Small intestine histomorphometry of beef cattle with divergent feed efficiency

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Abstract

Background: The provision of feed is a major cost in beef production. Therefore, the improvement of feed efficiency is warranted. The direct assessment of feed efficiency has limitations and alternatives are needed. Small intestine micro-architecture is associated with function and may be related to feed efficiency. The objective was to verify the potential histomorphological differences in the small intestine of animals with divergent feed efficiency.

Methods: From a population of 45 feedlot steers, 12 were selected with low-RFI (superior feed efficiency) and 12 with high-RFI (inferior feed efficiency) at the end of the finishing period. The animals were processed at 13.79 ± 1.21 months of age. Within 1.5 h of slaughter the gastrointestinal tract was collected and segments from duodenum and ileum were harvested. Tissue fragments were processed, sectioned and stained with hematoxylin and eosin. Photomicroscopy images were taken under 1000x magnification. For each animal 100 intestinal crypts were imaged, in a cross section view, from each of the two intestinal segments. Images were analyzed using the software ImageJ. The measurements taken were: crypt area, crypt perimeter, crypt lumen area, nuclei number and the cell size was indirectly calculated. Data were analyzed using general linear model and correlation procedures of SAS.

Results: Efficient beef steers (low-RFI) have a greater cellularity (indicated by nuclei number) in the small intestinal crypts, both in duodenum and ileum, than less efficient beef steers (high-RFI) (P < 0.05). The mean values for the nuclei number of the low-RFI and high-RFI groups were 33.16 and 30.30 in the duodenum and 37.21 and 33.65 in the ileum, respectively. The average size of the cells did not differ between feed efficiency groups in both segments (P ≥ 0.10). A trend was observed (P ≤ 0.10) for greater crypt area and crypt perimeter in the ileum for cattle with improved feed efficiency.

Conclusion: Improved feed efficiency is associated with greater cellularity and no differences on average cell size in the crypts of the small intestine in the bovine. These observations are likely to lead to an increase in the energy demand by the small intestine regardless of the more desirable feed efficiency.

Keywords: Bovine, Duodenum, Functional workload, Ileum, Intestinal epithelium, Intestinal crypts, Residual feed intake
elucidated, it is likely to be controlled by a combination of factors including physiological [6-8], genetic [9-11] and behavioral mechanisms [3,8,12]. Residual feed intake (RFI) is a feed efficiency measure, first defined in beef cattle by [13] and largely used to study the biology of feed efficiency and to verify the effectiveness of indirect indicators of feed efficiency [14-18]. Differences in RFI reflect variation among animals’ background energy requirements, which are largely influenced by the visceral organs [3,19].

The gastrointestinal tract is an important energy sink using a disproportionate amount of energy in proportion to its weight [20]. For instance, when compared with muscle tissue, which accounts for six times more body weight than the gastrointestinal tract, the gastrointestinal tract presents two and half times higher fasting heat production [21]. The gastrointestinal tract appears to alter its mass and metabolism in accordance to dietary intake within and across physiological stages of maintenance, growth, fattening or lactation [22]. The small intestine, in particular, possesses the adaptive capacity to alter form and function in response to changes in digestive demand [23] to reach the nutrient needs for the animal using variable amounts of energy and protein according to the background requirements and production level [24].

It has been described that incremental starvation produces progressive small intestine atrophy in mice [25] and structural changes to the mucosa of rats, which include disappearance of some villi and a reduction in the size and number of crypts [26]. In contrast, studies related to re-feeding and feeding for ad libitum intake indicate histological changes in small intestine epithelium [27], while studying the feeding response in starved snakes, reported that the thickness of the intestinal mucosa increased three times after 48–72 h of re-feeding.

Therefore, the small intestine responds rapidly and dramatically to changes in functional workload, such as starvation or feeding for ad libitum intake. These changes include modifications in the intestinal micro-architecture. Beef cattle with different feed efficiency may have differences in their small intestine architecture, which could be associated with differences in feed intake. The objective of this study was to conduct histomorphometrical evaluation of the bovine small intestine (duodenum and ileum) to characterize the histological patterns in response to divergent feed efficiency.

Methods

Animals, experimental design and sample collection

Housing and experimental conditions were previously described in detail by [29]. Briefly, individual feed intake was measured daily during the 140 d of the experiment. Animals were divided in 3 pens of 15 steers each. Animals were weighed and ultrasound was performed, for assessing subcutaneous fat deposition, every 28 d until slaughter. Steers were fed a high-moisture corn-based diet for ad libitum intake. Steers were handled and monitored meeting or exceeding the recommendations of the Canadian Council of Animal Care guidelines (1993). All procedure protocols were approved by the University of Guelph’s Animal Care Committee. The determination of RFI was done through a regression of dry matter intake on mid-experiment body weight, average daily gain and end-experiment backfat thickness, as described by [30]. From the population of 45 crossbred steers, the 24 animals with extreme feed efficiency were selected: 12 with high-RFI (inferior feed efficiency) and 12 with low-RFI (superior feed efficiency). Animals were processed at 13.79 ± 1.21 months of age. The gastrointestinal tract was collected within 1.5 h after slaughter; two segments of 20 cm were gently harvested from duodenum (immediately distal to the pylorus) and ileum (immediately proximal to the ileocecal valve) [31].

Sample processing and histomorphometry

Fragments of duodenum and ileum were first washed in a 0.9% saline solution. Tissue fragments were pinned in cardboard and then fixed in 10% neutral phosphate buffered formalin under moderate agitation for 24 h and processed for 8.45 h in a tissue processor (Renaissance TP*: Ventana Medical Systems Inc.; Tucson, U.S.A.). Fixed samples then were embedded in paraffin. Paraffin blocks were sectioned at 5 μm thickness using a microtome (Shandon finesse Microtome 325*: Thermo Electron Corporation; Waltham, U.S.A.) and stained with hematoxylin and eosin according to the method described previously by [32].

Histological images were taken using bright field at 1000x magnification (under immersion oil) with a Leica DMLB microscope (Leica Microsystems Inc.*; Wetzlar, Germany) equipped with a video camera QICAM Fast 1394 (Qcapture®, Surrey, BC, Canada) connected to the computer-based image analysis software QImaging (Qcapture®, Surrey, BC, Canada). Histological measurements were made with Image® imaging analysis software (U.S. National Institutes of Health, Bethesda, Maryland, USA). For each steer 100 crypts were measured, in a cross section of both segments (duodenum and ileum), Figure 1. The measurements taken were crypt area (CA; μm²), crypt perimeter (CP; μm), crypt lumen area (LA; μm²) and nuclei number (NN). In addition, the average cell size (CS; μm²) was determined by subtracting the crypt lumen area from the total crypt area and then dividing this value by the nuclei number, which represents the number of mucosal cells present...
on each transversal image of the crypt (Figure 1). It was observed a separation of the epithelium from the underlying lamina propria, Figure 1. This artifact did not compromise the architecture of the intestinal crypts, which were the target structures for this study. All the pictures were taken and assessments were made by the same observer, who was blinded as to which feed efficiency group the samples belonged to.

Statistical analysis
Data were analyzed using SAS® software (SAS Institute, Cary, NC, USA). Means of the two feed efficiency groups were tested using the general linear model procedure and compared using T-test, according to the following model:

\[ Y_{ij} = \mu + \text{Group}_i + \epsilon_{ij} \]

where, \( Y_{ij} \) is the dependent variable (RFI and histomorphometrical measures), \( \mu \) is the overall mean effect; \( \text{Group}_i \) is the fixed effect of feed efficiency group and; \( \epsilon_{ij} \) is the residual error. Pearson correlation was determined within each group using the correlation procedure. For all analyses data were considered statistically significant when \( P \leq 0.05 \) and were considered a trend towards significance when \( 0.10 \geq P > 0.05 \).

Results
The mean value for the low-RFI and high-RFI groups were -0.53, and 0.64 kg/d (\( P < 0.001 \)), respectively. This represent a difference in daily dry matter feed intake of 1.17 kg more feed intake for the cattle with inferior feed efficiency to achieve the same performance as the steers with superior feed efficiency without differences on subcutaneous fat deposition, as a result of the adjustment for backfat thickness in the RFI prediction model.

The descriptive statistics composed by mean, standard deviation, coefficient of variation, minimum and maximum values, of the histomorphometrical measures is presented in Table 1. It is interesting to note that the different measurements obtained in both duodenum and ileum presented similar variability, as indicated by the coefficient of variation.

Table 2 reports the comparisons of the means for all the histomorphometry traits relative to feed efficiency group (low-RFI and high-RFI). Perimeter and area of the crypt (CP; CA) in the duodenum showed a tendency (\( P \leq 0.10 \)) to be larger for the more efficient animals (low-RFI group), which was not seen in the ileum crypts (\( P \geq 0.10 \)). The cell size (CS) did not differ between the RFI groups in both segments (\( P \geq 0.10 \)). Nuclei number (NN) was significantly greater in the low-RFI group than the less efficient animals (high-RFI) in both segments (\( P \leq 0.05 \)).

Correlations between the measurements in each segment and within each the RFI groups are shown in Table 3. Negative correlations were observed between CA, CP and NN in duodenum with low-RFI (\( P \leq 0.05 \)), the same measures in the ileum were not associated with any of the feed efficiency groups (\( P \geq 0.10 \)). Feed efficiency in the less...
efficient cattle (high-RFI group) appeared to be positively correlated with CS in both duodenum (P ≤ 0.10) and ileum (P ≤ 0.05).

Discussion
The small intestine is an organ with intense metabolic rate, using 17 to 25% of whole-body oxygen consumption [33], with a tremendous capacity to adjust function, size and shape according to the physiological demand in ruminants [22]. The intense metabolic rate of the small intestine is mostly due to the energy expenditure for biochemical processes by the intestinal cells [34] and is also due to constant and continuous epithelium renewal [35-37] to maintain or to cope with variations in workload [38,39]. The later factor is associated with changes in tissue structure [40,41]. Additionally, the workload of the small intestine is particularly increased in cattle fed with diets rich in starch [42] as in the present study.

The similar coefficients of variation, observed on duodenum and ileum measurements, of CA, CP, NN and the CS in the cross section view of the crypt suggests a comparable homogeneity of the same measures in both intestinal segments, which was also observed by other authors [43,44]. This similarity also indicates the consistency of the assessments conducted by a single observer. It is also interesting to notice that the mean values for CS were of larger magnitude in the duodenum in comparison to the ileum. Conversely, the values for NN were higher in the ileum. These results are in agreement with the findings made by [45] studying the cellular dynamics of avian intestine. This author reported that the small intestine possess a negative association between cell size and number of cells in its different segments, where the proximal part (in the case of this study duodenum) had a larger but fewer cells, in contrast to the distal parts, where the ileum could be included, which had smaller but more numerous cells.

The fact that the number of cells, represented by nuclei number, was higher (both in the duodenum and ileum) and the crypt area and perimeter of the duodenum were positively associated with improved feed efficiency, based on the correlation analysis, indicates a more metabolically active small intestine in cattle with superior feed efficiency. Similarly, [24] studying bulls of different breeds, described that a more efficient and higher growth rate breed of cattle had more cells in all small intestinal segments analyzed than the less efficient and lower growth rate cattle breed. We can infer that a greater cellularity and the lack of difference on cell size may associated with larger villi or a more intense repos-
indicative that a more metabolic active intestine not only leads to a better absorption of nutrients [46] but also to a better energetic efficiency. In addition, the correlations of histomorphometrical measures in duodenum (CA; CP; NN) and feed efficiency in more efficient beef steers (low-RFI) also support this argument.

Despite the fact that our results for CS did not differ between feed efficiency groups (P ≥ 0.10), a study by [47] described that the small intestine responds to differences in feed intake by altering organ visceral mass via an increase in the size of cells (hyperplasty). We observed a positive correlation between feed efficiency in the high-RFI group and the CS in the ileum, which suggests that an inefficient steer may have larger mucosal cells in this segment. This finding requires further investigations. On the other hand, [48] described that increased intestinal workload through changes on dietary protein level resulted in a quadratic change in the small intestinal mucosa. The DNA concentration increased when the protein levels were 8.5% to 10.7% (dry matter), resulting in a hyperplasia that is in line with the present findings.

The histomorphometric results of this study indicate that more efficient beef steers (low-RFI group) have increased metabolic activity in the small intestine, which is associated with improved feed efficiency in cattle [24] and also in other species [47,49,50]. Despite the fact that this increase is associated with a greater energetic demand [38], increasing the maintenance requirements [51]; the cost-benefit of this more functional small intestine results in more animal growth (productivity) per unit of feed intake. Expenditures with tissue plasticity [52] and cellular biochemical processes in small intestine are known to be largely influenced by the animal’s different physiological states [39] and also by changes according to the level of intake and diet composition via changes in the visceral organ mass [53]. The present study indicates that there is also variation in these expenditures due to individual variation and that such variation is associated with feed efficiency. Finally, the histomorphometrical associations found in here have a potential for further technical improvements (i.e. automated imaging analysis) that may result in a tool for indirectly assessing feed efficiency in the bovine. This could have immediate applications on breeding programs, where there is a possibility of evaluating the progeny of bulls through sampling their offspring at slaughter.

**Conclusion**

There are differences in small intestine micro-architecture of beef cattle with divergent feed efficiency. Improved feed efficiency was associated with greater cellularity in the small intestine crypts and no differences in average cell size, both in duodenum and ileum, as indicated by the nuclei number in the intestinal crypts and the direct associations between crypt area and crypt perimeter with feed efficiency. It is logical to suggest that the benefits of a more metabolically active small intestine are greater than the energetic costs associated with the increased workload, which leads to improved feed efficiency. Further studies aiming to develop imaging analysis techniques for optimizing these measures are warranted and may lead to solutions for improvement of feed efficiency in beef cattle.

**Abbreviations**

RFI: Residual feed intake; CA: Crypt area; CP: Crypt perimeter; LA: Crypt lumen area; NN: Nuclei number; CS: Cell size; Mu: Mucosa; Mm: Muscularis mucosae; lc: Intestine crypt; Lu: Intestine crypt lumen; Nu: Nuclei.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

The hypothesis was developed by YM and KS. The study was designed by YM, SM and KS. A pilot study and the development of methodology was done by YM, SY and BC. SY trained YM for tissue processing and slides preparation. YM processed the samples and prepared slides. BC trained AF for microscopy imaging. AF performed the imaging work and measurements and drafted the manuscript. YM performed all statistical calculations. All authors read, revised, provided suggestions and approved the final manuscript.

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