Ultrasonographic examination of the oesophageal groove reflex in young calves under various feeding conditions

U. Braun, C. Brammertz

Department of Farm Animals, Vetsuisse Faculty, University of Zurich

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Summary

The oesophageal groove reflex was examined in 6 milk-fed Holstein Friesian calves once weekly during the first 17 weeks of life. Additionally, the effect of different feeding methods (bucket, different nipple positions and openings), different milk temperatures (20, 30, 39, 45 °C) and milk replacer concentrations (100, 125, 150 grams/litre of water) on oesophageal groove closure was investigated. The reticulum and abomasum were examined ultrasonographically using a 5.0-MHz convex transducer before, during and after feeding, and the oesophageal groove reflex was considered to be functional when milk was seen entering the abomasum during feeding. The reflex was consistently induced throughout the study period in all calves at all examinations and under all experimental conditions. However, it should not be assumed that feeding technique can be neglected in unweaned calves because suboptimal feeding management has been linked to various digestive disorders.

Keywords: calf, ultrasonography, oesophageal groove reflex, abomasum, milk, milk replacer, milk temperature, feeding method

Überprüfung des Schlundrinnenreflexes bei Kälbern unter verschiedenen Bedingungen mittels sonographischer Untersuchung

In der vorliegenden Untersuchung wurde die Auslösung des Schlundrinnenreflexes mittels Milchtränke bei 6 Holstein-Friesian Kälbern von der ersten bis zur 17. Lebenswoche einmal wöchentlich untersucht. Darüber hinaus wurde der Einfluss verschiedener Tränkemethoden (Eimertränke, Sauger am oberen Eimerrand, Sauger am Eimerboden, Sauger mit kleiner bzw. grosser Öffnung), unterschiedlicher Milchtemperaturen (20, 30, 39, 45 °C) und verschiedener Milchaustauscherkonzentrationen (100, 125, 150 g MAT pro Liter Wasser) auf den Schlundrinnenreflex überprüft. Haube und Labmagen wurden vor, während und nach dem Tränken mit einem 5.0-MHz-Konvexschallkopf sonographisch untersucht. Der Schlundrinnenreflex wurde als ausgelöst betrachtet, wenn das Einfliessen der Milch in den Labmagen während des Tränkens nachgewiesen werden konnte. Die Untersuchungen zeigten, dass der Schlundrinnenreflex beim Trinken von Milch bei den wöchentlichen Untersuchungen während der ersten 17 Lebenswochen bei jedem Kalb und bei jeder Untersuchung ausgelöst wurde. Darüber hinaus wurde der Schlundrinnenreflex auch bei den verschiedenen Tränkemethoden, bei den verschiedenen Milchtemperaturen und bei den verschiedenen MAT-Konzentrationen bei jedem Kalb und bei jeder Untersuchung ausgelöst. Trotz dieser Ergebnisse darf nicht der Schluss gezogen werden, dass die Tränkebedingungen für den Schlundrinnenreflex nicht wichtig sind und deshalb vernachlässigt werden können. Vielmehr ist davon auszugehen, dass ein nicht optimales Tränkemanagement über längere Zeit zu vielfältigen Störungen der Verdauungsfunktionen führen kann.

Schlüsselwörter: Kalb, Sonographie, Schlundrinnenreflex, Labmagen, Milch, Milchaustauscher, Milchtemperatur, Tränkemethode

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Introduction

In unweaned calves, the oesophageal groove reflex causes the ingested milk to bypass the reticulorumen and to flow directly into the abomasum through the oesophageal groove and reticulo-omasal orifice (Newhook and Titchen, 1974; Wise et al., 1984). This reflex causes the two lips of the groove to contract and rotate to form a tube while the reticulo-omasal orifice relaxes (Kaske, 2005). Ultrasonography is a non-invasive and straightforward technique for indirectly monitoring the oesophageal groove reflex; a functional reflex is confirmed ultrasonographically when milk is seen entering into the abomasum during feeding (Wittek et al., 2005; Braun and Gautschi, 2012; Braun et al., 2013). Complete or partial failure of the oesophageal groove reflex can result from unnatural housing and feeding conditions (Kaske, 2005) or from neonatal disorders such as diarrhoea (Dirksen, 2006). Milk given to a calf by forced intubation feeding is deposited in the reticulorumen (Schipper et al., 1984; Dirksen and Baur, 1991) but overflow into the abomasum occurs after a sufficient amount has been given (Chapman et al., 1986). During normal feeding of a healthy calf, approximately 10% of the ingested milk reaches the reticulorumen (Ruckebusch and Kay, 1971) from where it is actively transported through the omasum into the abomasum within three hours without adversely affecting the forestomachs (Lateur-Rowet and Breukink, 1983). If milk remains in the rumen for an extended period of time, bacterial fermentation of the milk sugars occurs leading to a disorder called ruminal drinker syndrome (Dirksen, 2006). The milk in the rumen of these calves can be seen ultrasonographically (Gautschi, 2010; Braun and Gautschi, 2013). Conditions required for a functional oesophageal groove reflex include normal health status of the calf, voluntary nursing, normal smell and taste of the milk and contact of the milk with the relevant chemoreceptors in the oral cavity and oesophagus (Dirksen, 2006). Furthermore, visual, auditory and olfactory stimuli are believed to be involved in the oesophageal groove reflex (Abe et al., 1979). To our knowledge, the oesophageal groove reflex in older calves and the effects of various feeding methods on this reflex in healthy calves have not been determined. The purpose of this study was therefore to investigate whether the oesophageal groove reflex functions consistently during the first 17 weeks of life and to examine various factors affecting the reflex. In a first experiment, a functional reflex was investigated in milk-fed calves during the first 17 weeks of life. In a second experiment, the effects of milk temperature, concentration of milk replacer, feeding system and size of the opening of the artificial nipple on groove closure were investigated.

Animals, Material and Methods

Animals

Six Holstein Friesian bull calves, a maximum of 2 days of age and weighing 41.5 ± 4.60 kg, were enrolled in the study. The results of haematological and biochemical examinations were normal and testing for bovine virus diarrhoea antigen (Microsynth, Balgach) was negative.

Table 1: Body weight, meal size and duration of feeding in 6 Holstein Friesian bull calves during the 17-week study period (means ± standard deviations or medians, ranges in brackets).

Week	Body weight (kg)	Meal size (litre)	Duration of feeding (min)
1	41.5 ± 4.6 (33 – 46)	2.5 (2.0 – 2.5)	9.7 ± 2.5 (7.2 – 11.2)
2	44.1 ± 4.8 (35 – 48)	2.5 ± 0.3 (2.0 – 3.0)	8.6 (6.5 – 17.4)
3	48.8 ± 6.0 (46 – 58)	3.0 (2.5 – 3.0)	5.9 ± 2.4 (3.4 – 8.2)
4	53.3 ± 6.8 (43 – 63)	3.1 ± 0.4 (2.5 – 3.5)	4.8 ± 1.8 (3.0 – 7.4)
5	58.8 ± 5.8 (49 – 65)	3.5 ± 0.3 (3.0 – 4.0)	3.6 ± 1.0 (2.4 – 5.3)
6	64.7 ± 5.8 (56 – 74)	4.0	2.7 ± 0.5 (2.1 – 3.3)
7	71.2 ± 5.1 (65 – 78)	4.0	3.2 ± 1.0 (2.3 – 4.6)
8	76.5 ± 5.7 (69 – 84)	4.0	3.5 ± 1.4 (2.2 – 6.2)
9	82.4 ± 7.1 (72 – 92)	4.0	3.7 ± 1.5 (2.1 – 6.3)
10	88.5 ± 8.5 (75 – 97)	4.0	3.4 ± 1.4 (2.3 – 5.6)
11	96.1 ± 9.8 (82 – 107)	4.0	3.2 ± 0.4 (2.4 – 3.5)
12	103.3 ± 10.8 (88 – 115)	4.0	3.4 ± 1.0 (2.3 – 4.4)
13	110 ± 11.6 (96 – 123)	4.0	3.3 ± 0.9 (2.0 – 4.3)
14	117.8 ± 11.2 (107 – 130)	4.0	3.3 ± 1.1 (2.3 – 5.2)
15	124.7 ± 11.9 (109 – 136)	4.0	3.3 ± 0.9 (2.2 – 4.5)
16	131.1 ± 11.8 (114 – 142)	4.0	3.1 ± 0.8 (2.1 – 4.1)
17	139.6 ± 13.5 (120 – 152)	4.0	3.2 (3.1 – 4.5)

Table 2: Study design for the examination of the oesophageal groove reflex during the first 17 weeks of life in 6 Holstein Friesian bull calves.

Weeks (days)	Experiment
1 to 17 (0 – 120)	One ultrasonographic examination per week during feeding of cow's milk (39 °C) fed from a bucket with an artificial nipple near the bottom
4 (22 – 28)	Three ultrasonographic examinations during feeding of different concentrations of milk replacer (100, 125 and 150 g/litre of water)
5 (29 – 35)	Three ultrasonographic examinations during the feeding of cow's milk of different temperatures (20, 30 and 45 °C)
6 (36 – 42)	Two ultrasonographic examinations during feeding of cow's milk (39 °C) from a bucket and with a nipple that required a strong sucking reflex
7 (43 – 49)	Two ultrasonographic examinations during feeding of cow's milk from a nursing bottle and a nipple with a small (1 mm) and large opening (8 mm)

The calves were kept in individual pens, had visual contact with other calves and were fed twice daily. The calves were weighed once a week and the amount of milk fed per day was adjusted to 12% of body weight up to the age of five weeks (Tab. 1). Thereafter and until the end of the study, they received 8 litres of milk per day divided into two feedings. Hay and water were offered ad libitum and after day 60, the calves received 75 g of a concentrate containing yeast twice daily to stimulate ruminal development (Prima ProRumin Combi IPS, UFA AG, Herzogenbuchsee).

Ultrasonographic examination

Ultrasonographic examination of the reticulum, rumen, anterior blind sac of the rumen and abomasum was carried out as described earlier (Gautschi, 2010; Braun and Gautschi, 2012) in the standing calf using a 5.0-MHz convex transducer. The position, size and content of these organs were assessed before, during and after feeding milk.

Monitoring the oesophageal groove function for the first 17 weeks of life

The oesophageal groove reflex was examined once a week for the first 17 weeks of life (Tab. 2). Based on earlier studies (Braun and Gautschi, 2012; Braun et al., 2013), functioning of the reflex was confirmed ultrasonographically when milk was seen entering the abomasum during feeding. For feedings, a 10-litre bucket with a rubber nipple was used. The nipple was attached near the bottom of the bucket (Calf feeding bucket, Hauptner Instrumente GmbH, Dietlikon-Zürich). The examinations were alternated among different locations (in the barn or in one of four examination rooms) to avoid conditioning of the reflex. Each feeding consisted of 50 of the daily amount of milk fed, which was heated to 39 °C using an immersion heater (Milk heater, Hauptner Instrumente GmbH) before feeding. The duration of feeding was recorded. The results of these examinations from weeks 4 to 7 served as controls for the experimental examinations conducted in the same weeks (see Tab. 2).

Effect of milk replacer concentration on oesophageal groove closure

The effect of milk replacer concentration on oesophageal groove closure was examined on three different days of week 4 (Tab. 2). Concentrations of 100, 125 and 150 g milk replacer (UFA 207 plus universal milk replacer, UFA AG, Sursee) per litre water heated to 41 °C were used; each concentration was fed once on one of the 3 days. The concentration recommended by the manufacturer was 100 g/litre water and the milk replacer was fed as in the controls.

Effect of milk temperature on oesophageal groove closure

The effect of milk temperature on oesophageal groove closure was examined on three different days in week 5 (Tab. 2). Cow's milk heated to 20, 30 and 45 °C using an immersion heater was fed once on one of the 3 days. The milk was fed as in the controls.

Effect of bucket feeding on oesophageal groove closure

The effect of bucket feeding on oesophageal groove closure was examined once in week 6 (Tab. 2) by feeding cow's milk in a bucket without a nipple.

Effect of nipple position on oesophageal groove closure

The effect of nipple position on oesophageal groove closure was examined in week 6 (Tab. 2) with a rubber nipple attached to the top of the bucket and connected to a rubber hose, which reached to the bottom of a 10-litre bucket (Calf feeder, Etro simple, Etro AG, Rorschacherberg). This system demanded strong sucking movements from the calf.

Effect of size of nipple opening on oesophageal groove closure

The effect of the size of the opening of the artificial nipple was examined twice on different days in week 7. On one day, an opening of 1 mm was tested in one feeding and on another day, an opening of 8 mm was tested in one feeding (Tab. 2). Cow's milk heated to 39 °C was fed from a 2-litre nursing bottle (Hauptner Instrumente GmbH). The small opening consisted of a 1-mm cross-shaped orifice of the newly purchased nipple. The larger opening was made with an 8-mm skin biopsy punch (Biopsy Punch®, 8 mm, Stiefel, Germany).

Duration of feeding

A digital stop watch was used to determine the duration of feeding forin each calf.

Statistical analysis

Means, medians and standard deviations were calculated using the IBM® SPSS® Statistics 20 program (IBM Corporation, New York, USA). Normality of distribution was examined with the Shapiro-Wilk and Kolmogorov-Smirnov tests. Mean, standard deviation and range were given for normally distributed data and median and range for data with non-normal distribution. Differences between durations of feeding were analysed using a paired t-test for data with normal distribution and the Wilcoxon rank-sum test for data with non-normal distribution. Differences were considered significant at $P < 0.05$.

Results

Ultrasonographic examination before, during and after feeding

The ultrasonographic findings of the reticulum, anterior blind sac of the rumen, rumen and abomasum before, during and after feeding were the same as those published earlier (Braun and Gautschi, 2012; Braun et al., 2013) and are only presented collectively here; they were described in detail elsewhere (Brammertz, 2014). The reticulum appeared as an empty crescent-shaped structure with an echoic wall before feeding. During ingestion of milk, the reticulum became displaced cranially and dorsally by the expanding abomasum until it could no longer be seen because of superimposition of the lungs. After the age of about seven weeks, reticular displacement during feeding became incomplete and parts of the organ remained visible throughout, and after the end of feeding. The abomasum was always adjacent to the ventral abdominal wall, its echoic wall was wavy, the caudal margin distinct and the abomasal leaves appeared as hypoechoic structures in all calves before feeding. During feeding, echoic milk was seen entering the abomasum in a pulsatile fashion in all calves at all examinations. The milk had a snow-storm appearance in the abomasum and the homogeneous content was first seen in the cranial part and then in the caudal part. The stomach expanded longitudinally and from side to side. The abomasal leaves appeared as undulating hypoechoic structures during feeding. Two minutes after the end of feeding, the abomasum appeared as a long and wide hollow organ with homogeneous echoic content.

Oesophageal groove reflex in the first 17 weeks of life

Oesophageal groove closure was seen in all calves throughout the study period based on ultrasonographic visualisation of milk entering the abomasum during feeding. Milk was not observed in the reticulum of any of the calves before, during or after feeding.

Effect of milk replacer concentration on oesophageal groove closure

The oesophageal groove reflex was functional in all calves when fed the three concentrations of milk replacer based on ultrasonographic visualisation of milk entering the abomasum during feeding. In contrast to cow's milk, milk replacer appeared hyperechoic and heterogeneous on ultrasonograms. Milk replacer was not observed in the reticulum of any of the calves before, during or after feeding.

Effect of milk temperature on oesophageal groove closure

The oesophageal groove reflex was functional in all calves at all three milk temperatures based on ultrasonographic visualisation of milk entering the abomasum during feeding. Milk was not observed in the reticulum of any of the calves before, during or after feeding.

Effect of bucket feeding on oesophageal groove closure

Four of the six calves drank milk directly from the bucket. After 5 minutes, the experiment was stopped and the two calves that refused bucket feeding were fed using the regular method. The ultrasonographic findings of the reticulum and abomasum were similar to the previous experiments. A small amount of milk, which appeared homogeneous and echoic, was in the ventral sac of the rumen immediately after bucket feeding seen in one calf, but the abomasum filled with milk and expanded to the inguinal region after feeding, as observed in the other calves.

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Effect of nipple position on oesophageal groove closure

The oesophageal groove reflex was functional in all calves when fed with a rubber nipple attached to the top of the bucket and connected to a rubber hose, which reached to the bottom of a 10-litre bucket.

Effect of size of nipple opening on oesophageal groove closure

The oesophageal groove reflex was functional in all calves fed with nipples with different sized openings based on ultrasonographic visualisation of milk entering the abomasum during feeding. Milk was not seen in the reticulum of any of the calves before, during or after feeding.

Duration of feeding

The mean duration of feeding was 9.7 minutes per meal in the first week, then gradually decreased to 3.6 min in week 5 ($P<0.01$) and ranged from 2.7 to 3.7 min/4 litres from weeks 6 to 17. The duration of feeding did not differ among different milk replacer concentrations, different milk temperatures, bucket feeding and different nipple positions (Tab. 3). The use of the nipple with the small opening was associated with a prolonged feeding time (7.4 min) and the use of the large opening with a shortened time (1.6 min) compared with the regular feeding method (3.2 min, $P<0.05$).

Discussion

The ultrasonographic findings of the reticulum and abomasum before feeding were the same as those described previously (Gautschi, 2010; Braun and Gautschi, 2012; Krüger, 2012; Braun et al., 2013) and the position and size of the organs corresponded to results of computed tomographic examinations (Schnetzler, 2012; Braun et al., 2014). The flow of milk into the abomasum during feeding was readily confirmed ultrasonographically (Gautschi, 2010; Braun and Gautschi, 2012; Krüger, 2012; Braun et al., 2013); the stomach usually filled from cranial to caudal and the content was homogeneous and echoic. Feeding was associated with complete or partial displacement of the reticulum by the expanding abomasum, which was wide and extended caudally to the umbilical or inguinal region when full. The ultrasonographic features of the clotting process were as described previously (Wittek et al., 2005; Miyazaki et al., 2009; Braun and Gautschi, 2012). Observ-

Table 3: Duration of feeding (minutes) under different experimental conditions (different milk replacer concentrations, different milk temperatures, different nipple positions, different nipple openings).

Experimental condition		Duration of feeding		Range
(week)	Experiment	Mean ± sd	Median	
Milk replacer concentration (week 4)	Control[1]	4.8 ± 1.8	–	3.0 – 7.4
	100 g/litre water	4.5 ± 1.6	–	2.5 – 7.1
	125 g/litre water	3.6 ± 0.6	–	3.0 – 4.6
	150 g/litre water	4.0 ± 0.8	–	3.0 – 5.3
Milk temperature (week 5)	Control	3.6 ± 1.0	–	2.4 – 5.3
	20 °C	3.4 ± 0.9	–	2.5 – 5.0
	30 °C	3.7 ± 1.4	–	2.1 – 5.6
	45 °C	–	3.4	3.1 – 11.5[2]
Bucket/nipple requiring strong sucking reflex (week 6)	Control	2.7 ± 0.5	–	2.2 – 3.3
	Strong suction	1.9 ± 0.7	–	1.4 – 3.1
	Bucket	–	1.8	1.2 – 8.4[3]
Nipple opening (week 7)	Control	3.2 ± 1.0	–	2.3 – 4.6
	Small	–	7.4*	5.5 – 19.4[4]
	Large	–	1.6*[5]	1.4 – 2.2

[1] Feeding time recorded for regular feeding method (see Animals, Material and Methods) during the same week
[2] 5 calves required 3.1 to 3.6 minutes; calf 3 required 11.5 minutes
[3] 3 calves required 1.2 to 2.1 minutes; calf 4 required 8.4 minutes
[4] 5 calves required 5.5 to 9.0 minutes; calf 2 required 19.4 minutes
[5] 5 calves required 1.4 to 1.6 minutes; calf 2 required 2.2 minutes.

ing ingested milk entering the abomasum is the technique of choice for confirmation of a functional oesophageal groove reflex (Braun and Gautschi, 2012; Braun et al., 2013). The interval between the start of nursing and the first appearance of milk in the abomasum was not measured precisely in the present study but was largely within the previously determined range from 10.2 to 24.4 sec (Braun et al., 2013). In the past, oesophageal groove closure was monitored using elaborate techniques involving radiography (Ørskov et al., 1970; Lawlor et al., 1971; Lateur-Rowet and Breukink, 1983), fluoroscopy (Chapman et al., 1986) or the addition of compounds to the milk that could be traced in the intestinal tract postmortem (Abe et al., 1979). In the present study, oesophageal groove closure was confirmed in all calves throughout the 17-week study period. Several studies have shown that groove closure can also occur as a conditioned reflex; the sight of the trough containing the milk induced the reflex in lambs (Ørskov et al., 1970), and the noise associated with feeding preparations and the smell of the milk were thought to do so in calves because their heart rate increased above the normal range before sucking began (Veissier et al., 2002). In the latter study, feeding took place twice a day at the same time, which could have contributed to the conditioning of the oesophageal groove reflex. To avoid this in the present study, feeding occurred at irregular times, was carried out by different caretakers and took place in different rooms.

The milk temperature had no effect on oesophageal groove closure; this was in agreement with the findings of another study, in which calves fed warm and cold milk had similar pH changes in the abomasum (Gränzer, 1978). Calf 3 of the present study had normal groove closure during ingestion of milk at 45 °C, although it paused often and took 11.5 min to finish the meal.

Likewise, the position of the artificial nipple did not affect oesophageal groove closure; the reflex was always induced with both nipples as well as with bucket feeding. Two forms of drinking have been described in bucket-fed calves; some calves were observed to carefully draw the milk from the surface and were referred to as „sippers“ while others immersed their mouths deeply into the milk and gulped it and were referred to as „gulpers“ (Wise et al., 1984). These patterns were also seen in the present study. Calf 4, a sipper, sucked milk from the surface of the milk in the bucket and took 8.4 min to complete the feeding. Calves 1, 5 and 6 were gulpers and immersed their entire muzzle including the nostrils into the milk and required only 1.2 to 2.1 min (1.6 ± 0.5 min) to finish. Milk leaking into the reticulorumen was observed in 95% of gulpers and 17.5% of sippers (Wise et al., 1984) but this relationship was not clear in our study; calf 1, a gulper, had a large amount of milk in the abomasum and a small amount in the reticulorumen after feeding, indicating incomplete groove closure.

The size of the opening of the artificial nipple did not affect the oesophageal groove reflex, and both sizes were consistently associated with induction of the reflex. When the large opening was used, the milk flowed into the calf's mouth by hydrostatic pressure and little sucking was required. When the small opening was used, one calf (no. 2) took numerous breaks and required 19.4 min to finish compared with 7.1 min in the other calves. It can therefore be concluded that the oesophageal groove reflex functions regardless of whether feeding is continuous or interrupted.

The functioning of the oesophageal groove reflex in calves fed milk replacer was confirmed indirectly in a study showing that the abomasal pH increased from 1.4 to 6.0 within 3 min of the start of feeding (Constable et al., 2005). All concentrations used in this study induced groove closure. Of note, cow's milk appeared echoic and homogeneous on ultrasonograms whereas milk replacer was echoic with hyperechoic stippling. Bucket-fed calves took 1.8 min (1.2 to 8.4 min) to finish compared with 2.7 ± 0.5 min in calves fed by the regular method ($P > 0.05$). In another study, bucket-fed calves ingested the milk significantly faster than calves fed using an artificial teat (2.6 versus 9.4 min, Veissier et al., 2002).

Conclusion

All feeding methods investigated induced the oesophageal groove reflex. However, this does not mean that feeding management can be neglected. Failure of the oesophageal groove reflex cannot be excluded in calves undergoing chronic feeding irregularities or in sick calves, causing ruminal drinker syndrome. The duration of sucking should also receive appropriate attention; calves that ingest the milk very rapidly are often nervous and anxious after feeding and engage in non-nutritive oral activities such as licking other calves or their pen.

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Corresponding author

Ueli Braun
Departement für Nutztiere
Winterthurerstrasse 260
8057 Zürich
E-Mail: ubraun@vetclinics.uzh.ch

Ultrasonographic examination of the oesophageal groove reflex in young calves under various feeding conditions

U. Braun, C. Brammertz