cd: Caudal

using USG was found equally efficient.

After feeding whole milk, milk fortification using probiotics and herbs, we observed that these components in milk did not affect the clotting process in the abomasum but rather enhanced the process. Several clot formations were observed within 30 minutes of feeding which slowly clumped into a larger clot within an hr or two, the clot formation decreased within 3 to 4 hrs of feeding. However, whole milk clotting process was rather slow. There are reports that milk and milk replacers with Oral Rehydration Solution (ORS) had no effect on abomasal curd formation (Constable *et al.* 2009). Gastric emptying of the abomasum could be visualised in whole milk probiotics herb mixture in a faster pace. Several authors claim that clot formation in the abomasum could be readily assessed within 1 to 2 hrs of milk feeding when the pH is just sufficient for milk coagulation by chymosin and then decreased at 4 hrs (Miyazaki *et al.* 2009, Ahmed *et al.* 2001). Similar observation was seen in the present experiment except that the clot with whole milk probiotics and herb mixture was significantly reduced during the standard time.

Abomasal emptying or gastric emptying refers to the time span the chyme remains before passing to the intestine. Delay in this process will lead to gastrointestinal problems such as bloat, pain, poor appetite and oesophageal reflux (Pasricha and Parkman 2015). Such passage of the ingesta depends upon feed quality, climate, hormonal, individual factors such as feed intake and passage rate etc. (Okine *et al.* 1998, Zabielski *et al.* 1998). Abomasal bloat is common in farms feeding milk replacers, milk replacers with higher protein and fat or errors in management such as feeding cold milk, irregular feeding schedules, failure of passive transfer (Marshall 2009) and bacterial contamination (Aubry 2004, Glenn Songer and Miskimins 2005). Therefore, addition of probiotics and herbs in whole milk had effect on the clotting process and gastric emptying of chyme from the abomasum. It accelerated abomasal emptying thereby leaving very less opportunity for problems associated with digestion, diarrhoea or flatulence. Simultaneously, these products are rather cheap, non harmful and having antibacterial and antioxidant properties therefore, can be included in the diet of neonatal calves.

Overall, work gives us an insight how addition of various products in milk for calf consumption do affect milk clotting and abomasal emptying. This information may help to optimize nutritional planning of the calves while reducing risk associated with gastric problems and diarrhoea.

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