Contents lists available at ScienceDirect





# Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

# The effect of large milk meals on digestive physiology and behaviour in dairy calves



# Kristian Ellingsen <sup>a,\*</sup>, Cecilie M. Mejdell <sup>a</sup>, Nina Ottesen <sup>b</sup>, Stig Larsen <sup>b</sup>, Ann Margaret Grøndahl <sup>b</sup>

<sup>a</sup> Norwegian Veterinary Institute, PO Box 750 Sentrum, N-0106, Oslo, Norway

<sup>b</sup> Norwegian University of Life Sciences, Faculty of Veterinary Medicine and Biosciences, PO Box 8146 Dep, N-0033, Oslo, Norway

# HIGHLIGHTS

# GRAPHICAL ABSTRACT

- The abomasum of the newborn calf has a large ability for distension.
- Voluntary milk meals of 3.5 to 6.8 l did not cause milk to enter the rumen.
- Milk meals of 3.5 to 6.8 l did not cause behaviour indicating abdominal pain.
- Milk meals beyond 3 l can safely be given through a small aperture teat.



# A R T I C L E I N F O

Article history: Received 25 August 2015 Received in revised form 24 November 2015 Accepted 25 November 2015 Available online 26 November 2015

Keywords: Milk meal size Abomasal capacity Backflow Ingestion Indigestion

# ABSTRACT

It is commonly believed that young calves should not be fed more than about 2 l of milk per meal. If calves are fed beyond this volume, it is said that the capacity of the abomasum may be exceeded and that milk could enter the rumen. This can disturb the microbial flora/fauna of the rumen and increase the risk of indigestion, diarrhoea and reduced growth. The aim of this study was to examine the effect of large milk meals on digestive physiology and behaviour in dairy calves. Six calves (19-23 days of age at the beginning of the experiment) were fed 2 l of warm whole milk by teat bottle three times per day, which was the recommended Norwegian feeding regime at the time. The calves were given free access to hay, concentrates and water. During three morning feeding sessions, each separated by 48 h, all calves were offered larger meals. The offered amounts were calculated according to the within patient 3-level Response Surface Pathway (RSP) design. The milk given on the three test days contained a contrast medium (barium sulphate), and the animals were radiographed before, during and immediately after intake to reveal whether milk entered the rumen. Four out of the six calves drank more than 5 l in one meal and the highest voluntary intake was 6.8 l in one meal (13.2% of BW). Abdominal radiographs showed that the abomasum has a large ability for distension. Milk in the rumen was not observed in any of the calves, regardless of intake. The behaviour of the calves was observed for 2 h after each test session. No behaviour indicating abdominal pain or discomfort was observed regardless of intake. The results indicate that when warm whole milk is administered from a teat bottle, farmers can increase the amount of milk they offer their calves beyond the traditionally

\* Corresponding author at: Norwegian Veterinary Institute, PO Box 750 Sentrum, N-0106 Oslo, Norway.

E-mail address: Kristian.Ellingsen@vetinst.no (K. Ellingsen).

http://dx.doi.org/10.1016/j.physbeh.2015.11.025

0031-9384/© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

recommended portion size without risk of milk entering the rumen. Hence, farmers who want to feed their calves more milk can do so by increasing meal sizes, and not necessarily by introducing an additional meal. © 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

#### 1. Introduction

#### 1.1. Stomach development

During the first weeks of life, calves are functionally monogastric and milk is the primary source of nutrition. Upon drinking milk, the oesophageal (reticular) groove (sulcus retículi) is activated and the milk is shunted directly past the forestomachs to the abomasum [1]. A number of factors trigger this oesophageal reflex, including sucking behaviour, warm milk, the position of the calf's head while drinking [1] and familiarity with the feeding method [2].

For newborn and very young calves, milk in the forestomachs is usually unproblematic. The rumen, along with the reticulum and omasum, is not yet developed and empties into the abomasum within hours [3]. For calves with ruminal development (2-3 weeks and older) [4], large quantities of milk in the rumen may pose a problem. The lactose is converted to lactic acid and other organic acids, or the milk proteins may rot, which may lead to a change in pH, subsequently affecting the rumen microflora causing indigestion, diarrhoea and reduced growth [1].

Milk is thought to enter the rumen in one of two ways. The first is through insufficient closure of the oesophageal groove [5]. The second results from overfeeding fluids beyond the capacity of the abomasum, causing backflow into the reticulorumen [5–7]. While the scientific origin remains unclear, it is widely believed that the capacity of the abomasum is about 2 l, and that milk meal sizes beyond this volume will cause milk to enter the rumen. In a study from 2012, Flor et al. also calculated the abomasal volume to be less than 2 l based on computed tomography (CT) scans [8]. They did not, however, report the size of the offered milk meal or actual intake by the calf.

# 1.2. Milk feeding in dairy calves

Traditionally, dairy calf milk feeding systems have been based on daily feeding rates of 8-10% of body weight (BW) [9]. This feeding regime is highly restrictive compared to ad libitum feeding. Research has shown that calves allowed ad libitum access to milk from artificial teats drink 8-10 [ [10]. Calves allowed to suckle drink up to 12-15 l of milk per day at 2-4 weeks of age [11,12] (Grøndahl, unpublished). Khan et al. [13] recommend to feed dairy calves the equivalent of 20% of BW per day based on a comprehensive literature review. This recommendation is more in line with the natural milk intake level of dairy calves. The Norwegian milk feeding recommendations were also recently increased from 6 to 8 l per day [14].

Until 3-4 weeks of age, restrictive milk feeding can result in the calves being unable to meet their daily energy requirements [15], leading to chronic hunger [16]. One way of feeding the calf more milk is to introduce an additional meal. This increases the workload for farmers without automated milk feeders and is therefore often undesirable. Another way is to increase the meal size. Fear of exceeding the abomasal capacity causing milk to enter the rumen, however, is a major reason to limit the meal size.

# 1.3. Aim

We aimed to use radiography to examine the effect of voluntary intake of large milk meals on digestive physiology and determine how much milk a dairy calf can drink from a teat bottle in one meal before the abomasal capacity is exceeded and milk enters the rumen. We also wanted to use behavioural observations to detect any abdominal pain or discomfort resulting from large milk meals.

#### 2. Material and methods

#### 2.1. The experimental animals

Seven Norwegian Red calves (four heifers and three bulls) born four days apart were borrowed from a dairy farm south of Oslo, Norway. One of the heifer calves became ill before the test period and was therefore excluded. The animals were 19-23 days of age at the beginning of the experiment. All experimental animals were weighed upon arrival (day 0) and before each test session. The calves weighed between 39.5 and 48.5 kg at arrival. The calves were housed in a group pen (4 by 4 m) at the Norwegian University of Life Sciences in Oslo. The calves were fed according to the recommended Norwegian feeding regime at the time and received 2 l of warm whole milk three times per day at 8 am, 1 pm and 6 pm. The animals had free access to water, hay and concentrates.

All experimental procedures were in accordance with the regulations controlling experiments/procedures on live animals in Norway, and the study complies with the 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 owner after the experiment.

# 2.2. The test procedure

The study was performed as an open, non-randomized trial with a within patient 3-level RSP design [17]. On test days 3, 5 and 7 (days after arrival to the test facility), the calves' morning meal was replaced by milk containing the barium sulphate contrast (BaSO<sub>4</sub>) (Bracco, MIXOBAR® COLON (1 g/ml)) at a ratio of 6:1. The small aperture teat used allowed drinking at approximately 1.5 l/min. To standardize the rate of intake, the same rubber teat was used for all test sequences and all calves. Abdominal radiographs of the standing calves were taken before, during and immediately after administration of the barium sulphate contrast (Table 1).

Lateral-lateral abdominal computed radiography (Kodak DirectView CR 850) with a focus film distance (FFD) of 140 cm using a grid (potter bucky) on standing calves was performed. Exposure factors were 90 kV and 32 mA-s.

A pilot study was performed in one calf (different from the experimental animals) to assure that milk in the rumen could be distinguished

#### Table 1

Table 1					
Day-by-day	schedule	of milk	amounts	and	actions

Age of calves (days)	Day at test facility	Amount of milk given (litres)		[	Action
		8 am	1 pm	6 pm	
19–23	0	-	2	2	Transportation from farm
20-24	1	2	2	2	Habituation to new housing
21-25	2	2	2	2	Habituation to new housing
22-26	3	Test	2	2	X-ray after morning meal +
		meal <sup>a</sup>			observation
23-27	4	2	2	2	-
24-28	5	Test	2	2	X-ray after morning meal +
		meal <sup>a</sup>			observation
25-29	6	2	2	2	_
26-30	7	Test	None	None	X-ray after morning meal +
		meal <sup>a</sup>			observation

<sup>a</sup> Test meals were decided based on the RSP design.

from milk in the abomasum, as shown by Lateur-Rowet and Breukink [3] in calves and Phillipson [18] in lambs. One hundred millilitres barium sulphate was administered to the rumen through an oesophageal tube (Fig. 1A). Immediately thereafter, the calf was allowed free intake of warm whole milk mixed with the contrast through a small aperture teat, as with the experimental calves. The abomasum and rumen could easily be discerned on radiographs after intake of 4 l of milk (Fig. 1B).

On each of the three test days, continuous live behavioural observations were carried out in each calf during 2 h starting immediately after the test meal, to reveal signs of abdominal pain or discomfort according to a predefined ethogram (Table 2). The ethogram was based on Bourne [19]. All behavioural observations were carried out by the same observer (1st author, ethologist and researcher with experience from behavioural observations and pain assessment in animals). For practical reasons, the observer could not be blinded to the performance of the calves during trials.

Diarrhoea (defined as abnormal frequency and fluidity of faecal evacuations) was recorded for each calf individually.

The RSP design consisted of three dose levels, in which all the calves were started on the same dose at the first design level, but thereafter participated in the study based only on own obtained results. The grades of negative reactions (milk detected in the rumen or failure to ingest the offered amount) determine the dose for the subsequent levels. All calves were given 4.0 l at the first RSP level, and the meal size was subsequent-ly changed individually based on the obtained results, i.e. milk detected in the rumen or failure to ingest the amount offered. The minimum and maximum meal size were set to be 1.0 and 7.0 l, respectively.

#### 3. Results

All calves drank volumes far exceeding their normal meal size. Milk in the rumen was not detected, regardless of intake. Four of the six calves drank more than 5 l in one meal, while two of them drank more than 6 l when the highest amounts were offered (Table 3). Mean intake from the teat bottle in one meal was 3.8 (8.1% of BW), 4.9 (10.2% of BW) and 5.4 (10.8% of BW) litres of milk on Level 1, 2 and 3, respectively. The highest voluntary intake recorded in the study was 6.8 l (13.2% of BW).

On Level 1, five of the six calves drank all of the 4 l they were offered. One calf drank only 2.6 l (65% of the amount offered). This particular calf had a coughing spell during the meal and thereafter lost interest.

Fig. 2A (radiograph of the cranial abdomen of a standing calf) shows the abomasum before intake of the contrast milk, while Fig. 2B–D shows the gradual abomasal extension after ingestion of 2, 4 and 6 l of contrast milk, respectively.

#### Table 2

Ethogram used to detect signs of abdominal pain or discomfort.

Behaviour	Description	Categories or units
Dull appearance <sup>a</sup>	Passive, unresponsive and uninterested in surroundings	No or yes
Vocalisation	Any kind of vocal expressions	Number of vocalisations 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	Number of kicks per animal
Getting up/lying down	Partly or fully stands up or lies down	Number of times calf partly of fully stood up or laid down
Rapid, breathing	Rapid, shallow breathing	Number of bouts and duration per bout (second)
Bruxism	Grinding teeth	Number of bouts and duration per bout (second)
Hunched stance	Standing with head low and back arched	Number of bouts and duration per bout (second)

<sup>a</sup> Recorded every 30 min.

With regards to the behavioural indicators of abdominal pain or discomfort, a low frequency of licking at the abdomen (performed by two calves) and lying down/getting up (performed by all calves) were observed. The highest number of lying down/getting up behaviours performed by one calf was 5 times within one observation period. No diarrhoea was observed in any of the experimental animals. Table 4 shows the accumulated observations for all six calves per level. The numbers in brackets show the number of animals that carried out the behaviour.

# 4. Discussion

The abomasum has large ability for distension. Voluntary intake of up to 6.8 l of warm whole milk in 3 weeks old calves did not cause milk to enter the rumen and no behaviour indicating abdominal pain or discomfort was observed.

#### 4.1. Methodological issues

The RSP method was chosen because it effectively narrows down the dose window to pinpoint an exact dosage [17,20]. The method permits the dose level to be adjusted based on the amount of milk in the rumen,



Fig. 1. Lateral cranial abdominal radiography of the 15 days old pilot calf (head oriented to the right) after administration of 100 ml of barium sulphate contrast using an oesophageal tube (A) and after consuming 4 l of contrast milk (BaSO<sub>4</sub>) (B). Note that the rumen (white arrow) and reticulum (black arrow) can be differentiated from the abomasum. Some barium sulphate contrast has passed to the small intestine.

# Table 3

Individual milk amounts ingested and offered in litres and per cent of body weight for the six calves in the experiment.

Animal	Amount ingested/offered (litres)		Intake in per cent of body weight			
	Test day 3	Test day 5	Test day 7	Test day 3	Test day 5	Test day 7
1	2.6/4.0	3.0/3.0	3.5/3.5	5.7	6.3	7.2
2	4.0/4.0	5.0/6.0	5.3/6.5	10.3	12.3	12.8
3	4.0/4.0	5.3/6.0	5.5/6.5	8.5	10.7	10.7
4	4.0/4.0	6.0/6.0	6.8/7.0	7.8	11.9	13.2
5	4.0/4.0	4.8/6.0	4.7/5.5	8.2	9.5	9.0
6	4.0/4.0	5.5/6.0	6.4/6.5	7.8	10.7	12.0
Mean intake	3.8	4.9	5.4	8.1	10.2	10.8
(SD)	(0.6)	(1.0)	(1.2)	(1.5)	(2.2)	(2.4)

#### Table 4

The accumulated occurrence and/or frequency of behaviours related to abdominal pain in calves. The numbers in brackets show the number of animals that carried out the behaviour.

Behaviour	Test day 3	Test day 5	Test day 7
Dull appearance	0	0	0
Vocalisation	0	0	0
Licking at the abdomen	3 (1 calf)	4 (2 calves)	0
Biting at the abdomen	0	0	0
Kicking at the abdomen	0	0	0
Getting up/lying down	14 (6 calves)	10 (5 calves)	19 (5 calves)
Rapid, breathing	0 bouts	0 bouts	0 bouts
Bruxism	0 bouts	0 bouts	0 bouts
Hunched stance	0 bouts	0 bouts	0 bouts

allowing us to determine the volume at which the abomasal capacity would be exceeded. Between- and within-patient RSP designs are generally analysed with isotonic regression analysis [17,20]. However, no meal size in the current study turned out to cause milk to enter the rumen. This means that there were no quantiles to estimate and hence isotonic regression could not be applied to determine an optimal dose level based on abomasal capacity.

Milk is easily digestible and abomasal emptying in young calves normally starts within a few minutes [21]. Radiographs were taken during and immediately after the milk intake when backflow is most likely to occur. Ruminal emptying can take up to 48 h [3]. To avoid confusion from barium sulphate potentially remaining in the forestomachs, test sessions were performed with 48 h intervals. Teat feeding, and the use of a small aperture teat, are widely recommended in the literature [22–25], and was also the method of choice in this experiment. Compared to non-sucking methods, teat feeding has advantages like stimulating the oesophageal reflex, and causing fewer sequential openings and closings of the oesophageal groove [26].

# 4.2. Optimal milk feeding

Voluntary intake as high as 5–6 l of milk in one meal did not cause milk to enter the rumen. This does not mean that we can recommend feeding milk meals of that size to dairy calves on a daily basis. The test meals were offered as single meals on three separate occasions, and we did not test how much the calves would have drunk if offered this amount three times a day. Neither were the long-term effects of large



**Fig. 2.** Cranial abdominal radiograph (head oriented to the right) taken at test day 7 before administration (A) and after sucking 2 (B), 4 (C) and 6 (D) litres of contrast milk (milk:barium sulphate contrast 6:1). A: Note a small amount of residual barium sulphate evident in the cranioventral aspect of the abdomen consistent with the location of the abomasum (arrow). B/C/ D: Note that radiopaque material is present in the abomasum and no visualization of the rumen. D: The lateral abdominal radiographs of standing calves are acquired using horizontal beam. The fluid level refers to an interface between fluid and gas (gas-fluid interface, white arrow) in the stomach. In addition to the gas-fluid interface there are occasionally visible fluid-fluid interfaces (black arrow). The double interface is most likely due to radiographs taken immediately after the calves stopped drinking, before the fluid finds its true level, causing layering of the fluid.

meals investigated. However, it has been found elsewhere that feeding milk ad libitum [9] or in large quantities [27] results in higher growth rates without compromising health or reducing solid feed intake after weaning. For instance, in a study by de Passillé et al. [12], calves were allowed to suckle twice daily. The amount drunk by the calves increased from 6.5 kg per day in week 1 to 12.5 kg per day in week 9, indicating large milk meals without adverse effects. Also, in the study by Appleby et al. [10], the average size of the calves' first milk meal of the day was 4.7 l (range 1.7–8.0 l) with favourable outcomes. Our results support these findings by showing that the abomasum has a large ability for distension and that voluntary intake does not cause milk to enter the rumen.

Based on this study, it could be argued that calves can be fed one single, large meal per day rather than several smaller ones. This was not tested, but is probably not recommendable. Large, infrequent meals have been found to have negative effects on calf metabolism and decrease insulin sensitivity [28]. There are also several studies reporting positive effects on weight gain and calf health and welfare as a result of an increased number of milk meals [29–31].

# 4.3. High milk intake and pain behaviour

Behavioural observations were included to detect signs of abdominal pain or discomfort resulting from the large milk meal. Despite the fact that the calves on several occasions drank milk amounts exceeding 10% of BW, no behaviour indicating abdominal pain or discomfort was observed. Some calves were observed licking at their abdomen. The behaviour was carried out as part of the grooming and was not directed exclusively at the abdomen. Most calves would lie down and get up again during the 2-hour observation periods, but not in a rapid sequence as seen in colic behaviour [19]. On several occasions, the calves rised to eat hay, drink water or as a response to an external stimuli like people looking into the pen. The lack of behaviours indicating pain implies that although high amounts of milk were ingested, the calves are able to control intake in such a way that pain is avoided.

No diarrhoea was observed in any of the experimental animals. However, as barium sulphate is an antidiarrhoeal agent, the contrast could have hidden any laxative effects of increased amounts of milk, thus making it hard to draw conclusions from the current study.

In the current study all calves were given warm whole milk through a small aperture teat in a standardized way. Hence potentially influential factors related to milk type or feeding technique were not investigated. For instance, it is currently unknown whether feeding large volumes of milk replacer would yield the same results. Teat feeding, and the use of a small aperture teat, is widely recommended in the literature. Future studies should therefore be aimed at testing if larger volumes of milk can be problematic if administered e.g. through a teat with a larger opening or straight from an open bucket.

# 5. Conclusion

No optimal milk meal size could be determined based on the results of the current study. Nevertheless, voluntary meals of 3.5 to 6.8 l of warm whole milk given by a small aperture teat did not cause milk to enter the rumen, and behaviour indicating abdominal pain or discomfort was not observed regardless of intake. These results support other studies indicating that dairy calves can be fed milk meals beyond the traditionally recommended portion size without the risk of adverse effects.

# **Conflict of interest statement**

The authors declare that there is no conflict of interest associated with this manuscript.

#### Acknowledgements

The authors thank radiographers Lena Stenhaug and Bernadette Helmer at the Norwegian University of Life Sciences. We also thank Kalnes upper secondary school and the always generous Gunhild Dangstorp for letting us borrow the calves for the experiment, and Miriam Cohen for caring for the calves during the experiment. A final thank you goes to Tilde Sæther, whose student project on abomasal capacity spawned the idea of this study. The project was financed by Foundation for Research Levy on Agricultural Products (FFL) and Agricultural Agreement Research Fund (JA) through Norwegian Research Council (project number 190424) and the Norwegian Veterinary Institute (project number 10101).

#### References

- Ø.V. Sjaastad, K. Hove, O. Sand, Physiology of Domestic Animals, 2nd ed. Scandinavian Veterinary Press, Oslo, 2010.
- [2] M. Abe, K. Kondoh, H. Shibui, Effects of nipple or bucket feeding of milksubstitutes on rumen by-pass and on rate of passage in calves, Br. J. Nutr. 41 (1979) 175–181.
- [3] H.J. Lateur-Rowet, H.J. Breukink, The failure of the oesophageal groove reflex, when fluids are given with an oesophageal feeder to newborn and young calves, Vet. Q. 5 (1983) 68–74.
- [4] E.W. Swanson, J.D. Harris, Development of rumination in the young calf, J. Dairy Sci. 41 (1958) 1768–1776.
- [5] J.J.G.C.v.d. Borne, S.J.J. Alferink, W.J.J. Gerrits, Identifying ruminal drinking by measurement of respiratory quotient and methane production in preruminant calves, J. Anim. Sci. 82 (S1) (2004) 365 (W209).
- [6] B.P. Smith, Large Animal Internal Medicine, 5th ed. Mosby Elsevier, St. Louis, Mo., 2014
- [7] M.J. Akssenowa, Zur Physiologie des Magens der Wiederkäuer, Arch Tierernähr Tierzucht. 7 (1932) 295–304.
- [8] J. Flor, B. Linke, P. Sanftleben, Möglichkeiten der Computertomographie zur Beurteilung der Mägenentwicklung beim Milchkalb, Züchtungskunde. 84 (2012) 213–225.
- [9] J. Jasper, D.M. Weary, Effects of ad libitum milk intake on dairy calves, J. Dairy Sci. 85 (2002) 3054–3058.
- [10] M.C. Appleby, D.M. Weary, B. Chua, Performance and feeding behaviour of calves on ad libitum milk from artificial teats, Appl. Anim. Behav. Sci. 74 (2001) 191–201.
- [11] F.C. Flower, D.M. Weary, Effects of early separation on the dairy cow and calf: 2. Separation at 1 day and 2 weeks after birth, Appl. Anim. Behav. Sci. 70 (2001) 275–284.
- [12] A.M. de Passillé, P.-G. Marnet, H. Lapierre, J. Rushen, Effects of twice-daily nursing on milk ejection and milk yield during nursing and milking in dairy cows, J. Dairy Sci. 91 (2008) 1416–1422.
- [13] M.A. Khan, D.M. Weary, M.A.G. von Keyserlingk, Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers, J. Dairy Sci. 94 (2011) 1071–1081.
- [14] H. Overrein, A.C. Whist, K.M. Sølvberg, L.T. Nyhus, Godt kalveoppdrett det er bedre å bygge kalver enn å reparere kyr, Tine Rådgiving, 2015 [Norwegian].
- [15] H.M. Nielsen, I. Olesen, S. Navrud, K. Kolstad, P. Amer, How to consider the value of farm animals in breeding goals. A review of current status and future challenges, J. Agric. Environ. Ethics 24 (2011) 309–330.
- [16] A. De Paula Vieira, V. Guesdon, A.M. de Passillé, M.A.G. von Keyserlingk, D.M. Weary, Behavioural indicators of hunger in dairy calves, Appl. Anim. Behav. Sci. 109 (2008) 180–189.
- [17] S. Dewi, V. Kristiansen, S. Lindkær-Jensen, S. Larsen, Between- and within-patient nlevel response surface pathway design in dose-finding studies, Open Access J. Clini. Trials. 6 (2014) 63–74.
- [18] A.T. Phillipson, The movements of the puches of the stomach of sheep, Q. J. Exp. Physiol. Cogn. Med. Sci. 29 (1939) 395–415.
- [19] Bourne, D. Assessment of pain in ruminants. In: Wildpro the electronic encyclopaedia and library for wildlife. http://wildpro.twycrosszoo.org/S/00Man/ PainRumOverviews/PainGeneral/02Assessment\_PainRum.htm. 2013.
- [20] S. Dewi, T. Aune, J.A. Bunæs, A.J. Smith, S. Larsen, The development of response surface pathway design to reduce animal numbers in toxicity studies, BMC Pharmacol. Toxicol. 15 (2014) 18.
- [21] P.D. Constable, T. Wittek, A.F. Ahmed, T.S. Marshall, I. Sen, M. Nouri, Abomasal pH and emptying rate in the calf and dairy cow and the effect of commonly administered therapeutic agents, Proceedings of the 24th World Buiatrics Congress. Nice 2006, pp. 54–68.
- [22] M.B. Jensen, The effects of feeding method, milk allowance and social factors on milk feeding behaviour and cross-sucking in group housed dairy calves, Appl. Anim. Behav. Sci. 80 (2003) 191–206.
- [23] L. Lidfors, L. Isberg, Intersucking in dairy cattle review and questionnaire, Appl. Anim. Behav. Sci. 80 (2003) 207–231.
- [24] A.M. de Passillé, Sucking motivation and related problems in calves, Appl. Anim. Behav. Sci. 72 (2001) 175–187.
- [25] D.B. Haley, J. Rushen, I.J. Duncan, T.M. Widowski, A.M. de Passillé, Effects of resistance to milk flow and the provision of hay on nonnutritive sucking by dairy calves, J. Dairy Sci. 81 (1998) 2165–2172.

- [26] G.H. Wise, G.W. Anderson, A.C. Linnerud, Relationship of milk intake by sucking and by drinking to reticular-groove reactions and ingestion behavior in calves, J. Dairy Sci. 67 (1984) 1983–1992.
- [27] J.L. Uys, D.C. Lourens, P.N. Thompson, The effect of unrestricted milk feeding on the growth and health of Jersey calves, J. S. Afr. Vet. Assoc. 82 (2011) 47–52.
- [28] A. Bach, L. Domingo, C. Montoro, M. Terré, Insulin responsiveness is affected by the level of milk replacer offered to young calves, J. Dairy Sci. 96 (2013) 4634–4637.
- [29] D.J. Schingoethe, D.P. Casper, J.K. Drackley, F.C. Ludens, Increased solids intake and feeding frequency for calves in hutches during cold weather, J. Dairy Sci. 69 (1986) 1063–1069.
- [30] A.D. Kmicikewycz, D. da Silva, N.B. Litherland, Effects of a modified intensive milk replacer program fed two or four times daily on nutrient intake, calf growth and health, Minnesota Dairy Health Conference. St. Paul 2010, pp. 95–96.
- [31] T.J. Thomas, D.M. Weary, M.C. Appleby, Newborn and 5-week-old calves vocalize in response to milk deprivation, Appl. Anim. Behav. Sci. 74 (2001) 165–173.