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Application note for the use of a wireless device measuring reticular pH under practice conditions in a Swiss dairy herd

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A B S T R A C T

Subacute ruminal acidosis (SARA) is caused by ingestion of diets high in rapidly fermentable carbohydrates and/or deficient in physically active fiber, often suspected to occur in high yielding dairy cows. Although SARA has been defined as repeatedly occurring periods of low ruminal pH, both practical measuring methods and threshold values remain subject to research.

Precision livestock farming comprises use of real-time monitoring and managing systems for livestock farmers. Reticular pH can be monitored continuously on individual cow level by use of an indwelling bolus situated in the reticulum, linked to a wireless data transmission system. Practical experience in use of such boluses is scarce. The first aim of this field application was to test a commercially available bolus under field conditions in a dairy herd, and to assess the data obtained on herd level. The second aim was to suggest criteria indicative of SARA on herd level. The threshold for this investigation was set at pH $\leq$ 5.5 for $\geq$ 3 h, simultaneously occurring in $\geq$ 3 cows, by combining a testing strategy and a threshold from two peer-reviewed publications.

Of 15 boluses administered, 12 (80%) provided reliable data. Reticular pH was measured in 10-min intervals. Useful data were obtained during the first 80 days after bolus application. This was considerably lower than the 150 days as described by the manufacturer.

Based on pH measurements, a mean individual pH curve for each cow was created through grouping pH values for each time point over the entire period. The mean individual curve showed a circadian pH change with low pH values in the evening and variation of mean individual pH values among cows. No event of three cows simultaneously showing a reticular pH $\leq$ 5.5 during a $\geq$ 3 h interval was recorded. Maximum time span during which pH was $\leq$ 5.5 in an individual cow was 4 h and 40 min.

We conclude that use of the system on herd level is appropriate. Establishing a herd-level threshold for SARA based on continuous measuring systems requires integration of the mean individual pH curve, the individual minimal pH values, and the time span of low reticular pH.

1. Introduction

Smart Farming comprises soft- and hardware applications to optimize a variety of processes in modern agriculture. Within Smart Farming, precision livestock farming (PLF) refers to real-time monitoring and managing systems for livestock farmers [1]. Examples of sensing technologies are thermal and 3D cameras, accelerometers, positioning GPS and indwelling boluses. In cattle, PLF is used to monitor individual animals by continuous measurement of various parameters such as health, behavior, production, reproduction, and environmental impact [2,3]. In dairy cattle, monitoring individual animals can be justified with their economic value and their body size. Wearable sensors recording health variables ideally provide real-time data that are used as early warning system for farmers and veterinarians, thus preventing or mitigating severity and length of medical problems [4]. Advantages arising from the use of PLF on well-managed and high-yielding dairy farms may be an increase of welfare through better health. Optimal use of the resources furthermore contributes to the sustainability of farming [4,5]. In the present study, optimal use of resources refers to efficiently converting a high-energetic diet into milk without overstraining the digestive health of the individual. Many of the wearable technologies for cattle are still in the adoption phase [4]. They generate big data volumes which need to be processed and analyzed. Furthermore, PLF technologies should be evaluated for investment costs,

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their added value, and need of experts for data processing [5].

Subacute ruminal acidosis (SARA) is caused by ingestion of diets high in rapidly fermentable carbohydrates and/or deficient in physically active fiber. It is often suspected to occur in high yielding dairy cows [6] and intense beef production [7]. Detecting specific clinical signs of SARA on cow-level or herd-level is challenging [8]. Most signs are non-specific (e.g., reduced dry matter intake and milk yield, body condition loss) and influenced by individual prerequisites (sorting behavior, rumination, inflammatory responses leading to rumenitis). Liver abscess formation is reported to be a direct sequela of SARA-induced rumenitis [9]. Diagnosis intra vitam is possible via ultrasound examination. However, depending on localization and appearance of the abscesses, a definite diagnosis can be challenging, or be verified post mortem only.

Currently, testing ruminal juice pH value is the diagnostic standard to detect SARA. For this, rumenocentesis (transdermal puncture of the ventral rumen sac) is reported to be most accurate, followed by oro-ruminal rumen juice collection [10]. Although SARA is commonly suspected whenever prolonged periods of depressed ruminal pH occur repeatedly in individual animals with values between 5.2 and 6.0 [8, 11–13], both practical measuring methods and threshold values for herd level investigations are missing. Establishing herd level criteria for SARA are even more challenging than on individual animal level, as not all cows may be affected by SARA simultaneously, and individual susceptibility may vary among cows. For practical herd level SARA diagnostics, analysis of a subsample of 12 cows from a herd or diet group was suggested [8]. In accordance with the latter investigation, three cows simultaneously showing ruminal pH $\leq$ 5.5 may be used as criteria to effectively differentiate between herds with $\leq$ 15% or $>$ 30% prevalence of cows with a low ruminal pH.

However, performing ruminal pH measurement through rumenocentesis or oro-ruminal sampling is impractical, invasive, and compromises animal welfare [14]. Using these techniques, usually only one or few single measurements are available per cow, and assessing data obtained on herd level is limited.

There is no generally accepted definition of SARA with strict criteria for pH threshold and timespan of low pH. Gozho et al. [12] assumed in one experimental study with few animals that SARA was successfully induced when ruminal pH was $<$ 5.6 for 174 min in one and 187 min in another steer. Zebeli et al. [15] suggested in a modeling approach that the accumulated duration per day and cow of ruminal pH $<$ 5.8 should not last longer than 5.24 h.

Technical advances and increasing interest in PLF allow for continuous measuring of reticular pH through use of indwelling devices (boluses). One bolus per cow is administered orally and retained in the reticulum due to gravity.

In the reticulum, pH values may differ from the rumen. However, differences may be limited, as pH values were reported to depend rather on diet composition or dry matter intake (or both) than location [16].

Practical experience in use of boluses is scarce. There is no established procedure for data analysis and interpretation. The first aim of this field application was to test a commercially available bolus under field conditions in a dairy herd and to assess the data obtained on herd level. The second aim was to suggest criteria indicative of SARA on herd level. It was decided to combine the approaches by Garrett et al. [8] and Gozho et al. [12], thus, the threshold in the present herd was set at pH $\leq$ 5.5, simultaneously occurring in $\geq$ 3 cows for $\geq$ 3 h.

2. Material and methods

2.1. Herd characteristics

Approval for animal experimentation was granted by the competent authority of the Canton of Bern, Switzerland (permission number 33334, 15 dairy cows).

In May 2021, the boluses were applied in a dairy herd in the Canton of Bern, Switzerland. The herd consisted of 58 Holstein Friesian cows in the first third of their 1st to the 6th lactation in a year-round calving system. The average 305-day milk yield was 9 650 kg. Cows were kept in a freestall system with cubicles, feeding fence and limited access to a pasture area. They were milked by a robotic milking system (Lely Astronaut A4, Lely Switzerland, Härkingen, Switzerland). The cows were fed a partially mixed ration containing grass silage, corn silage, sugar beet pulp silage, hay, canola cake, corn gluten, salt and mineral premix, magnesium oxide, sodium bicarbonate and ammonia, supplemented by a balanced concentrate in the milking robot, quantity based on individual milk yield. The partially mixed ration was offered once per 24 h at 10 a.m. and pushed up to the feeding fence every hour until finished. The diet was checked routinely for nutrient balance, mixing quality and duration, forage-to-concentrate ratio, particle size length and sorting. The average daily herd milk yield had dropped from 35 kg/cow/day to 32 kg/cow/day during the previous year of bolus application, which made the farmer seek advice from the Herd Health Section of the Ruminant Clinic of the Vetsuisse Faculty in Bern, Switzerland. After ruling out various other causes such as infectious diseases, inappropriate husbandry or feeding management, macro- or micronutrient deficiencies, herd level SARA remained the most probable cause. Findings which supported this assumption were the following: i) severe liver abscess formation in two out of twelve cows slaughtered in 2020, ii) episodes of fever of unknown origin in twelve of 58 cows in the same period, iii) repetitive episodes of diarrhea in various individual cows with feces containing undigested feedstuff and mucin casts, and iv) episodes of milk fat depression on herd level.

Reticular boluses were then used as additional diagnostic tool (pH Plus Bolus, smaXtec animal care GmbH, Graz, Austria). Fifteen cows were randomly selected from the herd based on the following criteria: 21 to 120 days postpartum, after a normal delivery and puerperal phase, no sign of any disease other than SARA in the general examination at the day of application. The number of 15 was selected i) to fulfill the criteria of at least 12 cows per herd as suggested by Garrett et al. [8] including accommodation for animal loss, ii) to accommodate for technical failure of boluses, iii) to fulfill the instruction of the manufacturer of at least 6–10% of all cows being equipped.

2.2. Bolus application

One bolus (Fig. 1) was applied per cow. Before application, the

Fig. 1. New bolus before application (cylindrical, 132 mm × 35 mm).

boluses were activated, cross-referenced with the cow's individual ear tag number, and connection to the base station was ensured. Proper functioning of the device was confirmed, and it was calibrated according to the manufacturers' instruction in a buffer solution at pH 7.00 (Reagecon Buffer Solution 10702550, Reagecon, Shannon, Ireland). After the general examination of cows in a self-locking grid, heads were restrained manually by the person applying the bolus. Mouths were opened and boluses were applied using the appropriate applicator (1.34-inch balling gun). Boluses were carried to the base of the tongue, and cows swallowed the bolus voluntarily. Cows were then monitored for adverse reactions during two hours. The owner was advised to report any concern regarding animal health during the entire study period.

2.3. Mode of operation of the boluses

Boluses measured pH values every 10 min and transmitted data via sub-GHz radio (STM32, complying with the requirements of the Low Range Wide Area Network, LoRaWAN) to a base station. The base station was positioned within LoRaWAN data transmission range (max. 99 feet) and at an inaccessible height. There, data were converted for use in an end user device. An additional data repeater that transmitted data to the base station was installed to increase the transmission range to 325 feet ideally, and in our case to assure functionality in the barn where obstacles were present. Bolus device save data for up to six days in order to still be available in case of temporal transmission failure.

According to the manufacturer, the accuracy of the pH data is within ± 0.2 pH points for up to 90 days after activation, and ± 0.4 between 90 and 150 days after placement [17]. A recent study revealed that the confidence interval of the mean pH difference measurements was - 0.33 to - 0.25, suggesting a systematic negative bias [18], i.e. that real pH values are higher than measured ones. The accompanying software provides an alarm function related to risk for SARA (pH $\leq$ 5.8) and to the drop of the individual mean value of the pH to $\leq$ 5.8 for $>$ 300 min. However, these values only partially rely on research data [15], do not represent a gold standard, and were ignored during the present study.

2.4. Data management and analysis

Individual cow pH data were analyzed using Microsoft Excel ® (Microsoft, Redmont, USA) and 'R' (R Studio, R Core Team, Vienna, Austria).

Mean individual cow-level circadian pH curves (MIpH) were calculated. For this, mean cow-level pH were calculated for each cow separately for each time point of a 'mean' day, i.e., mean pH was calculated of all pH values obtained at 00:00 a.m. across the study period of 150 days, mean pH of all pH values at 00:10 a.m., 00:20 a.m. and following. Corresponding plots were constructed. Testing positive for SARA at herd level was defined as three or more cows showing simultaneous reticular pH of $\leq$ 5.5 during a minimum of three consecutive hours. No conversion factor was used to address the difference between reticular and ruminal pH [16,19].

3. Results

The activation of the boluses was mostly uneventful, and so were connection to the base station, calibration, initialization and collection of data. Of the 15 initially selected cows, one cow had to be replaced before bolus application due to clinical parameters deviating from the norm which indicated presence of upper respiratory disease unrelated to SARA. None of the cows showed adverse reactions due to bolus application.

Twelve of the 15 indwelling boluses (80%) provided reliable data during the 150 days as proposed by the distributor. Due to the lack of a gold standard, reliability here does not refer to gold standard values. Data were checked for erroneous values, missing values, circadian pattern, mean and standard deviation. One of the boluses was placed in the rumen and never reached its final destination in the reticulum. Data of this bolus was excluded from analysis. Wrong placement in the rumen was obvious at visual inspection of the data plot due to the lack of the circadian sinusoid curve of the pH value. Data of two other boluses were excluded due to failure of pH measurement, as boluses started sending permanently identical pH values after 81 and 101 days, respectively. Data of 12 boluses were used for data analysis over 150 days.

A total of 193,095 measurements were recorded. Twenty measurements were missing due to technical failure.

Of all 193,095 measurements, 0.302% were pH values $\leq$ 5.5. Of all measurements, the lowest single value was 4.67, the highest single value was 7.22. At cow-level, mean pH was 6.20 ± 0.08 (mean ± SD of the means; min: 6.08; max: 6.34). At cow-level, proportion of measurements of pH $\leq$ 5.5 were a minimum of 0% and a maximum of 1.897% (mean: 0.302%). Mean overall herd level pH was 6.21. At 24 measurement time points, two cows' pH was $\leq$ 5.5 at the same time. No event of three cows simultaneously showing a reticular pH $\leq$ 5.5 during *a* $\geq$ 3 h interval was recorded throughout the study period. Maximum time period of continuous pH values $\leq$ 5.5 in a single cow was 4 h and 40 min. Statistical quantities are summarized in Table 1.

Reticular pH was consistently highest at approximatively 9 a.m. and lowest at approximatively 9 p.m. Transition from high to low pH values between 9 a.m. and 9 p.m. was deemed steady by visual inspection (Fig. 2). Comparing mean pH plots of the 12 cows, variation of mean individual reticular pH was approximately within 0.3 pH points. Mean individual pH curve plots are shown in Fig. 2. Episodes of low pH are shown exemplarily for two episodes of the same cow in Fig. 3. The total cost per cow per diagnostic day was 7.40 USD.

4. Discussion

One objective of this application note was to test a commercially available bolus for reticular pH measuring under field conditions on herd level. Field use is feasible, given that technical fitting was simple, and application to the cows was free of adverse effects. In accordance with Gasteiner et al. [20], using this indwelling pH measurement system enabled long-term measurement of the reticular pH. However, of the 15 boluses administered, 12 (80%) provided useable data. Incorrect bolus positioning in the rumen (1 bolus) and failure of accurate pH measurement (2 boluses) occurred. Positioning cannot be influenced during field use of the indwelling boluses in non-fistulated cows and is not

Table 1
Statistical quantities used and respective units (if applicable).

Item	Number and unit (if applicable)
Cows enrolled	15
Boluses per cow	1
Number of boluses providing analyzable data	12
Observation period	150 days
Measurement interval	10 min
Total number of measurements	193,095
Number of lost measurements	20
Used definition of SARA[a]	pH $\leq$ 5.5 for $\geq$ 3 h, simultaneously in $\geq$ 3 cows, adapted from Garrett et al. [8] and Gozho et al. [12]
Mean overall herd level pH	6.21
Highest/ lowest measured pH	4.67/ 7.22
% of cow-level pH $\leq$ 5.5	mean 0.302% (min: 0.000%, max: 1.897%)
Max. timespan of pH $\leq$ 5.5 in individual cow	4 h 40 min
Local area network coverage radius	325 feet/ 99.06 m
Bolus length/diameter	132 mm/35 mm
Weight per bolus	223 g
Cost per bolus/cow/day	7.40 USD

[a] Subacute ruminal acidosis.

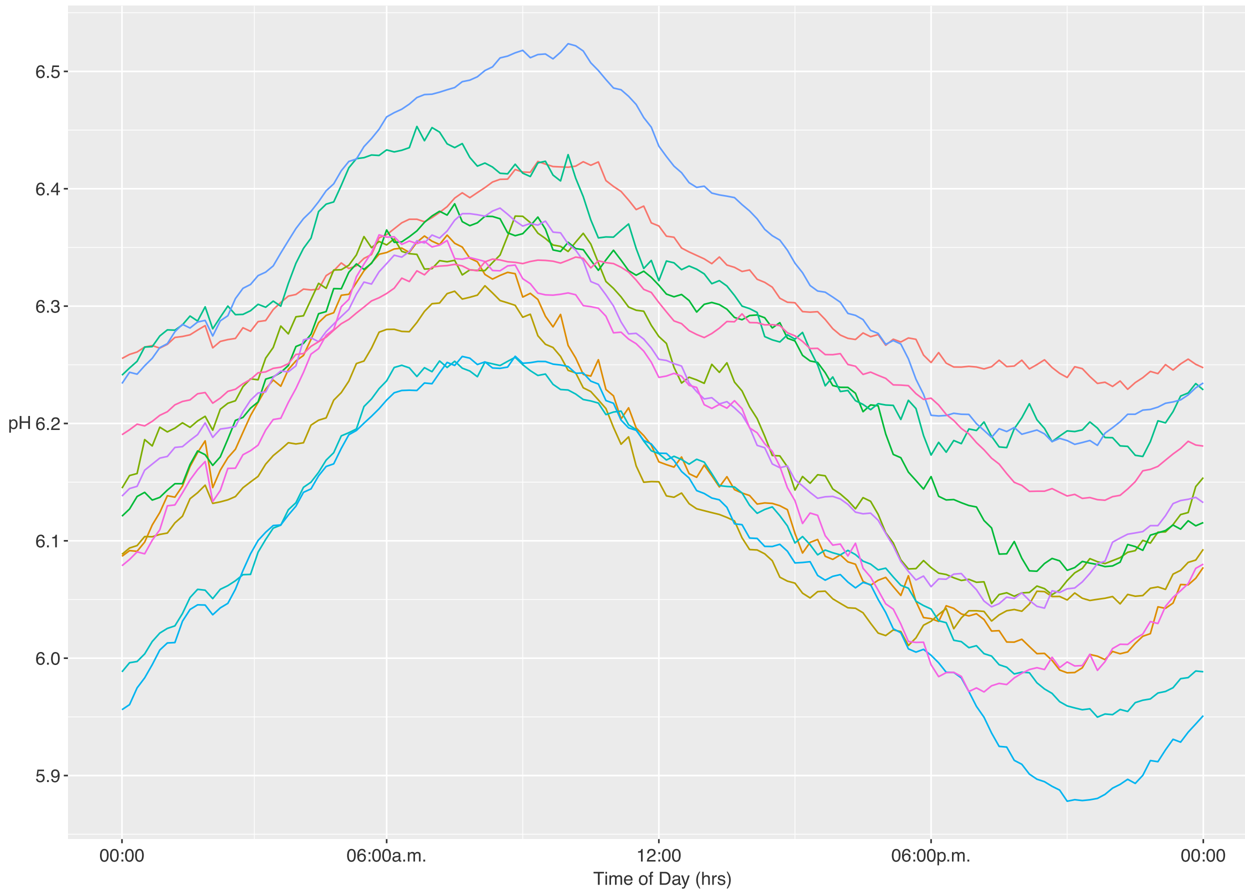

Fig. 2. Representation of circadian reticular pH through calculation of mean reticular pH on the level of the individual cow in twelve Swiss dairy cows during a 24-h period. Measurements were performed in 10-min intervals by use of indwelling boluses after oral application during a period of 150 consecutive days.

promptly setting off an alert by default in the provided software. The time of failure of pH measurement in two boluses (starting at 81 and 101 days after administration, respectively) approximately coincided with the decrease of measurement accuracy from day 90 described in the bolus datasheet, provided by the manufacturer. In our herd, applying additional independent pH measuring systems was not feasible. Therefore, we could not verify differences in measurement precision between devices. In summary, we suggest that a useful time interval for correct interpretation of field data of the boluses is until day 80 post-application rather than until 150 days as described by the manufacturer.

A time interval of 80 days is sufficient to obtain a diagnosis on herd level. However, boluses are not suitable for long-term pH surveillance exceeding 80 days. Interpretation of the large amount of data on herd level is challenging.

Regarding pH measurement inaccuracies of individual boluses, equipping multiple cows simultaneously is beneficial. Twelve boluses provided close to 200,000 measurements, of which 0.3% were pH values $\leq$ 5.5. The mean individual pH curve (Fig. 1) showed the circadian pH rhythm in accordance with results of other publications [21,22], and confirmed the occurrence of low pH values in the evening. In our herd, cows were provided partially mixed ration once daily at around 10 a.m and the ration was pushed up to the feeding fence every hour. Forage intake thereafter was followed by reticular pH drop until 9 p.m. The drop was probably enhanced by provision of a fresh ration once per 24 h only. Results by Jonsson et al. [22] show a similar diurnal pattern in an ad libitum feeding system, but time of provision of a new ration cannot be compared due to lack of information. We suggest this MIpH curve to be provided by default in the software.

Additionally to the diurnal change, the curve shows the individual variation of MIpH of approximately 0.3 points among cows. This is in accordance with findings of Denwood et al. [21] who found substantial variation in the individual animal characteristics of pH when measuring with the same bolus system. In recent literature, individual compositional differences in the rumen microbiome were given as a possible explanation [23,24]. Furthermore, individual differences regarding rumen epithelial permeability to volatile fatty acids and in blood flow through the active absorptive epithelium of the rumen [25] may contribute to the MIpH curves being unique in each cow. In our field study, investigations targeting potential causes of MIpH differences were not possible.

Secondly, we aimed at suggesting criteria indicative of SARA on herd level. No event of three cows simultaneously showing a reticular pH $\leq$ 5.5 during an interval $\geq$ 3 h was recorded, despite data being collected in a population at risk. The criteria of 3 cows from a group of 12 with a pH $\leq$ 5.5 over a period $\geq$ 3 h represents the hypothetical approach of this study, which is based on peer-reviewed literature [8,12]. Denwood et al. [21] concluded that description of continuous pH data should be based on deviations from an expected rhythm rather than on some defined pH threshold. One major limitation of both our approach and that of others is the missing histological postmortem evaluation of respective tissues that should be merged with clinical and diagnostic data obtained. Furthermore, establishing pH thresholds and time-spans indicative of SARA combining both individual animal and herd level data might be needed, and should be subject to future research. Loss of data through incomplete data transmission to the end user device or omitted recording at the bolus or base station was negligible. Data loss may have been theoretically introduced through failure of data recording at bolus level, through an individual cow being outside of the coverage of the LoRaWAN range or through solid items blocking the transmission for more than 6 days. However, the loss of the 20 data

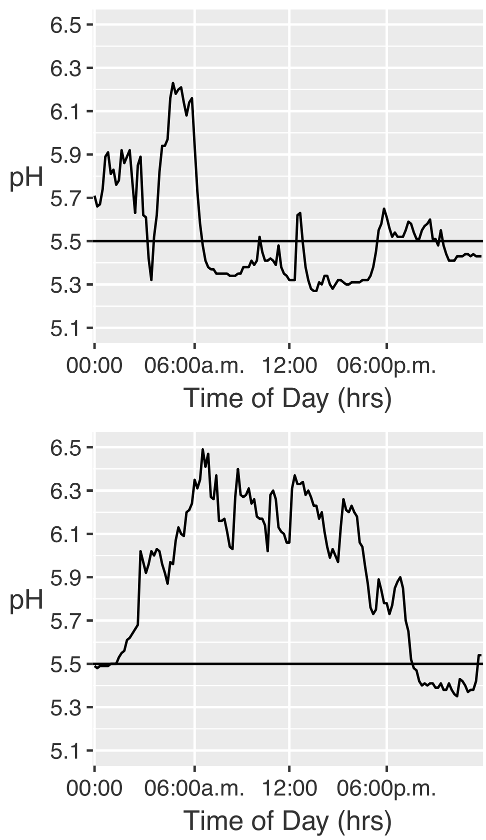

Fig. 3. Representation of individual reticular pH of one Swiss dairy cow on two different days by use of a reticular bolus. Values falling below threshold pH (represented by the horizontal line) indicate reticular pH $\leq$ 5.5 and would be interpreted as indication of subacute ruminal acidosis if occurring simultaneously in 3 cows for a period $\geq$ 3 h according to the suggested criteria in this study.

points cannot be attributed to one of the possibilities in the frame of this study.

Ideally, facing growing performance of future PLF [26], the data obtained from the bolus system should then be integrated with incoming information from other systems (e.g. robotic milking system, accelerometer, weather data) that are applied to monitor the respective herd and the health and productivity of the herd's individuals.

5. Conclusions

We conclude that continuous monitoring of reticular pH is applicable for herd level investigations of SARA. Furthermore, we suggest to use data until day 80 after correct intrareticular positioning. Creation of circadian MIpH curves is suitable to gain insight in herd-level dynamics. Accordingly, creating MIpH curves by default by the accompanying software should be implemented. Establishing a threshold for SARA based on continuous measuring systems needs consideration of the MIpH curve, the individual minimal pH values, and the time span of the possible insult. Clinical evidence of the defined threshold should be based on histomorphological correlates of the ruminal mucosa.

Declaration of Competing Interest

None of the authors of this paper has a financial or personal relationship with people or organisations that could inappropriately influence or bias the content of the paper, in particular not with manufacturing or distributing companies related to the indwelling boluses.

CRediT authorship contribution statement

E. Studer: Conceptualization, Investigation, Writing – original draft. **M. Alsaaod:** Conceptualization, Methodology, Resources, Writing – review & editing. **A. Steiner:** Conceptualization, Funding acquisition, Writing – review & editing. **J. Becker:** Formal analysis, Supervision, Validation, Writing – review & editing.

Data availability

Data will be made available on request.

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