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Estimation of minimum tolerated milk temperature for feeding dairy calves with small- and large-aperture teat bottles: A complementary dose-response study

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ABSTRACT

At birth, calves are functionally monogastric and remain so for the first weeks of life. Milk in the rumen may cause indigestion, diarrhea, and reduced growth. Calves are often fed cold milk from a large-aperture teat, but warm milk and sucking behavior are believed to trigger the esophageal reflex. The aim of this study was to use radiography to estimate the lowest milk temperature that can be given to dairy calves at high and low intake rates without causing milk in the rumen. Our hypothesis was that cold milk drunk at high speed would cause insufficient closure of the esophageal groove and hence milk in the rumen. Fifteen Norwegian Red calves, 9 to 27 d of age, weighing between 45.5 and 71.0 kg, were tested according to the response surface pathway design. Each calf was offered 4 L of milk from both a small- (2 mm) and a large-aperture (19 mm) teat. The milk contained barium sulfate, and radiography was applied before, during, and after the milk meal. Following radiography, the calves were returned to a group pen and observed for 2 h using continuous live behavioral observation to detect signs of abdominal pain or discomfort. Starting with a low number of subjects and increasing this number with increasing design levels reduces the sample size without reducing the statistical power. The minimum milk temperature was estimated to be 8°C. No behavioral signs of pain or discomfort were observed, but shivering was noted in several calves drinking 8°C milk. These results strengthen the argument that calves can be fed large milk meals without risk of causing milk in the rumen, even cold milk drunk at high speed.
Key words: milk feeding, esophageal groove, milk temperature, drinking speed, digestive physiology

INTRODUCTION

Calves are functionally monogastric at birth and remain so for the first weeks of life. During this period, calves' forestomachs are underdeveloped, and ruminal fermentation alone is unable to provide sufficient energy for the growing calf (Blowey, 2004). Hence, milk digestion occurring in the abomasum is the primary source of nutrition. When a young calf drinks milk, it triggers the esophageal reflex. The reflex causes the muscular walls of the esophageal (reticular) groove (*sulcus reticuli*) to contract, turning the open canal into a closed tube. This attribute shunts the milk past the forestomachs directly into the abomasum (Sjaastad et al., 2010). Several factors trigger this esophageal reflex, including sucking behavior, warm milk, the position of the calf's head while drinking (Sjaastad et al., 2010), and absence of stress (Blowey, 2004). However, unfamiliarity with the feeding method (Abe et al., 1979; Blowey, 2004), cold milk, and high drinking speed may cause insufficient closure of the esophageal groove or exceed its capacity (Wise et al., 1984; Blowey, 2004), allowing milk to enter the rumen.

Milk in the forestomachs is usually unproblematic for newborn and very young calves. Any milk in the rumen, such as after feeding with an esophageal tube, as well as in the reticulum and omasum, empties into the abomasum within hours (Lateur-Rowet and Breukink, 1983). For calves that are 2 to 3 weeks and older with a ruminal microflora, large quantities of milk in the rumen may pose a problem (Swanson and Harris, 1958). The milk sugar may be converted to organic acids, and the proteins will rot, changing the ruminal microflora. This may subsequently cause indigestion, diarrhea, and reduced growth (Sjaastad et al., 2010).

A study by Ellingsen et al. (2016) showed that meal sizes up to 6.8 L of milk, representing 13.2% of calf BW, did not cause backflow to the reticulorumen. In that study, milk feeding was optimized in terms of low drinking speed (small-aperture teat) and temperature

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of 38°C. In practice, some farmers may be less accurate when heating milk to feed their calves, and others may manipulate the teat opening to speed up the feeding.

This experimental study on milk feeding has resulted in 2 publications. Holand et al. (2017) provides detailed descriptions of the study design and the novel combination of the response surface pathway with Latin square design, in an effort to introduce and demonstrate additional procedures to strengthen and generalize the response surface pathway methodology. The current publication is complementary to Holand et al. (2017), focusing on the biological and applied aspects of dairy calf feeding, targeting animal scientists and producers. The aim of this study was to use radiography to estimate the lowest milk temperature that can be given to dairy calves at high and low intake rates without causing milk in the rumen. Our hypothesis was that cold milk drunk at high speed would cause insufficient closure of the esophageal groove and, hence, milk in the rumen. Additionally, we observed behavioral indicators of abdominal pain or discomfort resulting from cold milk or high drinking speed.

MATERIALS AND METHODS

Design and Randomization

The study was performed as a randomized single-center study with a 2 × 2 Latin square design (Holand et al., 2017). Each calf participated twice, with the aim of receiving 4 L of milk once via a small- (**Sm**, 2-mm) and once via a large-aperture (**La**, 19-mm) teat of the bottle. In accordance with Latin square design, the calves randomly received the milk in the sequence Sm-La or La-Sm. To control for the influence of a possible sequence effect, a washout period of 2 d between the 2 feedings was included. Within each of the 2 aperture teat groups Sm and La, the study was performed as independent randomized between-patient response surface pathway-designed trials (Holand et al., 2017), with milk temperature as the interventional variable and milk in rumen as response. The predefined milk temperature window in the design was from 8 to 38°C, with a starting temperature of 23°C, in the first design level. The milk temperatures at design levels 2 and 3 were based on the results obtained at design levels 1 and 2, respectively. Further methodological and statistical details are given in Holand et al. (2017).

Experimental Animals

The study sample consisted of 9 bull calves and 6 heifer calves of the Norwegian Red dual-purpose cattle breed. To minimize the age gap, both bull and heifer calves were included consecutively. The animals originated from 2 dairy farms, 1 in Oslo, Norway, and 1 in Sarpsborg, south of Oslo. Calves were housed at the Norwegian University of Life Sciences (Oslo). Clinical examination of the calves was performed on their respective home farms immediately before transportation and again daily during the habituation period. Only animals in good health showing no signs of clinical disease (e.g., lameness, coughing, dullness, low BCS) at the time of transportation were included in the study. All calves were transported to and from the test facility in a horse trailer. One-way transportation time was 1 h for the Sarpsborg calves and 15 min for the Oslo calves. For practical reasons, the calves were transported, housed, and tested successively according to design level. Calves from the same farm were housed together in group pens. The calves had free access to hay (high-quality first cut), water, and concentrate (Formel Kalv, a commonly used calf feed in Norway; Felleskjøpet Agri, Oslo). They were fed 2 L of whole milk 3 times a day, as was the Norwegian recommendation at the time (Overrein et al., 2015) and the practice at their home farms. Milk meals were provided at 0800, 1300, and 1800 h. To habituate calves to lower milk temperature, all milk except at the time of testing kept a temperature of 23°C. The mean age of the calves was 18.5 d and varied between 9 to 27 d at the beginning of the experiment. The mean BW was 54.9 kg, ranging from 45.5 to 71.0 kg upon arrival at the test facility.

The calves were habituated to the new housing and the new milk temperature for 2 d after arrival, and randomized to treatment sequence. The first feeding trial was performed on the third day, followed by observation and a washout period of 48 h. The second feeding trial followed on d 5, with subsequent observation (Table 1).

All experimental procedures were in accordance with the regulations controlling experiments and procedures on live animals in Norway, and the study complied with policies relating to animal ethics. Due to the nature of the experiments, permission from the Norwegian Animal Research Authority was not required. All calves were returned to their home farms after the experiment.

Test Procedure

The milk used in the trial was unpasteurized whole milk, with the contrast agent barium sulfate ($BaSO_4$; Mixobar Colon, Bracco SpA, Milan, Italy; 1 g/mL) added at a ratio of 6:1. To standardize the intake rate, the same 2 rubber teats were used for feeding all the animals. The rate at which the bottle emptied was timed using a stopwatch. A small hole was made in the

Table 1. Day-by-day schedule of milk amounts fed to study calves and actions performed as part of the experiment

Day at test facility	Amount of milk given (L)			Action
	0800 h	1300 h	1800 h	
0	—	2	2	Transportation to test facility
1	2	2	2	Habituation: housing and milk temp
2	2	2	2	Habituation: housing and milk temp
3	4[1]	2	2	X-rays + observation
4	2	2	2	Washout period
5	4[1]	—	—	X-rays + observation + transportation to home farm

[1]Teat aperture and temperature based on response surface pathway design.

large-aperture teat bottle to prevent vacuum and allow for a more even milk flow. The small-aperture teat bottle emptied at 1.5 L/min, the large at 3.2 L/min, when filled with water and turned upside down. At the time of testing, the duration of milk consumption was measured from the point when the calf started drinking to the point when it ceased drinking, using a stopwatch. Abdominal radiographs were taken before, during, and 10 min after intake of the meal for investigation of milk in the rumen (Figure 1). Lateral-lateral abdominal digital radiography (Acroma ceiling-mounted system, Växjö. Sweden; Medivet software and digital radiography plates, Engelholm, Sweden) was performed on standing calves with a focal-film distance of 140 cm, using a grid. Exposure factors were 85 kV and 20 mAs. Radiographs showing contrast milk in the reticulorumen have been previously demonstrated (Ellingsen et al., 2016). Any potential milk in the rumen was recorded as "Milk" (radiopaque material clearly visible in the rumen), "Trace of milk" (droplets of radiopaque material in the rumen), or "No milk" (no radiopaque material visible in the rumen). Possible onset of diarrhea was monitored throughout the experimental period. Following each of the 3 daily meals, fecal registrations were performed for all calves using a 3-point scale: 0 = No diarrhea, 1 = Mild diarrhea, and 2 = Severe diarrhea. If no severe diarrhea was observed, the second feeding with the other aperture teat was started 2 d later.

Behavioral Observations

Milk in the rumen undergoes rapid fermentation and may cause acute colic pain within 15 to 30 min of feeding (Blowey, 2004). Therefore, each calf was observed using continuous live behavioral observation for 2 h immediately after each of the 2 test meals, to reveal signs of abdominal pain or discomfort according to a predefined ethogram (Table 2), based on Bourne (2013). All behavioral observations were carried out by the same observer (first author, an ethologist and scientist with experience of behavioral observations and pain assessment in animals). For practical reasons, the observer could not be blinded to the performance of the calves during trials. Behavior data were not statistically analyzed but were used as background information related to classification for the next design level.

Statistical Analysis

The sample space of the milk temperature may be expressed as $\Omega T = \{DL \leq \ldots \leq DU\}$. Let μd represent minimum milk temperature for young calves, and assume μ_d is covered by Ω_D, with further details in Holand et al. (2017). Milk in rumen is ordinal in interventional variable, and the probability increases monotonically over the interventional levels. Isotonic regression is the

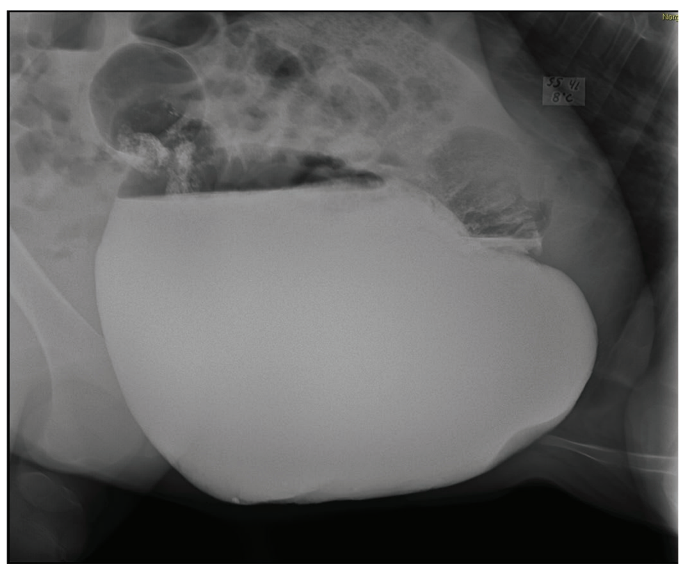

Figure 1. Cranial abdominal radiograph (head oriented to the right) taken after the calf had drunk 4 L of 8°C contrast milk (milk: barium sulfate contrast 6:1) from a large-aperture teat bottle. Note that radiopaque material is present in the abomasum and none is visible in the rumen.

Table 2. Ethogram used to detect signs of abdominal pain or discomfort in calves after milk feeding

Behavior	Description	Categories or units
Dull appearance[1]	Passive, unresponsive to pen mates, and uninterested in surroundings when awake	No or yes
Vocalization	Any kind of vocal expressions	Number of vocalizations per animal
Licking at the abdomen	Turns head and licks at the abdomen	Number of licks per animal
Biting at the abdomen	Turns head and bites at the abdomen	Number of bites per animal
Kicking at the abdomen	Kicks at the abdomen with hind leg	Number of kicks per animal
Getting up or lying down	Partly or fully stands up or lies down	Number of times calf fully stood up or lay down, or this sequence was started but interrupted
Rapid breathing	Rapid, shallow breathing, >60 breaths/min	Number of bouts[2] and duration per bout (s)
Bruxism	Grinding teeth	Number of bouts[2] and duration per bout (s)
Hunched stance	Standing with head low and back arched	Number of bouts[2] and duration per bout (s)

[1]Recorded every 30 min.

[2]Separate bouts are defined by an interval of 10 s or more between instances of the behavior.

suggested model for analyzing the material (Hamilton et al., 1977; Stylianou and Flournoy, 2002). Continuously distributed variables are expressed by mean values, standard deviation (SD) in brackets, and 95% confidence interval (Altman, 1991). The prevalence of diarrhea was analyzed by the use of contingency cross tabulation (Agresti, 2002). The duration of milk consumption was compared using a paired-samples *t*-test (Altman, 1991).

RESULTS

We did not detect milk in the rumen in any of the animals, independent of milk temperature and aperture size of the teat bottle. Hence, we estimate the minimum milk temperature to be 8°C.

Mean milk consumption was 3.8 L (SD = 0.6) with the small- and 3.6 L (SD = 0.8) with the large-aperture teat bottle. With the small aperture, one calf drank 2 L, another 3 L, and the remaining 13 drank 4 L of milk. By changing to the large-aperture teat, 2 calves drank 2 L, 1 drank 2.5 L, 1 drank 3 L, and the remaining 11 drank 4 L of milk. The difference was not significant.

Mean duration of milk consumption was 160 s (SD = 22) for the small-aperture teat and 111 s (SD = 53) for the large (Table 3). The reduction was significant ($P < 0.01$). Duration of milk consumption decreased slightly in both groups with decreasing milk temperature. However, the 2 groups developed differently with regard to amount. The amount of milk consumed slightly decreased with decreasing milk temperature when drinking from the small-aperture teat. With the large-aperture teat, the amount of consumed milk increased with decreasing milk temperature.

With the small-aperture teat, milk temperature positively correlated with milk consumption and slightly with duration (Table 4). We detected only a slight but positive correlation between duration and milk consumption. This pattern remained nearly unchanged in the partial correlation analysis. Using the large-aperture teat, the milk temperature negatively correlated with milk consumption. This correlation was unchanged in the partial analysis. However, milk consumption significantly correlated negatively with duration of milk consumption and remained unchanged in the partial analysis.

Although not included in the ethogram, shivering was observed in several calves at the time of drinking. Four of the animals each licked at their abdomens once. All animals performed lying down and getting up behavior 2 to 3 times during the first 2 h after the test meal.

Only mild degree diarrhea was recorded in the study sample. We recorded this 3 times with the small-aperture teat and 5 times with the large. In 2 of the 3 instances with the small-aperture teat, we also recorded it with the large. We registered the occurrence of diarrhea twice following the first test meal and 6 times following the second.

DISCUSSION

In this experiment, we fed young calves milk at 23, 13, and 8°C with small- and large-aperture teats. Despite the suboptimal feeding regimen, we observed no milk in the rumen. This demonstrates that the esophageal groove closes efficiently even when calves drink cold milk at high speed. Apart from shivering at the time of drinking, we observed no behavioral signs of pain or discomfort. The current findings strengthen the conclusion by Ellingsen et al. (2016) that calves can be fed large milk meals without the risk of causing milk in the rumen.

Table 3. Comparison of milk temperature groups with regard to duration and volume of milk consumption; results expressed as means with 95% CI

Milk temperature	Duration of milk consumption (s)		Milk consumption (L)	
	Small opening	Large opening	Small opening	Large opening
23°C (n = 3)	165	122	4.0	3.3
	106–223	52–193	3.3–4.7	2.3–4.3
13°C (n = 5)	161	102	4.0	3.4
	130–192	47–157	3.5–4.5	2.6–4.2
8°C (n = 7)	158	112	3.6	3.8
	138–178	65–158	3.1–4.0	3.1–4.4

Milk Temperature

To ensure triggering of the esophageal reflex, it is generally recommended that calves be provided with warm milk (approximately body temperature, or 38°C; Overrein et al., 2015). In our experiment, however, 8°C milk was sufficient to induce a functional esophageal groove. This indicates that a low milk temperature per se does not cause milk in the rumen. According to Moran (2002), maintaining a consistent milk temperature when feeding calves is more important than the temperature itself. Calves are creatures of habit, and maintaining a standardized feeding regimen, including temperature, has been found vital to ensuring that the esophageal groove is well closed (Abe et al., 1979; Blowey, 2004).

With the small-aperture teat and thus low drinking speed, 13 of the 15 calves drank all 4 L offered. With the large-aperture teat, the high milk flow combined with the low temperature resulted in only 11 of the 15 calves finishing the entire meal. This may indicate that calves are less tolerant of receiving cold milk at high speed. Also, it was obvious that the calves found the cold milk less tempting. Latency to drink, although not formally timed, increased with decreasing milk temperature. Greater effort was also required to make the calves interested in the teat and maintain drinking. In addition, several animals fed the 8°C milk shivered for several minutes after ingesting the milk, indicating low body temperature. Energy expenditure used to heat ingested cold milk can lead to reduced growth (Flipot et al., 1972) and may pose a significant strain at low ambient temperatures (CalfCare.ca, 2019).

Drinking Speed

A milk flow rate of 1 L/min is suggested to be optimum for calves (de Passillé, 2001), to avoid spilling milk into the rumen (Wise and Anderson, 1939; Wise et al., 1984; Blowey, 2004). As with low milk temperature and contrary to our hypothesis, the esophageal grooved closed efficiently even at high drinking speed. It is important to note, however, that the relationship between aperture size and drinking speed is not linear. The large-aperture teat resulted in a significantly higher drinking speed than did the small-aperture teat in the current experiment, but calves are able to reduce the speed of ingestion from large-aperture teats (de Passillé, 2001), thus potentially reducing the risk of esophageal spillover.

Although not found to cause milk in the rumen, we do not recommend feeding calves with large-aperture teats. A large-aperture teat does not sufficiently cover the calf's sucking need. This need gradually wanes dur-

Table 4. Multiple and partial correlation patterns between milk temperature (Temp) and volume and duration of milk consumption for both small and large teat openings; results expressed as Pearson correlation coefficients

Teat opening	Variable	Multiple correlation			Partial correlation		
		Temp (°C)	Consumption (L)	Duration (s)	Temp (°C)	Consumption (L)	Duration (s)
Small	Temp (°C)	1	0.31	0.11	—	0.30	0.07
	Consumption	0.31	1	0.15	0.30	—	0.12
	Duration	0.11	0.15	1	0.07	0.12	—
Large	Temp (°C)	1	−0.23	0.07	—	−0.24	−0.11
	Consumption	−0.23	1	−0.65*	−0.24	—	−0.65*
	Duration	0.07	−0.65*	1	−0.11	−0.65*	—

*$P < 0.05$.

ing the 10 min following the meal (de Passillé, 2001; Nielsen et al., 2018) and is independent of the level of satiety (Hammell et al., 1988). Hence, we urge farmers with manual milk feeding not to cut the top of the teat off to save time during feeding.

Pain Behavior and Diarrhea

Behavioral observations were included in the study to detect signs of pain or discomfort resulting from cold milk or intake at high speed. Although not included in the ethogram, shivering was observed in several calves at the time of drinking. Shivering is autonomous muscle contractions with the sole function of producing heat (thermogenesis) to maintain the core body temperature (Sjaastad et al., 2010). When shivering, the animal will probably experience discomfort due to feeling cold.

Although 4 of the 15 calves licked at their abdomens, the frequency (once per calf within the 2-h observation period) was too low to indicate pain. The same is true for getting up and lying down behavior. Although it may suggest colic pain if preformed repeatedly (Bourne, 2013), the observed occurrence of getting up and lying down (on average performed 2–3 times by each calf) was spread across the 2-h observation period and hence cannot be said to be indicative of pain. These findings are similar to the behaviors reported after slow ingestion of large milk meals at 38°C (Ellingsen et al., 2016), meaning that cold milk and high drinking speed do not cause abdominal pain or discomfort.

Diarrhea is commonly reported in conjunction with low milk temperatures (Gleeson and Fallon, 2007). In the current study, mild diarrhea was observed more often following ingestion from the large-aperture teat, indicating that a high drinking speed is unfavorable to the animals, as discussed by McInnes et al. (2015). However, in 2 cases with the large opening, we classified the diarrhea as “unrelated” to treatment, meaning diarrhea was detected after inclusion but before intake of the first test meal. Hence, drawing conclusions regarding diarrhea from the current experiment is difficult.

CONCLUSIONS

Radiographs showed no milk in the rumen, regardless of milk temperature and aperture size. In addition, no behavioral signs of pain or discomfort, apart from shivering at the time of drinking, were observed. Hence, the lowest estimated milk temperature that can be fed to dairy calves without causing milk in the rumen is 8°C. These results strengthen the argument that calves can be fed large milk meals without risk of milk in the rumen, even cold milk drunk at high speed.

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