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*†

*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
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, Tierver- suchsgesetz 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., Markness, 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). 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 $0.05 \leq P < 0.10$. 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
TARU is a reliable and repeatable method. Mean RMT increased from 4.7 ± 0.19 mm in the MG diet to 5.3 ± 0.17 mm in the HG diet ($P < 0.01$), whereas the mean reticular pH decreased continuously with every week of HG feeding, from 6.8 ± 0.01 in the MG diet to a mean of 6.5 ± 0.01 ($P < 0.01$) in the HG diet (Table 1). Total DMI increased from 20.1 ± 0.81 kg in the MG diet to 25.0 ± 0.75 kg in the HG diet ($P < 0.01$), with a peak in HG wk 2. Rumen contractions increased from 2.3 contractions/2 min ± 0.2 in the MD diet to 2.8 contractions/2 min ± 0.2 in the HG diet ($P < 0.02$), with a numerical peak in HG wk 3. There was no difference in visually assessed rumen fill between feeding phases ($P = 0.15$). Milk yield increased from 31.5 ± 0.58 kg/d in the MG diet to 33.7 ± 0.53 kg/d in the HG diet ($P < 0.01$; Table 1). Although RMT and reticular pH showed a clear congruent association over the duration of the complete experiment, the Pearson correlation coefficient between RMT and mean reticular pH was negligible ($r = 0.13$, $P = 0.12$, $n = 155$). This can be largely explained by the narrow range of concentrate inclusion levels tested in this study (40–60%, DM basis), which led to only moderate differences in reticular pH. Mirmazhari-Anwar et al. (2013) found a higher correlation between ruminal pH and RMT ($r = 0.818$, $P < 0.01$) in bulls fed diets varying in concentrate levels from 5% up to 96%, which consequently led to a more extreme range in ruminal pH (5.2–7.1). In our study, the RMT increased with the duration of HG feeding, reaching an average difference of +1.3 mm in HG wk 4 compared with MG. Mirmazhari-Anwar et al. (2013) described a median increase of approximately 2.4 mm during the 7 wk on diets ranging from 5% to 96% concentrate. These findings suggest that the longer cows are exposed to high-concentrate feeding, the more rumen mucosa proliferates. Future research is needed to evaluate whether RMT reaches a plateau at a certain

### Table 1. Effect of high-grain feeding on daily mean DMI, milk yield, reticular pH, rumen mucosa thickness, and rumen contractions in 6 Simmental milking cows (100 ± 16.4 DIM) during the feeding phases: 1 wk of 40% concentrate on a DM basis (moderate grain; MG) and 4 wk of 60% concentrate on a DM basis (high grain; HG)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Feeding phase</th>
<th>Phase</th>
<th>SEM</th>
<th>P-value</th>
<th>Cow</th>
<th>Phase × cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI (kg/d)</td>
<td>MG</td>
<td>HG wk 1</td>
<td>HG wk 2</td>
<td>HG wk 3</td>
<td>HG wk 4</td>
<td>0.76</td>
</tr>
<tr>
<td>Milk yield (kg/d)</td>
<td>20.1c</td>
<td>24.9b</td>
<td>26.0a</td>
<td>24.6b</td>
<td>24.4b</td>
<td>0.54</td>
</tr>
<tr>
<td>Mean reticular pH</td>
<td>31.5a</td>
<td>33.5c</td>
<td>33.8b</td>
<td>33.3c</td>
<td>34.4ab</td>
<td>0.012</td>
</tr>
<tr>
<td>Rumen mucosa thickness (mm)</td>
<td>6.79a</td>
<td>6.63b</td>
<td>6.56c</td>
<td>6.50d</td>
<td>6.29c</td>
<td>0.177</td>
</tr>
<tr>
<td>Rumen contractions (/2 min)</td>
<td>4.68cd</td>
<td>4.70d</td>
<td>5.05c</td>
<td>5.57b</td>
<td>6.90a</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*Superscript letters indicate significant ($P < 0.01$) changes between the feeding phases.
time or ratio of concentrate feeding and to what extent diminished cell function and parakeratosis occurs (Černík et al., 2011; Steele et al., 2011).

We initially aimed to set a threshold for RMT that corresponds to a certain pH in the reticulum; however, regression analysis between RMT and mean pH revealed a negligible result ($R^2 = 0.015$). Mirmazhari-Anwar et al. (2013) suggested that a threshold of 7.3 mm RMT was indicative of pH < 5.5 in the rumen of Holstein-Friesian bulls fed up to 96% concentrate. Considering our findings, this threshold seems only meaningful for cattle receiving more than 75% concentrate in their diet, which is almost impossible with current feeding practices of dairy cattle. Indeed, RMT was higher in bulls (approximately 6.0–7.2 mm) than in cows (4.7–6.0 mm) in the current study during the weeks when Mirmazhari-Anwar et al. (2013) fed approximately the same amount of concentrate (44–64% DM) as in our study. These results suggest that cattle breed, age, and sex might have different initial RMT levels. To compare RMT between individual cows, we considered the interaction of cow × feeding phase. Considering individual cows, we found that at MG, the RMT already ranged from 3.7 to 6.0 mm, and ruminal pH ranged from 6.4 to 7.2 (Figure 3). Four cows had an increase in RMT ($P < 0.05$) from MG to HG, and 2 cows showed only a trend ($P < 0.08$) between MG and HG wk 4. One possible explanation for the different RMT and pH levels of our cows could be the different number of lactations. Older cows (>3 lactations) had higher pH and RMT ($P < 0.01$) in MG than younger cows (2 or 3 lactations). This effect was consistent for the HG weeks as well ($pH P < 0.03$; RMT $P < 0.01$; Figure 3). Moreover, the number of lactations was highly positively correlated with mean reticular pH ($r = 0.73$, $P < 0.01$) and, to a small extent, with RMT ($r = 0.38$, $P < 0.01$). This suggests that older cows, or cows with a higher number of lactations, might be less susceptible to high-grain feeding and corresponding pH decreases due to an already-thickened rumen mucosa. It has previously been discussed in the literature that primiparous cows are more susceptible to SARA than multiparous cows (Humer et al., 2015) due to their naivety to high-concentrate feeding. Another influencing factor on RMT could be the higher BW of the older cows (824 ± 43.3 vs. 681 ± 84.0 kg in young cows), which also had a moderate positive correlation with mean reticular pH ($r = 0.50$, $P < 0.01$). Moreover, older cows had a slightly lower DMI in relation to their metabolic BW (15.8 ± 0.66 vs. 17.8 ± 0.66 kg of DMI/BW$^{0.75}$; $P < 0.01$), which could have contributed to higher pH levels (Stone, 2004).

In conclusion, we were able to visualize the RMT in lactating dairy cows and its increase with concentrate feeding using a linear ultrasound probe, which is also applicable by veterinary practitioners using a rectal probe with adequate frequency. This study suggests that RMT measurement via TARU, adjusted for the age or lactation number of the individual cow, has the potential to act as a noninvasive diagnostic tool for SARA. More RMT measurements in larger herds with an increased risk of SARA will be needed to establish practical RMT threshold levels for predicting the risk of SARA.

**Figure 3.** Changes in daily mean reticular pH and rumen mucosa thickness (RMT) during the feeding phases: 1 wk of 40% concentrate on a DM basis (moderate grain; MG) and 4 wk of 60% concentrate on a DM basis (high grain; HG) in the diet of 6 Simmental milking cows (100 ± 16.4 DIM). Young cows (2 or 3 lactations) are indicated by -□-, and old cows (>3 lactations) are indicated by -△-. Means of young and old cows are indicated with corresponding standard errors of the means.
ACKNOWLEDGMENTS

This study was part of the K-project “ADDA—Advancement of Dairying in Austria” funded by the Austrian Research Promotion Agency (FFG, Vienna, Austria) under the scope of the COMET program. Special thanks go to Österreichische Veterinärmedizinische Gesellschaft (Vienna, Austria) for financially supporting the diploma thesis included in this project, Königshofer Futtermittel GmbH (Ebergassing, Austria) for providing the concentrate feed, and the clinical unit for herd health management for ruminants of the Vetmeduni Vienna for providing the ultrasound scanner.

REFERENCES


