J. Dairy Sci. 101:1–5
https://doi.org/10.3168/jds.2017-13682


# *Technical note:* Changes in rumen mucosa thickness measured by transabdominal ultrasound as a noninvasive method to diagnose subacute rumen acidosis in dairy cows

**V. Neubauer,* E. Humer,* I. Kröger,* A. Meißl,* N. Reisinger,† and Q. Zebeli*[1]**
*Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria
†Biomin Research Center, Biomin Holding GmbH, 3430 Tulln, Austria

## ABSTRACT

Feeding high-grain diets leads to the release and accumulation of short-chain fatty acids in the rumen. The subsequent prolonged decline in ruminal pH can lead to subacute ruminal acidosis (SARA). Accumulation of short-chain fatty acids can cause proliferation of rumen papillae to increase absorption surface, subsequently leading to a thickening of the rumen mucosa. The aim of this study was to evaluate the appropriateness of continuous measurements of the rumen mucosa thickness (RMT) as a diagnostic tool for SARA in dairy cows compared with continuous measurements of ruminal pH. The study used 6 lactating Simmental cows switched from a moderate-grain (MG) diet with 40% concentrate (dry matter basis) for 1 wk to a high-grain (HG) diet with 60% concentrate (dry matter basis) for 4 wk. Reticuloruminal pH was recorded with indwelling sensors throughout the trial. Rumen mucosa thickness was measured by transabdominal ultrasound at 4 d during the MG diet and 23 d during the HG diet. Mean RMT increased from 4.7 ± 0.19 mm in the MG diet to 5.3 ± 0.17 mm in the HG diet, whereas daily mean reticular pH decreased from 6.8 ± 0.01 in the MG diet to 6.5 ± 0.01 in the HG diet. Older cows (>3 lactations) had increased RMT, associated with higher reticular pH throughout the experiment. The higher RMT and pH level in older cows underlines their lesser susceptibility to SARA during high-grain feeding. In conclusion, RMT can successfully be measured using linear ultrasound probes, commonly used by veterinary practitioners as rectal probes. By combining noninvasive RMT measurements with the lactation number of the individual cows in a herd, this study suggests that RMT is a viable option for diagnosing SARA. Further research, using a larger number of cows with different lactations numbers, is needed to establish a cut-off RMT indicating the risk of SARA.
**Key words:** dairy cow, high-grain diet, rumen mucosa, ruminal acidosis, transabdominal ultrasound

## Technical Note

High-yielding dairy cows are typically fed diets containing large amounts of starch. These diets are rapidly fermented to large amounts of short-chain fatty acids (**SCFA**) in the rumen, providing enough energy to support high levels of milk production. However, accumulation of large amounts of SCFA can lead to sustained pH depression and SARA. Subacute ruminal acidosis is a common digestive disorder that causes economic losses in the dairy and beef industries (Enemark, 2008). The diagnosis of SARA has been a highly discussed topic for years due to the nonpathognomonic clinical signs of SARA. Continuous reticuloruminal pH measurements with indwelling rumen boli are currently considered to be the most effective diagnostic tool (Penner et al., 2006; Neubauer et al., 2017). However, costs for such pH boli are still high for constant herd surveillance, and therefore research for alternative methods is needed. Previous studies have shown that high-concentrate diets can lead to increased rumen papillae length and width (Zitnan et al., 2003; Černík et al., 2011). Mirmazhari-Anwar et al. (2013) reported increasing rumen mucosa thickness (**RMT**) when using a transabdominal rumen ultrasound (**TARU**) in cannulated bulls fed diets increasing in concentrate level (5–96% DM). Therefore, they suggested TARU as a possible diagnostic tool for SARA. The background of using this approach is that SCFA—in particular butyrate (Mentschel et al., 2001)—trigger rumen epithelium growth and increase absorption surface and capacity (Penner et al., 2011; Steele et al., 2011). To the authors' knowledge, studies measuring RMT and ruminal pH at the same time, in response to prolonged feeding of high-grain diets, are not available for dairy cows. Therefore, the objective

Received August 13, 2017.
Accepted November 2, 2017.
[1]Corresponding author: qendrim.zebeli@vetmeduni.ac.at

of this study was to evaluate the suitability of continuous RMT measurements as a diagnostic tool for SARA, compared with the most effective diagnostic tool of continuous ruminal pH measurements in dairy cows fed moderate- and high-grain diets.

In this study, 6 lactating (100 ± 16.4 DIM; 3.7 ± 1.86 lactations, mean ± SD) Simmental cows (BW = 753 ± 98.4 kg) kept together in a freestall barn were used. All procedures involving animal handling and treatment were approved by the institutional ethics committee of the University of Veterinary Medicine (Vetmeduni) Vienna and the national authority according to section 26 of the Law for Animal Experiments, Tierversuchsgesetz 2012- TVG (GZ: BMWFW-68.205/0098-WF/V/3b/2016). Cows were first fed a moderate-grain (**MG**) diet containing 60% forage and 40% concentrate (DM basis) for 7 d (d 1–7). Starting on d 8, cows were offered a high-grain (**HG**) diet with 40% forage and 60% concentrate (DM basis) and fed on this level for 29 d (d 8–36). The forage portion consisted of mixed-grass hay and grass-silage (50:50 on a DM basis), and the concentrate was based on barley (63%), soybean meal (15%), corn (9%), solvent-extracted canola meal (8%), and mineral-vitamin supplements (DM basis). Diets were provided as a TMR ad libitum. Fresh feed was offered at 0730 and 1500 h. Feed intake of each individual cow was recorded using feeders equipped with electronic weighing scales (Insentec B.V., Marknesse, the Netherlands). Cows had free access to water and a salt block. Chemical composition of the TMR was 43.7% DM, 91.5% OM, 15.5% CP, 42.1% NDF, 24.8% ADF, 2.2% ether extract, and 18.7% starch for the MG diet and 45.7% DM, 92.3% OM, 16.9% CP, 33.8% NDF, 19.4% ADF, 2.4% ether extract, and 27.8% starch for the HG diet (DM basis). Cows were milked twice per day (0700 and 1700 h) in a tandem milking parlor where milk yield was recorded automatically (Alpro Milking, DeLaval Inc., Kansas City, MO). Reticular pH was measured continuously throughout the complete trial using indwelling pH boli (Smaxtec Animal Care GmbH, Graz, Austria) placed into the reticulum via oral insertion (Klevenhusen et al., 2014). The boli were administered 3 mo before the start of the experiment and had a manufacturer-guaranteed life span of 5 mo. Rumen mucosa thickness was measured at 4 d (d 4, 5, 6, and 7) during the MG diet and 23 d (d 8–19, 21, 22, 24, 25, 27, 29–32, 35, and 36) during the HG diet by TARU using a linear probe with 6.0 MHz (DigiPrince DP-3300, Mindray Bio-Medical Electronics Co. Ltd., Shenzhen, China). The ultrasound probe was positioned following the suggestions of Mirmazhari-Anwar et al. (2013), who observed that the intercept of the lines from the third lumbar vertebra and the costal-bone-cartilage border provided the best results (Figure 1). The examination window was clipped and cleaned with alcohol, and lubrication gel was applied to ensure the same examination position and proper conduction. The ultrasound scan was frozen between rumen contractions when there was no movement of the scanned layers (Figure 2). Rumen mucosa thickness was directly measured on the frozen image using the distance measurement function of the ultrasound scan (Figure 2). The RMT measurement procedure was conducted in triplicate for each cow and day, taking measurements of 3 positions on each scan. For analysis, the mean RMT value per day and cow was used. Immediately after RMT measurement, a clinical examination of the rumen was performed, including rumen fill, rumen layering, rumen contractions, and abdominal wall tension according to Baumgartner (2014). Rumen examination, positioning of the ultrasound probe, and measurements of RMT were always performed by the same trained person at the same time of day (1600 h). Statistical analyses were performed using the software package SAS (version 9.2.; SAS Institute Inc., Cary, NC), including only data collected on days when RMT was assessed. An ANOVA was performed (PROC MIXED of SAS) to analyze differences in RMT, rumen contractions, reticular pH, DMI, and milk yield between the feeding phases [MG (d 1–7), HG wk 1 (d 8–14), HG wk 2 (d 15–21), HG wk 3 (d 22–28), and HG wk 4 (d 29–36)], between individual cows (cows 1–6), and between the different parity groups [young (2 or 3 lactations) and old (>3 lactations)]. Feeding phases, cows, and parity as well as the cow × phase and parity × phase interactions were included as fixed effects. To consider repeated measurements for 1 cow on different measurement days within a feeding phase, first-order autoregressive variance–covariance structure was used. Approximation of the degrees of freedom was conducted using the Kenward-Roger approach. Results are presented as least squares means and standard error of the mean. Comparisons among the least squares means were performed with the PDIFF option and considered significant at $P < 0.05$ and a trend at $0.05 \leq P \leq 0.10$. For the ordinal scale variable rumen fill, a contingency table was used. Differences between feeding phase or individual animals were analyzed using chi-squared test. Correlations between RMT and reticular pH, DMI, milk yield, parity, and rumen contractions were analyzed using the Pearson correlation coefficient (PROC CORR of SAS).

Overall, mean RMT was 5.2 ± 0.18 mm for all cows over the experiment. The relatively small mean standard deviation (±0.68 mm) of the 3 RMT measurements per cow and day throughout the experiment showed that