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Technical note: The development of a methodology for ruminal and colon tissue biopsying of young Holstein dairy calves

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ABSTRACT

The objectives of this study were to develop a methodology for biopsying the rumen and colon of young dairy calves and to collect suitable quality tissue samples for microscopic and gene expression analysis. Six Holstein dairy bull calves (45.0 ± 1.5 kg birth weight) were ruminally cannulated during the second week of life and weaned at the end of wk 6. Ruminal and colon tissue samples were collected at the end of wk 5, 6, 7, 8, and 12. Calves were not sedated but were restrained in a chute for sampling. The endoscope (100 cm length, 9.8 mm diameter) was introduced through the rumen cannula to harvest ruminal tissue. Endoscopic biopsies of the rumen with endoscopic biopsy forceps were unsuccessful 85% of the time because they were unable to shear the ruminal tissue. Thereafter, an Allis clamp was used to retrieve the blind sac through the rumen cannula to perform direct tissue biopsying with surgical scissors. To biopsy the colon, the lubricated distal tip of an endoscope was slowly inserted into the calf's anus. A total of 6 colon tissue samples (12.6 ± 0.74 mg) were collected per calf per time point from the distal colon 30 to 40 cm from the calf's anus using endoscopic biopsy forceps, which were inserted through the instrument channel. A new forcep was used between sites and calves. Between calves, the outside of the endoscope was washed with 4% chlorohexidine and rinsed with water and the instrument channel was washed with distilled water and 70% ethanol. Colon and ruminal samples were processed for histological measurements, and RNA was isolated and sequenced. High-quality RNA (RNA integrity number 8.8 ± 0.08) was collected from samples, and light and electron microscopy was performed on samples. In conclusion, endoscopic biopsying can be used for tissue harvest in the colon of young calves. However, it was found that collecting ruminal tissue by retracting the rumen from the cannula and taking samples with surgical scissors was more successful than an endoscopic biopsy. This method allows for tissue collection of the same animal throughout time, which can help the research community investigate the effect of weaning regimens, feed rations, and age on the structure and function of the gastrointestinal tract.
Key words: endoscopic biopsy, colon, rumen, microscopy

Technical Note

The gastrointestinal tract (**GIT**) is of great importance in dairy production systems because it is involved in many biological processes and its function and health can be easily influenced by both internal and external factors. The GIT has multiple roles that include, but are not limited to, absorption, metabolism, nutrient delivery, and barrier function (Steele et al., 2016). Young calves experience dramatic structural and metabolic adaptations of the GIT. It has been reported that weaning increases GIT permeability (Wood et al., 2015) and negatively influences cell-mediated and humoral immunity of calves (Mackenzie et al., 1997; Hickey et al., 2003) and therefore may lead to increased health risks for calves. The effect of weaning on the rumen has been researched extensively, but little is known about its effect on the lower gut.

Gastrointestinal tract development of the calf has been reported in multiple publications using serial slaughtering (Warner et al., 1956; Lane et al., 2002). However, this approach is not ideal to study temporal responses because the sampling time point for the animal is limited to 1. Large variations also exist among animals, and repeated measurements can increase statistical power, reduce animal numbers, and reduce the time it takes to conduct the research given that fewer animals need to be recruited (Dell et al., 2002). Developing a method to collect GIT tissue in the same animal throughout time using an endoscope will enable researchers to investigate the effect of weaning regimens, feed rations, and age on the GIT postweaning as well as long-term responses.

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Ruminal cannulation remains the best approach to access the rumen because it is minimally invasive and allows for long-term, sequential sampling (Hecker, 1969). However, cannulation can be a limitation when large numbers of animals are required. Ruminal tissue is normally obtained by partially evacuating the ventral sac, retracting the ventral sac to the cannula, and then clipping the ruminal tissue using surgical scissors (Steele et al., 2011). As an alternative approach, McRae et al. (2016) introduced an endoscope through the esophagus of sedated, noncannulated sheep in a dorsally recumbent position at 45° with the head upright. They were able to biopsy the anterioventral region of the rumen using single-use biopsy forceps in sheep fasted for 4 or 24 h. Furthermore, Sasikala et al. (2017) reported that when endoscopic biopsying of the reticulum was attempted via the nasal route in nonsedated but restrained cattle, the cattle tolerated the procedure well and biopsies were successful. However, they also reported that it was possible to visualize the reticular mucosal surface only after 36 h of fasting, which is a limitation given that it may influence performance and other metabolic processes the study may be evaluating. Ultimately, both methods (McRae et al., 2016; Sasikala et al., 2017) have disadvantages when access to the ruminal epithelium for long-term sequential sampling is desired due to either sedation or fasting.

Endoscopic biopsy of the colon is commonly performed in humans to diagnose intestinal diseases such as intestinal tuberculosis (Kim et al., 1998) and cancer (e.g., Lieberman et al., 2000; Schoenfeld et al., 2005) as well as in companion animals (e.g., cats, dogs) to diagnose intestinal diseases (Washabau et al., 2010). Histology is also used to examine the tissue samples obtained via endoscopy because it allows for a more precise diagnosis, especially in inflammatory and neoplastic diseases in humans (Geboes et al., 2013) and companion animals (Washabau et al., 2010). However, currently no data or literature exist around endoscopic biopsying of the colon of ruminants to investigate development throughout time. Thus, the primary objective of this study was to develop a methodology for biopsying the rumen and colon of young dairy calves. The secondary objective was to determine whether the samples were suitable for microscopic and gene expression analysis.

To meet our objectives, 6 Holstein dairy bull calves (45 ± 1.5 kg birth weight) were obtained from a commercial dairy farm and housed in individual pens at the Metabolic Unit, University of Alberta, Edmonton, Canada. The calves were cared for and handled in accordance with the Canadian Council on Animal Care (CCAC, 2009) regulations, and the institutional Animal Care and Use Committee approved all experimental procedures (UCACS protocol no. 00002010). Calves received colostrum replacer the first day of life and then 3 L of milk replacer solution twice daily for the first week of life. From the second week onward, milk replacer was fed according to 15% of BW/d in 2 equal volumes twice daily. During weaning, calves were restricted to 50% of milk for wk 6 and fully weaned at the end of wk 6. Texturized calf starter (23.4% CP, 4.5% crude fat, 15.1% NDF, 9.3% ADF), straw (4.1% CP, 1.0% crude fat, 75.7% NDF, 53.9% ADF), and water was offered ad libitum at 0700 h and when required at 1900 h. In the second week of life, all calves were fitted with rubber ruminal cannulas (2.8 cm diameter; Lesmeister and Heinrichs, 2004).

Ruminal and colon tissue biopsies were taken at the end of wk 5, 6, 7, 8, and 12. The calves were not sedated but were restrained in a calf chute during sampling. For ruminal tissue sampling, the distal tip of the endoscope (100 cm length, 9.8 mm diameter; GIF-Q140, Olympus, Tokyo, Japan) connected to a light source and processor (CLV-U40 and CV-140, Olympus) was introduced into the rumen through the cannula. The biopsy was attempted at the blind sac (Figure 1A) using Captura hot biopsy forceps (Figure 2B; 2.4 mm diameter; HDBF-2.4-230-S, Cook Medical, Bloomington, IN). The blind sac was the chosen site of sampling because it could be visualized with the endoscope; the other sites were submerged with digesta. Sampling without visualization of the biopsy site was not attempted because the pressure of the endoscope or forcep against the tissue risked puncturing through the tissue. With 5 attempts using the Captura hot biopsy forceps, samples could be collected only 15% of the time. Alternatively, alligator jaws with needle biopsy forceps (2.4 mm diameter; DBF-2.4-230SP-S, Cook Medical) were used to attempt sampling the blind sac. Five sampling attempts were made per calf per forcep type. The outside of the endoscope was washed with 4% chlorohexidine and rinsed with water between calves. The instrument channel was washed with double distilled water and 70% ethanol between calves. Both endoscopic biopsy forceps were able to excise the rumen epithelium only 15% of the time, which we suspect is due to strong connective tissue. Even though the success rate was low using endoscopic biopsying in the rumen, it still can be used as a means of tissue sampling in live animals (McRae et al., 2016; Sasikala et al., 2017). Franz et al. (2006) used an endoscope for visualization of the rumen in calves that were fasted for 12 h via a rumen cannula and through the esophagus. The authors reported that when the endoscope was introduced through the esophagus in nonsedated calves, it failed in 3 of 9 calves because the calves moved too much. In addition, even though the calves were fasted for 12 h, visualization of