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Technical note: Three-dimensional imaging of rumen tissue for morphometric analysis using micro-computed tomography

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ABSTRACT

Rumen development in calves has been evaluated by measuring papillae length, width, and density using microscopy for over 50 yr. Although common in the literature, disadvantages to this method exist, such as large variations in rumen papillae size and shape, small numbers of total papillae being measured, and the time required. The objective of this study was to develop a more effective technique for assessing rumen papillae using micro-computed tomography (micro-CT) and to compare this technique with microscopy. Rumen tissue was collected from the ventral sac of 20 postweaned bull calves at 55 d of age, immediately fixed in 10% neutral buffered formalin for 48 h, and stored in 70% ethanol at 4°C before the contrast enhancement. After evaluation of contrast-enhancement protocols, it was determined that mercury chloride provided the most pronounced contrast for accurate micro-CT imaging based on relative density of the papillae. A 1-cm^2 tissue section from the ventral sac of all bull calves was tensioned on a rapid prototyped curved plastic holder and imaged at 45 μm resolution for 56 min using a GE Locus Explore micro-CT (General Electric, Milwaukee, WI). MicroView V2.2 software (General Electric) was used to create a 3-dimensional virtual model of the entire sample. The length and width of papillae were measured 3-dimensionally and compared with measurements of papillae under the light microscope taken from the same region. The length and width measurements using micro-CT (2.47 ± 0.12 and 0.55 ± 0.01mm) compared with light microscope (2.96 ± 0.03 and 0.86 ± 0.01 mm) were significantly smaller. The difference may reflect a more accurate determination in the base of the rumen tissue with micro-CT or the specificity of mercury chloride to bind only to intact rumen tissue. The mean number of papillae per centimeter squared viewed using micro-CT was 128.5 ± 33.9 with a total surface area of 681.8 ± 112.4 mm^2 and volume of 156 mm^3 per sample. Micro-CT data demonstrated that surface area and volume are positively associated and that papillae length was negatively associated with papillae per centimeter squared and positively associated with total volume of tissue section. This study represents the first time that micro-CT has been being used to assess morphology of rumen tissue. Micro-CT has the potential to improve the accuracy and efficiency of rumen tissue measurements; however, more standardization of each factor involved in tissue preparation, imaging, and location of papillae measurements is required.
Key words: development, morphology, micro-computed tomography

Technical Note

The ruminant forestomach is primarily known for its role in microbial fermentation, although it also plays a major role in host defense, nutrient absorption, and metabolism (Gäbel et al., 2002). Thousands of finger-like projections called papillae line the ruminant forestomach and function to increase the surface area of absorption of the end products of ruminal fermentation (Steele et al., 2011a). During the lifespan of the ruminant, the microarchitecture of the rumen papillae undergoes dramatic adaptations, especially during the transition of weaning. This development involves a combination of rapid cellular differentiation in the rumen lining resulting in increased rumen papillae size, thus more surface area for the absorption of ruminal fermentation end products (Baldwin et al., 2004). Inadequate rumen papillae development and dry feed intake before and after weaning is often associated with decreased weight gain, performance, and welfare (Khan et al., 2011). Taking this into consideration, identifying feeding regimens and functional nutrients that encourage rumen papillae growth and maturation may improve production performance, as well as the health and welfare of the young ruminant during the transition of weaning.

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The development and adaptations of the rumen is commonly assessed by rumen mass (Baldwin et al., 2004) and relative gene expression techniques (Penner et al., 2011; Steele et al., 2011a,b); however, the gold standard lies in assessing rumen papillae length, width, and density using microscopy (Lesmeister et al., 2004). Two-dimensional microscopic measurements are an indirect method of assessing the surface area for absorption and have been employed in ruminant physiology and nutrition for over 50 yr. The rumen consists of multiple compartments (caudal dorsal blind sac, cranial dorsal sac, cranial ventral sac, and the caudal and ventral portions of the ventral and dorsal blind sac), but most studies evaluating rumen papillae morphology focus only on specific sites, namely the ventral or dorsal sac where papillae are most developed (Lesmeister et al., 2004; Benschop and Cant, 2009). Furthermore, due to the time-consuming nature of the methodology, generally less than 20 papillae length and width measurements per site are measured. Large variations in papillae measurements between studies exist, which may reflect the diverse treatments or the timing of sampling (Lesmeister et al., 2004; Hill et al., 2005; Gorka et al., 2009; Roth et al., 2009). Nevertheless, most studies do not provide a detailed description of their methodology which may contribute to the discrepancies in the scientific literature.

Perhaps the largest source of error in rumen papillae measurements comes from the inherent complexity of ruminal morphological changes during weaning—a process that remains largely undescribed. For example, the surface of the rumen during weaning displays contours not evident in mature ruminants (Figure 1a). Cross-sections of these contours examined under the light microscope (Figure 1b) show that the rumen epithelium at weaning displays a polyp-like structure where several immature papillae of different sizes and shapes protrude. This observation requires more investigation, as it likely contributes to the inconsistencies in the literature and, more importantly, could be an important event in rumen structural development. Furthermore, a closer look at rumen papillae after weaning under a stereoscope displays a large variation in rumen papillae size in adjacent papillae in the same section of rumen (Figure 1a). These 2 factors, rarely discussed in the literature, create significant challenges when collecting, analyzing, and interpreting papillae length and width measurements in ruminants during weaning.

A technical approach that may address the accuracy and time limitations of microscopic measurements of rumen papillae is to visualize rumen tissue in 3 dimensions using micro-computed tomography (**micro-CT**). X-ray tomography has been used extensively in medical research to create cross sections of 3-dimensional objects that can later be used to recreate a virtual model without destroying the original sample. Over the last decade, the field of x-ray tomography has been revolutionized through technical developments which allow for pixel sizes of the cross-section to be viewed in the micrometer range (Metscher, 2009). Micro-CT has proven to be a valuable technique for the imaging of bone structures; however, the visualization of soft tissue is still a challenge due to low x-ray contrast (Pauwels et al., 2013). By finding the optimal contrast enhancement it may be possible for multiple rumen tissue samples to be scanned simultaneously using a micro-CT to create 3-dimensional models of large tissues. These large sections could consist of thousands of rumen papillae which can be instantaneously measured for surface area. It further enables the technician to measure the length and width of individual rumen papillae in a 3-dimensional manner, improving the accuracy of measurements. In spite of its potential to the field of gastrointestinal research, a technique has not been developed to use micro-CT to measure gastrointestinal morphology. Therefore, the objective of our study was to develop a protocol for the measurement of rumen papillae length, width, and surface area using micro-CT and compare the results to the classical microscopic technique.

To meet this objective, the rumen tissue from the ventral sac of 20 bull calves was harvested on d 55 of life (7 d postweaning). All animal procedures for raising and euthanizing the bulls were approved by the Nutreco Canada Agresearch Animal Care Committee in accordance with the Canadian Council on Animal Care (1993) guidelines. Calves were slaughtered by captive bolt pistol and exsanguination and the rumen was immediately harvested, rinsed in tap water, and washed in PBS. A 3-cm^2 section of tissue was dissected from the ventral sac of the rumen and pinned to dental wax (Canemco Inc., Gore, QC), to prevent tissue folding, and submersed in neutral buffered formalin (10%) for 48 h at a volume of over 10:1. After 48 h of fixation the rumen tissue was transferred to a solution of 70% ethanol at a volume of 10:1 and stored at 4°C until staining for micro-CT.

To optimize the accuracy of the micro-CT imaging, a series of staining protocols were tested to determine the optimal contrast enhancement specific for rumen tissue. Tissue submerged in 70% ethanol was removed from the fridge, trimmed to an area of 1 cm^2 using a microtome blade, and mounted on a rapid prototype curved plastic holder (Figure 2). Plastic was used for the specimen holder to eliminate the chance of x-ray dispersion and provided a sealed humidified environment. The holder was designed using Solidworks software (Dassault Systemes, Waltham, MA) with the predetermined