Associations between rumen function and feed efficiency in beef cattle

By
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ABSTRACT

ASSOCIATIONS OF RUMEN FUNCTION AND STRUCTURE WITH FEED EFFICIENCY IN BEEF CATTLE

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University of Guelph, 2016

Rumen functional and structural parameters and their associations with feed efficiency were evaluated. Forty-eight crossbred feedlot cattle were assessed for feed efficiency using residual feed intake (RFI) adjusted for body ultrasound measurements. Rumen fluid and tissue samples were evaluated for microbial and VFA profiles, papillae histomorphometry, and rumen pH (RpH) and temperature (RT) and compared between efficient and inefficient cattle. Additionally, microbial and VFA profiles were compared on-farm and at slaughter. Efficient cattle have greater bacteria (P=0.04), lower methanogen concentrations (P=0.04), differences in multiple VFA (P<0.05) between on-farm and at slaughter, greater ventral blind sac papillae width (P=0.02), spend more daily time in optimal RpH (P=0.02) and have lower circadian RpH compared to inefficient cattle. Differences in VFA profiles or RT traits were not found across efficiency groups. This suggests rumen structural and functional parameters influence the efficiency of feed utilization and may serve as potential indicators for feed efficiency.

Key words: histomorphometry, microbiology, residual feed intake, rumen pH, volatile fatty acids
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<tbody>
<tr>
<td>ABW</td>
<td>Average Body Weight</td>
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<tr>
<td>ADF</td>
<td>Acid detergent fibre</td>
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<td>ADG</td>
<td>Average daily gain</td>
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<td>AP</td>
<td>Acetate:propionate ratio</td>
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<td>BKF</td>
<td>Average back fat</td>
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<tr>
<td>BW</td>
<td>Body weight</td>
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<td>d</td>
<td>Day</td>
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<tr>
<td>DM</td>
<td>Dry matter</td>
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<td>DMI</td>
<td>Dry matter intake</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization, United Nations</td>
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<td>FCC</td>
<td>Farm Credit Canada</td>
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<td>FCR</td>
<td>Feed conversion ratio</td>
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<td>g</td>
<td>Gram</td>
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<tr>
<td>GC</td>
<td>Gas chromatography</td>
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<tr>
<td>GHG</td>
<td>Greenhouse gas</td>
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<td>kg</td>
<td>Kilogram</td>
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<td>mmol/L</td>
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<td>Acronym</td>
<td>Description</td>
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<tr>
<td>ng</td>
<td>Nanogram</td>
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<td>nm</td>
<td>Nanometer</td>
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<td>oz</td>
<td>Ounce</td>
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<tr>
<td>MAR</td>
<td>Average marbling</td>
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<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>NDF</td>
<td>Neutral detergent fibre</td>
</tr>
<tr>
<td>PEG</td>
<td>Partial efficiency of growth</td>
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<tr>
<td>qRT-PCR</td>
<td>Quantitative real time-polymerase chain reaction</td>
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<tr>
<td>R²</td>
<td>Coefficient of determination</td>
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<tr>
<td>REA</td>
<td>Average rib eye area</td>
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<tr>
<td>RFI</td>
<td>Residual feed intake</td>
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<tr>
<td>RG</td>
<td>Residual BW gain</td>
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<tr>
<td>RIG</td>
<td>Residual intake and BW gain</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>RpH</td>
<td>Rumen pH</td>
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<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
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<tr>
<td>RT</td>
<td>Rumen temperature</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical Analysis Software</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<td>VFA</td>
<td>Volatile fatty acids</td>
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<td>vs.</td>
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<td>°C</td>
<td>Degrees Celsius</td>
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<td>%</td>
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<td>µm</td>
<td>Micrometer</td>
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<td>µg</td>
<td>Microgram</td>
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CHAPTER 1: INTRODUCTION

The consistently rising global population leads to an increasing demand for livestock production, bringing challenges to the agricultural industry and recognition of the importance of applying more efficient and sustainable production systems (FAO, 2014). As feed costs are the largest production expense (Arthur et al., 2004), sustainable means to efficiently reduce feed costs are needed. Biological markers have been extensively researched (Nkrumah et al., 2006; Kelly et al., 2010; Gonano et al., 2014; Foote et al., 2016), due to their significant contribution to energy metabolism to determine potential indicators for feed efficiency. However, associations between rumen parameters with feed efficiency have not been critically evaluated. Until recently, studies assessing rumen functional parameters and feed efficiency have been emerging, suggesting that variation in rumen parameters exists (Guan et al., 2008; Carberry et al., 2014b; Kern et al., 2016a; McDonnell et al., 2016). However, further research is necessary as markers capable of differentiating feed efficiency in practice are still needed. The work reported in this thesis aimed to characterize feed efficiency in beef cattle using rumen functional and structural parameters, including microbial and volatile fatty acid (VFA) profiles, papillae histomorphometry, and rumen pH (RpH) and temperature (RT) to increase our understanding of the rumen physiology underlying feed efficiency and determine potential indicators capable of predicting feed efficiency.
CHAPTER 2: LITERATURE REVIEW

2.1. Feed efficiency and the beef industry

Canada is the world’s 11th largest beef producer, supplying 2% of world beef and 7th largest beef exporter contributing to 5.8% of the world export market. Additionally, total exports account for 73% of beef produced in Canada, which contributes approximately $1.2 billion in annual profit (FCC, 2014). Feed costs remain as the largest expense in beef cattle production contributing to 50% of herd cost variation (Miller et al., 2001). Additionally, with large export systems and the growing demand for beef, the environmental impact of the industry must be considered. Methane enteric emissions alone represent 30 to 50% of total GHG emitted from the livestock sector, with 80% of that portion contributed by ruminant enteric emissions (Gill et al., 2010). The increasing demand for beef and environmental stewardship of beef production has increased the demand for new strategies to reduce feed costs. Therefore, improving feed efficiency can improve the sustainability and profitability of agriculture.

2.2. Measures and physiological indicators for feed efficiency

2.2.1. Measures for feed efficiency

Feed efficiency is the conversion of feed energy used for growth and maintenance (Koch et al., 1963) and has potential for improvement which can provide major financial implications and improvement of environmental stewardship of agricultural systems. Several methods exist to measure feed efficiency in cattle, each pertaining to benefits and disadvantages.

A traditional measure of feed efficiency is feed conversion ratio (FCR) which is the ratio of dry matter intake (DMI) to average daily gain (ADG), or the inverse, gross feed efficiency (Ahola and Hill, 2012). FCR is known to be moderately correlated with residual feed intake (RFI) (Lancaster et al., 2009) and is a reasonable determination for performance in a feedlot.
herd; however, selection for feed efficiency using FCR has not been widely used, due to its correlation with growth rate and mature size, leading to selection of greater mature size and increased feed requirements (Herd and Bishop, 2000).

The partial efficiency of growth (PEG) is the residual of actual DMI and expected DMI for maintenance (Arthur et al., 2001; Nkrumah et al., 2004) and is known to be strongly correlated with RFI (Lancaster et al., 2009). However, selection for PEG may not be as independent of growth and body size as responses to selection for RFI, as PEG is strongly correlated with DMI. Additionally, weak correlations have been observed between ADG and body weight (BW) (Arthur et al., 2001; Nkrumah et al., 2004).

Residual BW gain (RG) is the difference between actual gain and predicted gain based on body weight, intake, and composition, and calculated by computing the residual from a multiple regression model regressing ADG on both DMI and BW (Berry and Crowley, 2012). However both RG and RFI are independent of growth rate which proposes issues in commercial use, as efficient animals may consume less but have lower growth rate.

A novel feed efficiency measure has emerged similar to RG that is not affected by differences in feed intake, known as residual intake and body weight gain (RIG). RIG measures can identify animals with higher ADG leading to a shorter duration in the feedlot and lower expected daily DMI while adjusting for differences in maintenance (Berry and Crowley, 2012).

Residual feed intake is an emerging method for evaluating feed efficiency. Koch et al. (1963) first proposed the concept of RFI in beef cattle, which was originally established by Titus (1928) in laying hens. RFI is defined as the difference between actual DMI and expected DMI computed by a regression approach based on BW and ADG over a performance period (Arthur et al., 2001; Nkrumah et al., 2004). Recently RFI has been adapted to adjust for body composition using ultrasound assessments, providing RFI values unaffected by body
composition variation (Lancaster et al., 2009; Montanholi et al., 2009; Kelly et al., 2011). Additionally, RFI is moderately heritable and genetically independent of growth and body size making it a more desirable measure for feed efficiency (Crews, 2005).

2.2.2. Metabolic parameters associated with feed efficiency

Specific measures regulated by metabolic processes may influence feed efficiency (Kolath et al., 2006; Mader et al., 2009). Additionally, manipulation of maintenance requirements can offer up to 20% of improvement in efficiency. For instance, service functions for high energy expending organs and maintenance of individual tissues or cells account for 36 to 50% and for 40 to 50% of maintenance energy expenditure, respectively (Baldwin et al., 1980); and the variation in feed intake can cause substantial differences in maintenance requirements. Multiple metabolic parameters have been assessed for associations with feed efficiency to determine potential indicators for this trait such as blood plasma metabolites (Kelly et al., 2010; Montanholi et al., 2010; Kelly et al. 2011; Gonano et al., 2013), milk and colostrum composition (Montanholi et al., 2013a), heat radiation using infrared thermography (Montanholi et al., 2009; Montanholi et al., 2010), and feeding behaviour (Adam et al., 1984; Nkrumah et al., 2006; Kelly et al., 2010; Montanholi et al., 2010). Additionally, associations of fertility traits with feed efficiency have been measured (Awda et al., 2013; Fontoura et al. 2016; Montanholi et al., 2016) to understand the impact of improved feed efficiency on bull reproductive function and determine biological markers that can improve both feed efficiency and fertility. Further research on mechanisms regulating growth and energetic efficiency is necessary as a cost-effective and reliable indicator for feed efficiency has yet to be adapted and applied in the industry.

Studies have shown that efficient cattle are associated with greater diet digestibility (Richardson and Herd, 2004) and RFI is known to be correlated with apparent digestibility of
DM (Nkrumah et al., 2006). Richardson and Herd (2004) revealed lower RFI is associated with higher digestibility and estimated 10% of the biological variation of RFI is due to digestibility. Studies on non-ruminants have shown that differences in digestibility are unimportant (Luitig, 1990), however current research on the rumen microbial population structure and abundance, volatile fatty acid (VFA) concentrations, and rumen pH (RpH) have been reviewed between efficiency groups in cattle (Hernandez-Sanabria et al., 2012; Fitzsimons et al., 2014a; Myer et al., 2015) and suggested associations exist between rumen metabolism and the efficiency of feed utilization.

2.3. Rumen microbiology

2.3.1. Rumen bacteria, protozoa, and fungi

The diverse microbial community in the rumen is critical for generation of energetic substrates utilised for growth and maintenance (Hungate, 1966). The three taxonomic microbial groups involved in the rumen fermentation process include bacteria, protozoa, and fungi (Van Soest, 1982). Bacteria and fungi collectively contribute to 80%, and protozoa contributes to 20% of degradative activity (Dijkstra and Tamminga, 1995) using adaptive strategies involving enzymatic activity, colonization of feed particles, and synergetic interaction of species for fermentation (Wang and McAllister, 2002).

Assessment of the rumen microbial ecology and function involves total DNA extraction commonly performed by a bead-beating method (Walter et al., 2000; Guan et al., 2008), followed by culture-independent methods including polymerase chain reaction-denaturing gel electrophoresis (PCR-DGGE) to illustrate microbial community structure, 16S rRNA gene sequencing analysis, and quantitative real time-PCR (qRT-PCR) to quantify microbial abundance (Meiju et al., 2012). Several studies have evaluated associations of the rumen
microbiology with feed efficiency (Guan et al., 2008; Hernandez-Sanabria et al., 2010; Hernandez-Sanabria et al., 2012; Carberry et al., 2012; Myer et al., 2015).

Using PCR-DGGE to generate dendrograms, Guan et al. (2008) revealed efficient and inefficient steers had a bacterial profile similarity of 91% and 71%, respectively. This suggests that the bacterial population composition is related to capacity of feed utilization efficiency. Additionally, these bacterial groups were correlated with specific breeds (Guan et al., 2008). Hernandez-Sanabria et al. (2012) revealed a greater similarity profile percentage in efficient (70.5%), compared to inefficient cattle (66.3%) and found that specific bacteria phylotypes are correlated with efficiency group and diet energy level, suggesting the microbial community structure is associated with divergent efficiency and diet (Hernandez-Sanabria et al., 2012). Contrastingly, Hernandez-Sanabria et al. (2010) revealed no correlation between bacterial community similarity and RFI groups. Previous studies evaluating the influence of feed efficiency and diet energy variation on the ruminal community structure and showed that specific bacterial species vary with diet, and the bacterial genus, prevotella, is more abundant in efficient cattle (Carberry et al., 2012).

Use of PCR-DGGE and 16S rRNA gene sequencing brings limitations, as such techniques solely identify microbial species available within the 16s rDNA libraries which contain only major microbial species. Novel sequencing technologies have recently been used, including taxonomic microbial abundance, diversity measures, and principle coordinate analysis to characterize the rumen microbiome of divergent efficiency groups (Myer et al., 2015) and revealed no association between bacterial diversity and feed efficiency; however, microbial population taxonomic abundance, and bacterial phylotype units varied with divergent efficiency suggesting that microbial population characterization by relative taxonomic abundance and phylotype unit classifications can serve as possible markers for feed efficiency.
2.3.2 Rumen methanogens

The majority of rumen archaea are methanogens and are necessary for maintaining the fermentation balance (Van Soest, 1982). Quantification of rumen methanogens also involves DNA isolation and qPCR. As increased methanogens is associated with increased methane emissions (Popova et al., 2013), the evaluation of the methanogen population structure and its influence on feed efficiency has been emphasized (Zhou et al., 2009; Zhou et al., 2011; Carberry et al., 2014a; Carberry et al., 2014b).

Evaluation of variation in methanogenic composition using detection of methanogenic species groups revealed less species variation and lower community diversity in efficient compared inefficient animals (Zhou et al., 2009; Zhou et al., 2011). However, evaluating total methanogen abundance showed no differences with feed efficiency (Zhou et al., 2009; Zhou et al., 2011; Carberry et al., 2014a). Evidence has also shown that relative abundance of total and specific methanogen species is associated with diet type (Carberry et al., 2014a).

Abundance of the bacteria genotype, Methanobrevibacter smithii, varies with differing diet energy, and is greater in inefficient compared to efficient animals regardless of diet treatment (Carberry et al., 2014b). As the most abundant methanogenic species in the rumen is Methanobrevibacter smithii, this suggests that diet composition has an influence on methanogenic population structure and abundance.

Research concerning feed efficiency and enteric methane emissions has received contrary reports suggesting efficient cattle have measurably lower (Fitzsimons et al. 2013) or higher methane emissions (Freetly and Brown-Brandl, 2013; McDonnell et al., 2016). However, further research is necessary to determine the energetic and microbial influence on methane emissions and its association with metabolic efficiency and diet composition.
Recent studies have proposed that microbial communities in efficient animals are highly related and have similar metabolic pathways that may affect feed efficiency (Guan et al., 2008; Zhou et al., 2009; Zhou et al., 2011). The involvement of the rumen microbiome as a component influencing the efficiency of feed utilization by microbial ecology at the species, strain, or genotype level in the rumen may contribute to the difference in efficiency of feed utilization (Myer et al., 2005) and methanogenic ecology (Zhou et al., 2011; Carberry et al., 2014b). Such techniques can be utilized to understand these variations in microbial ecology and their relationship with host factors to improve feed efficiency. Further analysis of the rumen microbial community in cattle in alternative animal populations and diets will contribute to the current knowledge of the rumen microbial community ecology and function.

2.4 Rumen volatile fatty acid profile

Approximately 70% of total metabolic energy in ruminants is derived from the rumen epithelial absorption of major VFA including acetate, propionate, and butyrate, which are absorbed and utilized by the gut, liver, and peripheral tissues (Bergman, 1990). The remaining VFA collectively contribute to a small percentage of total VFA and are utilized among the microorganisms and metabolized by the epithelium. Studies assessing associations of rumen fluid VFA profiles with feed efficiency use esophageal tubing for sample collection (Guan et al., 2008; Fitzsimons et al., 2013; Fitzsimons et al., 2014a), followed by gas chromatography analysis and internal standards to determine molar concentrations of VFA (Guan et al., 2008).

Higher concentrations of butyrate and valerate and a tendency for greater total VFA concentration and acetate have been found in efficient animals (Guan et al., 2008); in addition, the evaluation of microbial profile band patterns have revealed microbial clustering in efficient cattle suggesting an association of microbial clustering with greater VFA production or active fermentation (Guan et al., 2008). In contrast, Hernandez-Sanabria et al. (2010) found lower
butyrate and isovalerate concentrations in efficient cattle. Additionally, McDonnell et al. (2016) found efficient had lower propionate and greater acetate:propionate (AP) ratio when offered grass silage compared to inefficient heifers, while no differences in VFA traits were found between efficiency groups when on pasture or consuming a total mixed ration. Comparing rumen VFA profiles of cattle consuming different energy diets showed greater total VFA and lower isobutyrate concentrations when animals were consuming a higher energy diet (Hernandez-Sanabria et al., 2012). Steele et al. (2011) reported that greater dietary energy level resulted in higher ruminal butyrate concentrations. These studies provide evidence that VFA profile variation exists with divergent feed efficiencies (Guan et al., 2008; Hernandez-Sanabria et al., 2010) and that diet composition may also be a contributing factor to the variation in VFA concentrations (Steele et al., 2011; Hernandez-Sanabria et al., 2012; McDonnell et al., 2016).

Alternative studies have found no differences in VFA concentrations collected from rumen fluid post-feeding in divergent efficiency groups (Fitzsimons et al., 2013; Fitzsimons et al. 2014a); however, AP ratio tended to be lower for more efficient cattle (Fitzsimons et al., 2013), and decreased AP ratio is associated with reduced methane production (Russel, 1993). This trend is concurrent with Lawrence et al. (2011) where propionic acid tended to be higher, and AP ratio tended to be lower in efficient compared to inefficient heifers. Studies interested in the rumen epithelial metabolic activity found that VFA concentrations were not associated with ADG or DMI, but were associated with specific gene expression suggesting that metabolic activity within the rumen epithelium is associated with transport mechanisms for energetic substrates (Kern et al., 2016a). Further research is necessary to determine associations between VFA profile variation and microbial function, as well as metabolic pathways associated with rumen epithelial transport of energy substrates.
Current methods illustrate measures of the VFA profile at single time points, while RFI is calculated from a performance period, potentially adding bias to the data. Additionally, time of collection affects the VFA profile, as VFA concentrations are dependent on both diet composition and time after feeding (Warner, 1966; Bergman, 1990; Bevans et al., 2005), suggesting a standard collection time for assessment in relation to feeding is necessary. In addition, rumen fluid sampling using esophageal tubing is vulnerable to saliva contamination, and the sample representation of the whole rumen VFA profile can vary (Duffield et al., 2004). Therefore, methodology should additionally be considered for rumen fluid VFA profile assessments and may suggest why studies observe differences in results.

### 2.5 Rumen histomorphometry

The rumen exhibits high metabolic demand as energetic requirements for feed consumption and rumination account for up to 30% of the available metabolic energy (Susenbeth et al., 1998). Therefore, the energy metabolism of the gut may influence the whole animal energy utilization efficiency (Ortigues and Doreau, 1995). Greater average body energy expenditure is associated with higher relative visceral organ weights (Baldwin et al., 1980); and the reticulo-rumen empty weight of efficient cattle are known to be 8% lighter compared to inefficient cattle (Fitzsimons et al., 2014b). The rumen epithelium additionally acquires high tissue turnover (Baldwin, 1998) and facilitates multiple pathways for VFA absorption (Gäbel et al., 2002) illustrating its substantial metabolic demand.

Several studies have evaluated rumen papillae epithelial histomorphometry using light microscopy (Dobson, 1956; Lavker and Matoltsy, 1970) and studied the relationship of morphological changes with different diet compositions (Steele et al., 2011; Steele et al., 2012) and performance traits (Kern et al., 2016a). Steele et al. (2012) observed an increase in stratum corneum thickness of the ventral sac when consuming a high energy diet compared to a low
energy diet, illustrating the epithelial morphological adaption when shifting dietary energy level. A study assessing the lower-tract investigated changes in small intestinal crypt cellularity based on nuclei number, and proposed increased cellularity is associated with more feed efficient cattle (Montanholi et al., 2013b). This suggests increases in energetic demand and functional activity in the lower-tract is due to differences in feed efficiency (Montanholi et al., 2013b).

Kern et al. (2016a) reported that papillae morphological characteristics were not associated with feed intake or ADG. However, specific gene expression in epithelial tissue and VFA concentrations have been reported (Baldwin et al., 2012; Kern et al., 2016a; Kern et al., 2016b). Similarly, fed cattle are known to have increased mitotic index, VFA concentrations, and rumen papillae width compared to fasted cattle (Resende-Junior et al., 2006; Lima et al., 2015). These studies suggest performance traits and transport mechanisms for VFA may be related to the rumen epithelial metabolic activity.

The epithelial absorptive capacity of VFA is dependent on intraruminal parameters and absorptive surface area (Melo et al., 2013). Differences in epithelial metabolic demand, which is supported with cellular proliferation in the rumen epithelium (Tamate and Fell, 1977), may vary with differing metabolic efficiency.

2.5 Rumen pH and temperature

2.5.1 Rumen pH

The acid-base equilibrium is described by the Henderson Hasselbalch equation where dissociation and protonization of sodium bicarbonate and VFA causes fluctuations in the RpH (Kohn and Dunlap, 1998). The RpH and VFA concentrations are negatively correlated, and VFA is known to increase post-prandial which indicates that an increase in VFA production leads to a decreased RpH (Warner, 1966; Kimura et al., 2012). The RpH is additionally
influenced by substrate removal and bicarbonate buffering through saliva and saliva production and buffering rate shows gradual fluctuation with feed intake level (Dijkstra et al., 2012).

Fitzsimons et al. (2014a) revealed a lower RpH in inefficient cows compared to efficient cows, as well as a negative correlation between RpH and RFI when assessed through collection via oral tubing post-prandial. Contrastingly, several studies assessing RpH with feed efficiency using similar methodology revealed no differences in RpH (Lawrence et al., 2011; Lawrence et al., 2013; Fitzsimons et al., 2014b; McDonnell et al., 2016) between efficiency groups (Fitzsimons et al., 2013).

The RpH fluctuates due to accumulation of energy substrates and the microbial community composition, therefore the RpH may capture the metabolic differences in ruminal kinetic and fermentation parameters in feed efficient and inefficient animals (Fitzsimons et al., 2014a).

2.5.2 Rumen temperature

Rumen temperature is a measure of temperature variation due to heat of fermentation (Hungate, 1966). Studies have assessed RT as a predictor of RpH in beef cattle (Wahrmund et al., 2012; Mohammed et al., 2014) based on the concept that RpH fluctuates with ruminal fermentation activity, causing variation in heat of fermentation (Van Soest, 1982). Additionally, correlations between RpH and RT have revealed associations between maximum observed RT with minimum RpH in dairy cattle on high grain diets (Alzahal et al., 2009; Kimura et al., 2012) suggesting potential use of RT to represent the heat of fermentation from fermentation activity.

Previous research has shown that RT increases with increased frequency of feeding, and such patterns were associated with decreased RpH; in the same study, RT was not correlated with ADG (Greter et al., 2014). Further assessments are necessary to determine differences in RT with rumen fermentation parameters and variations in metabolic efficiency.
2.6 Definition and Purpose

It was hypothesized that the variability in rumen metabolism across feed efficiency phenotypes may be described through functional and structural rumen assessments. The objective was to characterize rumen functional and structural parameters, including microbial and VFA profiles, papillae histomorphometry, and RpH and RT in the context of feed efficiency in feedlot cattle.
CHAPTER 3: ASSOCIATIONS BETWEEN RUMEN FUNCTION AND
FEED EFFICIENCY IN BEEF CATTLE

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Abstract

Characterizing feed efficiency through ruminal parameters is fundamental to understand the efficiency of feed utilisation in the bovine. The objective was to feature feed efficiency by assessing microbial and volatile fatty acid (VFA) profiles, rumen papillae histomorphometry, and rumen pH (RpH) and temperature (RT). Forty-eight crossbred feedlot cattle underwent a productive performance test to determine residual feed intake (RFI). Rumen fluid was first collected and a logger for RpH and RT recording were inserted 5.5 ± 1 d prior slaughter using esophageal tubing. At slaughter, the logger was recovered and rumen fluid and rumen tissue were sampled. Rumen fluid was analysed for microbial and VFA profiles. Histomorphometric analyses determined the rumen papillae epithelial and stratum corneum width. The relative daily time spent in specific RpH and RT ranges were determined. Polynomial regression analysis was used to characterize RpH and RT circadian patterns. Animals were divided into efficient and inefficient groups based on RFI to compare productive performance and ruminal parameters. Rumen fluid bacteria concentration was higher (7.8x10^{11} v. 4.3x10^{11} 16s rRNA copy/ml) and methanogen concentration was lower in efficient compared to inefficient cattle (2.3x10^{9} v. 4.9x10^{9} 16s rRNA copy/ml). Additionally, comparison of rumen fluid VFA and microbiology profiles revealed increased acetate, and decreased propionate, butyrate, valerate, and caproate concentrations at slaughter compared to on-farm. Efficient cattle also revealed increased ventral blind sac papillae width at papillae middle (139.4 vs. 119.5 µm) and tip areas (148.4 vs. 125.0 µm) compared to inefficient cattle. Efficient cattle spent 4.1% more time daily in optimal RpH range compared to inefficient cattle (5.6<pH<6.0) (8.5 vs. 4.4%). The circadian patterns revealed lower RpH and no differences in RT at selected time points reflecting prior-, during, and post-prandial rumen function in efficient compared to inefficient cattle. Superior efficiency in cattle is linked to rumen features consistent with improved efficiency of feed utilization. The VFA concentrations and RT appeared to not influence the rumen metabolic
efficiency in this study; however, microbial abundance, papillae epithelial morphology, and RpH, may serve as indicators in differentiating feed efficiency in cattle. These findings can assist in improving current selection and nutrition programs and lead to financial and environmental benefits to the beef industry.

Keywords: histomorphometry, microbiology, residual feed intake, rumen pH, volatile fatty acids

**Implications**

Evaluating rumen functional and structural characteristics and their associations with feed efficiency is important to understand the rumen metabolism underlying efficiency of feed use. Our results revealed that feed efficient cattle have larger bacteria populations, smaller methanogen populations, thicker rumen papillae, and lower circadian rumen pH with extended time within optimal pH range. This evidence supports that feed efficient cattle have functional and structural rumen features consonant with improved feed energy utilization and rumen parameters may serve as indicators for feed efficiency, which may benefit genetic and nutrition programs and bring environmental and economic benefits to the beef industry.

**Introduction**

Improving feed efficiency can provide beneficial financial and environmental implications to agricultural systems, as the majority of greenhouse gases from agricultural production are due to cattle enteric emissions (Food and Agriculture Organization, 2014) and feed costs are a major cost in beef cattle farming. However, feed efficiency traits such as residual feed intake (RFI) lacks industry practicality. Despite RFI serving as an interesting concept to investigate
the biological basis underlying feed efficiency (Arthur et al., 2001; Montanholi et al., 2010; Gonano et al., 2014), few have evaluated associations between specific rumen functional and structural parameters with RFI until recently (Hernandez-Sanabria et al., 2012; Myer et al., 2015; McDonnell et al., 2016).

The production of volatile fatty acids (VFA) through microbial fermentation contributes to 75% of energy requirements in ruminants (Bergman, 1990). Additionally, greater VFA molar concentrations have been measured in efficient cattle (Guan et al., 2008). When considering total VFA concentration, differences exist among divergent efficiency groups (Hernandez-Sanabria et al., 2012). Evidence has shown that the majority of microbial profiles are similar among efficient cattle (Hernandez-Sanabria et al., 2012) and specific bacterial species are correlated with efficient steers (Guan et al., 2008). Additionally, studies have evaluated the rumen epithelial structure adaption with differing physiological state (Kern et al., 2016a). Such rumen functional parameters collectively contribute to the regulation of rumen pH (RpH) and temperature (RT) by microbial VFA production and epithelial absorption (Gäbel et al., 2002).

Therefore, it was hypothesized that the variability in rumen metabolism across feed efficiency phenotypes can be described through functional and structural rumen assessments. The objective was to characterize rumen functional and structural parameters including microbial and VFA profiles, papillae histomorphometry, and RpH and RT in the context of feed efficiency in feedlot cattle.

Materials and methods

Animal husbandry and experimental design

Experimental procedures followed the Canadian Council of Animal Care Guidelines (Canadian Council of Animal Care, 2009). A group of 48 crossbred beef cattle consisting of 32 steers (two lots of 16 steers) and 16 bulls were used. The average body weight (mean ±
standard deviation) for steers and bulls throughout the productive performance test were $476 \pm 65$ kg and $530 \pm 57$ kg, respectively. The average breed composition was 39.4% Angus, 44.9% Simmental and 15.7% other European breeds and crosses.

Each group of cattle was housed separately in indoor pens bedded with wood shavings and equipped with automated feeding stations (Insentec, BV, Marknesse, The Netherlands) with feed bunks filled at 0830 h daily at the Elora Beef Research Centre (University of Guelph). The as-fed ingredient composition of the diet was 52.5% high moisture ensiled corn, 42.3% haylage, 3.5% soybean meal, with premix composition described by Montanholi et al. (2009). The chemical composition was 53.8% DM, 13.9% CP, 22.2% NDF, 10.9% ADF, and 86.0% total digestible nutrients.

Animals underwent a 112 d productive performance test to determine DMI, body weight (BW), and ultrasound measurements for body composition determination, including ribeye area (REA), back fat thickness (BKF), and marbling score (MAR) (Montanholi et al., 2009). Rumen fluid was collected and RpH and RT data loggers were inserted $5.5 \pm 1$ d prior slaughter. Animals underwent transportation for approximately 30 min prior slaughter and were processed at $457.5 \pm 26$ d of age. At slaughter, rumen fluid and rumen sac tissues were collected and data loggers were recovered after evisceration.

**Residual feed intake determination**

Feeding events were individually summarized on a daily basis and records were screened for outliers and dates that were identified unfit for data use. Upper and lower limits for feed intake were applied on an individual basis to ensure specific inclusion of daily feed intake with $>98\%$ probability belonging to the normal distribution. The resulting individual daily feed intake records were then averaged and converted to a DM basis, which was used to calculate DMI and feed to gain ratio (FG; DMI/ADG).
Using the general linear model estimation described by Montanholi \textit{et al.} (2009), the following regression model was estimated and used to calculate RFI:

\[
DMI = 15.204 + 0.012(\text{ABW}) + 0.014(\text{ADG}) + 0.061(\text{REA}) + 0.354(\text{BKF}) - 0.917(\text{MAR}) + \text{subpopulation} + RFI
\]

The \(R^2\) for this model was 0.744. Average BW (ABW) and final-test body composition parameters were determined by a linear regression on days on test, with observations taken in 28 d intervals throughout the performance test. Individual intercepts and final-test values were calculated by computing the animals intercept and adding the slope for each trait multiplied by 112 d. The effects of the three subpopulations 32 steers (two lots of 16 steers) and 16 bulls were 0.00, -8.14, and -11.38.

\textit{Rumen pH and temperature}

Data loggers (T9 LRCpH, Dascor, Escondido, USA) for measuring RpH and RT were calibrated at pH 4.0 and 7.0 following the manufacturer protocol and programmed to record RpH and RT in 5 min intervals using the corresponding Data Logger Software (Version M5-v7.7.0, Dascor, Escondido, USA) prior to placement in the rumen. Briefly, animals were restrained in a squeeze chute and loggers were inserted aiming at the ventral sac using esophageal tubing and recovered at slaughter.

The RpH and RT data set was organized for average percentage time spent per day during specific RpH ranges (Nagaraja and Lechtenberg, 2007) including acidic (pH<5.6), optimal (5.6<pH<6.0), suboptimal (6.0≤pH<6.4), and high (pH≥6.4) RpH range; average RT during the RpH ranges were calculated. Additionally, overall time spent during RpH thresholds scaled from RpH 5.0 to 7.5 were graphed. The RpH value at the inflection point of the curve was determined by calculating the maximum and minimum value of the RpH threshold curve, and the corresponding slope was calculated using the line of best fit on the RpH threshold curve.
Rumen fluid and tissue sampling

Approximately 100 ml of rumen fluid was collected into a sterile cup (Specimen Container 4 oz, Fisherbrand™, Waltham, USA) 5.5 ± 1 d prior slaughter (on-farm samples) using an oro-ruminal probe (FLORA Ruminator; Profs-Products, Guelph, Canada), and within 1.98 ± 0.2 h after slaughter (at slaughter samples) by filtering digesta from the ventral sac using cheese cloth. The samples were submerged into liquid nitrogen, and kept at -80°C until microbial and VFA profile analysis. One 4 cm² sized tissue sample was excised from the ventral sac, ventral blind sac, and dorsal blind sac, then rinsed in PBS fluid and pinned to cardboard for fixation in 10% neutral phosphate buffered formalin. Then the rumen was emptied of digesta, separated from the other compartments, washed and weighed to determine empty rumen weight and empty proportional rumen weight as a percent of BW.

DNA extraction and qRT-PCR to determine microbial concentrations

Rumen fluid DNA extraction was performed following methods described by Guan et al. (2008). Briefly, rumen fluid samples were centrifuged (4°C, 19,000 x g for 5 min) with 0.5 g zirconium-silica beads (0.1 mm diameter). Then samples were homogenized using a bead-beating method (4,800 rpm for 3 min) (Mini Bead-Beater-8, BioSpec, Bartlesville, USA). Then DNA was purified (QIAamp Fast DNA Stool Mini Kit, Qiagen, Valencia, USA) and precipitated using ATE buffer and centrifuged (4°C, 16,000 x g for 1 min). Concentration and purity of DNA was measured at A260 nm and A280 nm wavelengths (Nanodrop 116 ND-1000 spectrophotometer, NanoDrop Technologies, Wilmington, USA). Extracted DNA samples were diluted to 10 ng/µl concentration and used as a template for quantitative PCR (qPCR) reactions to determine total bacteria, protozoa, fungi, and methanogen concentrations (16s rRNA gene number/ml rumen fluid). Using a fluorescent DNA stain and qPCR system (StepOnePlus qPCR system, Applied Biosystems, Foster City, USA), a fast and melting curve cycle program was used for quantifying microbial concentrations.
Rumen fluid volatile fatty acid profile analysis

Rumen fluid VFA concentrations for acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and caproate were analysed using methods described by Guan et al. (2008). Briefly, rumen fluid samples were centrifuged (0°C, 13,000 x g for 5 min). Using a gas chromatography (GC) vial, 25% phosphoric acid and internal standard was added. Samples were run using a GC system (Scion 456 GC with PVT injector and FID detector, Bruker, Bremen, Germany), which contained a GC column (30 m, 0.53 mm diameter, 210°C at 45°C/min) (Stabilwax®-DA Columns, Restek, Buckinghamshire, UK) with an injector and detector at 250°C. Then, VFA concentrations were quantified and concentration peaks were integrated for accuracy using chromatogram software (Compass Chromatography Data System, Bruker, Bremen, Germany). Rumen fluid samples were analysed in duplicate with repeats if duplicate values were >5% variation. The proportion of individual VFA to the total VFA concentration (µmol/ml; sum of individual VFA) was calculated.

Rumen papillae epithelium histomorphometry

Embedded rumen tissue was sectioned at 5 µm thickness and stained using hematoxylin and eosin. Using a light microscope (Primo Star light microscope, Zeiss, Oberkochen, Germany), microscope image capturing camera (AxioCam ERc 5s, Zeiss, Oberkochen, Germany) and software (AxioVision Rel. Version 4.8.2.0, Zeiss, Oberkochen, Germany), eight papillae from each of the three sacs of each animal were micrographed in three areas (Figure 1). Using an image processing software (ImageJ Pro Software, U.S. National Institutes of Health, Maryland, USA), the parameters measured were distance from connective tissue to outer stratum corneum (papillae width), and inner stratum corneum to outer stratum corneum (stratum corneum width). A total of four measurements of each parameter were performed on each micrograph consisting of two measurements on left and right sides of the papillae at 366.7 µm and 733.3 µm from the top of the image towards the centre on an 1100.0 µm height image (100X
magnification). The papillae width and stratum corneum width values were averaged in each rumen sac for individual animals (Figure 1).

**Statistical Analysis**

Data were analysed using SAS software v. 9.4 (SAS, Statistical Analysis System Institute, Cary, USA). Using the UNIVARIATE procedure variables were tested for normality based on the Anderson-Darling test, kurtosis, and skewness. Specific measures were transformed to reach normality using natural logarithm (DMI, acidotic RpH range, on-farm isovalerate, at slaughter isovalerate and caproate, and all microbiology variables), square root (at slaughter isobutyrate), reciprocal (RpH slope, ventral sac stratum corneum middle area width, and dorsal blind sac papillae base area width), and squared transformations (feed intake for circadian RT, and ventral blind sac papillae base area width). Data was back-transformed and presented with 95% confidence intervals.

The GLM SELECT procedure was used for model selection by identifying necessary fixed effects and covariates based on Bayesian information criterion values. Adjusted means were then determined and compared, across efficiency groups (feed efficiency groups contained the 50% most-feed efficient (efficient; n=24) and 50% least-feed efficient (inefficient; n=24) animals) using the selected model and the GLM procedure:

\[ Y_{ijkl} = \mu + \text{efficiency}_j + \text{subpopulation}_j + \text{breeds}_k + \epsilon_{ijkl} \]

where \( Y_{ijkl} \) is the trait of interest measured on the \( l \)th animal, belonging to the \( j \)th efficiency group, \( \mu \) is the overall mean for the trait, \( \text{efficiency}_j \) is the fixed effect of the \( j \)th efficiency group, subpopulation (individual trial populations) is the fixed effect of the \( j \)th subpopulation, breed is the fixed effect of the \( k \)th breed, where breed is the animals’ breed composition of the \( k \)th breed, and \( \epsilon_{ijkl} \) is the residual random effect associated with the \( l \)th animal.
A segmented polynomial was used to determine predicted curves for RpH and RT. Adjusted RpH and RT efficiency group means were determined on five-minute intervals using the MIXED procedure where covariates and fixed effects for RpH included: efficiency group, time, efficiency group x time, breed, and feed intake, and for RT included: efficiency group, time, efficiency group x time, breed, feed intake and feed intake x feed intake, where feed intake was included as a covariate to adjust for the influence of feed intake on predicted RpH and RT means.

According to the method of Munro et al. (2016), adjusted RpH and RT means were plotted and knots were selected at inflection points on each curve (RpH, 5 knots, RT; 7 knots, Figure 2) and used to calculate covariates at each time point. For both RpH and RT using the GLM procedure and the calculated covariates, segmented polynomials were estimated, and the selected (highest $R^2$) polynomial was used to predict RpH and RT curves for both efficiency groups. Detailed below are the selected segmented polynomials for RpH and RT:

$$\text{RpH}_{ij} = \mu + \text{efficiency}_i + \beta_1 T(i) + \beta_2 T^2(i) + \beta_3 Z_1(i) + \beta_4 Z_2(i) + \beta_5 Z_3(i) + \beta_6 Z_4(i) + \beta_7 Z_5(i) + \epsilon_{ij}$$

$$\text{RT}_{ij} = \mu + \text{efficiency}_i + \beta_1 T(i) + \beta_2 T^2(i) + \beta_3 Z_1(i) + \beta_4 Z_2(i) + \beta_5 Z_3(i) + \beta_6 Z_4(i) + \beta_7 Z_5(i) + \beta_8 Z_6(i) + \beta_9 Z_7(i) + \epsilon_{ij}$$

Where RpH$_{ij}$ and RT$_{ij}$ are the $j^{th}$ mean RpH and RT, recorded at time $T$ (time of measurement), within the $i^{th}$ efficiency group ($i$=efficient or inefficient cattle); $\mu$ is the overall trait mean; efficiency$_i$ is the fixed effect of the $i^{th}$ efficiency group and $\beta_1$ to $\beta_9$ are fixed regression coefficients on $T$ and $Z_1(i)$ to $Z_9(i)$ (for RpH) or $T$, $Z_1(i)$ to $Z_7(i)$ (for RT), within the $i^{th}$ efficiency group, where $Z_1(i)$ to $Z_9(i)$ were quadratic covariates calculated according to the equations described by Munro et al. (2016); and $\epsilon_{ij}$ is the random residual effect. Area under each RpH and RT curve was calculated using an on-line integral calculator (Integral Calculator, David Scherfgen, Bonn, Germany) and the difference between efficiency groups was determined.
Means at specific time points on the predicted RpH and RT curves were compared using a two-tailed t-test. Time point selection was based on the maximum and minimum value of inflection points, and the calculated mid-points in between. These time points represented RpH and RT prior-, during, and post-prandial (Figure 2). Results were considered significant when \( P < 0.05 \), and a trend approaching significance when \( 0.05 < P < 0.10 \). Multiple hypothesis tests adjustment tests were not performed, as the results were justified by biological evaluation.

Results

Productive performance traits

The overall mean ± SD and least square means of efficient and inefficient group productive performance traits are shown in Table 1. The DMI for efficient was 1.80 kg/d less than inefficient animals. Additionally, FG and DMI were lower for efficient compared to inefficient animals.

Rumen fluid microbial and VFA profiles

The overall mean ± SD and efficient and inefficient group least square means of rumen fluid microbiology traits for samples collected on-farm and at slaughter are presented in Table 2. The comparisons of microbial profiles revealed efficient had greater total bacteria and lower methanogen concentrations compared to inefficient cattle at slaughter. Microbial profiles on-farm showed no differences between efficiency groups. The overall mean ± SD and efficient and inefficient group means of the rumen fluid VFA profiles on-farm and at slaughter is displayed in Table 3, which showed no differences between efficiency groups.

Microbiology and VFA profiles on-farm were compared to profiles at slaughter, and the overall mean ± SD and means are shown in Table 4. Acetate was higher and propionate,
butyrate, valerate, and caproate concentrations were lower at slaughter compared to on-farm. Microbial profiles on-farm were not different from samples at slaughter.

Rumen structure and microstructure

The average empty rumen weight was 10.5 ± 1.8 kg where efficient and inefficient group means (upper, lower c.i.) were 10.6 (9.9, 11.2) and 10.3 (9.7, 11.0) kg (P=0.63), respectively. Additionally, the average empty proportional rumen weight was 3.5 ± 0.5% where efficient and inefficient group means 3.4 (3.3, 3.6) and 3.5 (3.3, 3.6) % (P=0.82), respectively. Therefore, no significant differences were observed between efficiency groups when comparing rumen weight traits.

Table 5 presents the overall mean ± SD and efficient and inefficient group means of each rumen sac assessed for base, middle, and tip areas for papillae and stratum corneum width. Observations from papillae width histomorphometry revealed that the ventral blind sac papillae middle and tip areas were greater in efficient compared to inefficient cattle. Additionally, the dorsal blind sac papillae width tip area tended to be greater in efficient compared to inefficient cattle (P<0.10).

Rumen pH and temperature

Table 6 displays the overall mean ± SD and efficient and inefficient group means for RpH and RT traits. Eight animals including 3 steers and 5 bulls, with RpH and RT loggers found in the reticulum were excluded from the dataset. When evaluating the daily time spent in RpH ranges, efficient spent 4.1% more daily time in optimal RpH range (5.6<pH<6.0) compared to inefficient cattle. There were no differences between efficiency groups when observing average RT during the RpH ranges.

Predicted curves of RpH (Figure 2A) and RT (Figure 2B) display that RT predicted means of efficient and inefficient groups prior-, during, and post-prandial did not differ. However,
RpH predicted means were lower for efficient compared to inefficient cattle at every time point selected for comparison. Observations of Figure 2 A show a decrease in RpH after major increases in feed intake in the morning and evening, followed by an increase overnight. Additionally, RT was observed to increase after major and minor increases in feed intake (Figure 2 B).

The difference in area under the RpH curve of predicted models including and excluding feed intake as a covariate was 0.26% for efficient and 0.07% for inefficient animals. Similarly, the difference in area under the RT curve was 0.03% for efficient and 0.01% for inefficient animals.

Discussion

Productive performance traits

This study evaluated associations between rumen function and structure with feed efficiency in feedlot cattle. Therefore, microbial and VFA profiles, papillae histomorphometry, and RpH and RT were characterized to evaluate the rumen metabolic differences between efficient and inefficient cattle. Cattle performance traits were evaluated, revealing a substantial difference of 1.80 kg/d in DMI between efficiency groups, illustrating the economic and socio-environmental importance of assessing efficiency in a commercial herd. Additionally, FG, DMI, and RFI results are consistent with previous studies on feedlot cattle (Arthur et al., 2001; Freetly and Brown Brandl, 2011).

Rumen microbiology

Rumen bacteria are the primary source of VFA production contributing to the majority of degradative activity (Van Soest, 1982) and were reported to have larger populations in efficient cattle in this study. Previous studies have identified specific bacteria groups that exist only in efficient cattle (Guan et al., 2008; Myer et al., 2015); proposing variation in rumen bacterial
traits exists with divergent efficiency. Our results suggest efficient cattle have larger bacteria populations which may assist with cellulosic substrate degradation and VFA production for host metabolic energy utilization (Van Soest, 1982).

When assessing microbial population band similarities between efficiency groups, protozoa and fungi showed no differences (Guan et al., 2008). Similarly, Carberry et al. (2010) found no differences in protozoa concentrations comparing beef heifers from different efficiency groups. However protozoa concentration was higher when increasing dietary energy consumption. Fungi concentrations are known to vary with high-fibrous diets, as they produce the most active fibrolytic enzymes (Wang and McAllister, 2002). The lack of association of protozoa and fungi with feed efficiency in this study may be due to their strong association with diet composition rather than efficiency of feed utilization.

Methanogenesis produces undesirable products including methane and CO$_2$ (Van Soest, 1982) and is associated with methanogen population size (Popova et al., 2013). This study revealed lower methanogen population size in efficient cattle. In contrast, previous studies have revealed no methanogen population size variation with feed efficiency (Zhou et al., 2009). However, evaluation of methanogen composition and population structure has shown differences at the methanogenic species level in inefficient cattle (Zhou et al., 2009). Additionally, changes in the microbial population structure can alter the microbial ecology and affect methane production (Popova et al., 2013). Our results suggest methanogen concentration variation exists among efficiency groups which may impact methane emissions; however, further insight on bacterial population structure is necessary to determine the impact on methane emissions (Popova et al., 2013).

Rumen VFA

Energetic products from microbial fermentation contribute to the majority of total metabolic energy in ruminants (Bergman, 1990). Fitzsimons et al. (2013) and McDonnell et
al. (2016) found no differences in VFA concentrations among efficiency groups which is consistent with the current study. In contrast, butyrate and valerate concentrations were higher in efficient compared to inefficient cattle (Guan et al., 2008). The microbial community adjustments to a diet are specific and influence the differences in VFA concentrations (Tajima et al., 2001). Additionally, research has shown blood plasma acetate concentration, which is influenced by the ruminal and endogenous pools, differ across physiological stages rather than efficiency groups in forage-fed heifers (Gonano et al., 2014). Further research on VFA across a range of diets is needed to further understand the possible associations between VFA and feed efficiency.

Rumen microbial and VFA profiles on-farm and at slaughter

Substantial differences in multiple rumen VFA concentrations were found comparing profiles on-farm and at slaughter. Commonly, cattle undergo extensive saliva polyrrhea during or post-transportation stress (Hogan et al., 2007). Additionally, studies have revealed increased VFA concentration post-transportation and throughout refeeding compared to non-transported cattle (Galyean et al., 1981). Stress responses may reflect poor VFA transport or absorption rates and increases in rumen fluid volume by saliva inflow in cattle under transport-stress and may be responsible for differences in VFA concentrations. Additionally, specific bacterial species concentrations vary post-transport stress (Xu et al., 2014), and total bacteria and protozoa concentration recovery post-refeeding is slower for post-transport compared to non-transport cattle (Galyean et al., 1981).

Acetate concentration was higher at slaughter compared to on-farm which may be due to the lack of morning feeding prior slaughter. The absorption of acetate tends to decline after feed restriction without complete withholding of feed (Zhang et al., 2013). Moderate feed restriction may impact epithelial absorption by reducing acetate-related metabolic pathways which acquire greater metabolic activity for transport and, therefore, reducing energy demand.
for epithelial transport (Aschenbach et al., 2009). Therefore, the rumen VFA profiles may be influenced by time of feeding and physiological responses relating to transport-stress.

**Rumen papillae histomorphometry**

The gastrointestinal tract acquires high metabolic function (Ortigues and Doreau, 1995). Studies comparing fed and fasted cattle have revealed increased rumen papillae width in fed cattle (Lima et al., 2015). In contrast, a lack of association between papillae morphology traits with ADG and average daily feed intake has been found. However, expression of genes has been correlated with VFA concentrations in the rumen suggesting a relationship between metabolic activity within the rumen epithelium and VFA production (Kern et al., 2016a). Montanholi et al. (2013b) revealed efficient have increased small intestine cellularity compared to inefficient cattle, suggesting improved efficiency is associated with increased metabolic demand in the lower-tract for greater growth per unit of feed intake.

Given that the rumen epithelium layers are abundant in mitochondria, contributing to the majority of the epithelial metabolic function (Baldwin, 1998), an increase in epithelial width suggests greater metabolic activity and functional activity in the papillae epithelium (Tamate and Fell, 1977). This study suggests an increased papillae width in efficient cattle, suggesting efficient cattle have higher epithelial metabolic activity, which is overcome by increased capacity for epithelial absorption.

**Rumen pH and temperature**

The RpH circadian pattern observed in this study is consistent with continuous RpH measurements in previous studies (Sato et al., 2012; Kimura et al., 2012). The difference in area under the curve for each of the predicted RpH and RT curves including and excluding feed intake were >0.5%. Therefore, this negligible difference supports that circadian RpH and RT
differences between efficiency groups are based on variation in metabolism, rather than differences in feed intake between efficient and inefficient cattle.

Studies have shown no differences in RpH between efficiency groups when measured by collecting rumen fluid orally prior- (McDonnell et al., 2016) and post-prandial (Fitzsimons et al., 2013) using an RpH meter; however, Fitzsimons et al. (2014a) revealed lower RpH in inefficient cattle from rumen samples post-prandial. In contrast, the current study revealed efficient cattle maintained a consistently lower circadian RpH prior-, during and post-prandial compared to inefficient cattle. The RpH results from this study propose that RpH may serve as an indicator for rumen metabolic efficiency and that superior efficiency may be related to enhanced feed utilization efficiency reflecting VFA production, leading to a lower RpH throughout the circadian period.

Efficient cattle of the current study also spent 4.1% more daily time in optimal RpH range compared to inefficient cattle. Specific bacterial species that primarily produce acidic products become dominant below pH 5.6, leading to reduced microbial activity and imbalanced fermentation (Nagaraja and Lechtenberg, 2007) suggesting RpH above 5.6 is more desirable for fermentation.

Evaluation of the circadian RT pattern was similar to continuous RT measures by Kimura et al. (2012). The RT predicted means comparison was not between efficiency groups at selected time points throughout the circadian pattern. Similarly, no differences were found when observing average RT during defined RpH ranges. The RT lacks a wide biological range and is known to increase due to the thermal increment of fermentation post-prandial, suggesting the circadian RT may be reflective of the increased thermogenic increment post-prandial due to increased fermentation activity (Walker and Forrest, 1964; Van Soest, 1982). However, continuous measurement of RT is reliable and precise, as the data was captured, despite the minimal RT biological range (Kimura et al., 2012).
Conclusion

The characterization of rumen functional and structural features has shown efficient cattle have larger bacteria, and lower methanogen concentrations, increased ventral blind sac papillae width, greater capacity to maintain optimal RpH, lower RpH throughout the circadian period, and no differences in VFA and RT compared to inefficient cattle. The rumen features of cattle with improved feed efficiency are associated with features supporting enhanced efficiency of feed utilization. This study additionally contributes to potential biomarkers for feed efficiency involving microbial abundance, papillae morphology and circadian RpH which are applicable to genetic and nutrition programs to improve the economic and environmental sustainability of beef cattle farming.

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CHAPTER 4: GENERAL DISCUSSION

Sustainable and efficient production systems are necessary for livestock sectors to meet global food demands (FAO, 2014). Determining practical biomarkers for feed efficiency can lead to improved metabolic efficiency, reducing feed costs and the environmental footprint of beef production (Arthur et al., 2004). To date, the relationship among rumen functional parameters and feed efficiency is conflicting (Hernandez-Sanabria et al., 2010; Hernandez-Sanabria et al., 2012; Carberry et al., 2012), and associations of rumen structural parameters with feed efficiency are lacking. It is suggested that the microbial community ecology (Guan et al., 2008; Hernandez-Sanabria et al., 2012; Myer et al., 2015), VFA profile (Guan et al., 2008; Hernandez-Sanabria et al., 2012) and RpH (Fitzsimmons et al., 2014a) is associated with divergent efficiency. Additionally, studies have revealed associations of rumen epithelial morphological adaptations to varying energy diets (Steele et al., 2011) and metabolic activity (Kern et al., 2016a). This study revealed associations of bacteria and methanogen abundance, papillae epithelial morphology, and circadian RpH with feed efficiency. Additionally, results suggest the VFA profile and RT were not associated with feed efficiency. Further studies are necessary to assess the associations between metabolic efficiency and diet composition on rumen functional and structural parameters. Results from this study have emphasized the importance of rumen functional and structural parameters for understanding the digestive biology underlying feed efficiency. Additionally, this study proposes potential biomarkers and contributes to the current knowledge of relationships between rumen fermentation parameters and feed efficiency.
CHAPTER 5: REFERENCES


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Table 1. Residual feed intake group (inefficient, efficient) means for productive performance traits, ultrasound traits and body weight (mean (95% confidence interval)).

<table>
<thead>
<tr>
<th>Traits (abbreviation; unit)</th>
<th>Mean ± SD</th>
<th>Inefficient</th>
<th>Efficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual feed intake (RFI; kg/d)</td>
<td>0 ± 1.3</td>
<td>1.0 (0.7, 1.4)</td>
<td>-1.0 (-1.4, -0.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Dry matter intake (DMI; kg/d)</td>
<td>9.19 ± 2.6</td>
<td>9.8 (9.1, 10.5)</td>
<td>8.0 (7.4, 8.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Feed to gain (FG; ratio)</td>
<td>5.51 ± 2.4</td>
<td>6.2 (5.7, 6.7)</td>
<td>4.8 (4.3, 5.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Backfat thickness (BKF; mm)</td>
<td>8.2 ± 2.9</td>
<td>8.0 (7.0, 9.0)</td>
<td>8.3 (7.3, 9.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>Rib eye area (REA; cm²)</td>
<td>92.7 ± 11.2</td>
<td>92.7 (89.7, 95.8)</td>
<td>92.8 (89.7, 95.8)</td>
<td>0.99</td>
</tr>
<tr>
<td>Marbling (MAR; score)⁴</td>
<td>8.2 ± 2.3</td>
<td>8.2 (7.4, 9.1)</td>
<td>7.8 (6.9, 8.6)</td>
<td>0.46</td>
</tr>
<tr>
<td>Mid-test body weight (kg)</td>
<td>304.2 ± 34.6</td>
<td>299.2 (286.2, 312.3)</td>
<td>309.2 (296.2, 322.2)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

⁴1=devoid and 11=abundant.

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Table 2. Residual feed intake group (inefficient, efficient) means for rumen fluid microbiology groups (mean (95% confidence interval)).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean ± SD</th>
<th>Inefficient</th>
<th>Efficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>On-farm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bacteria(^i)</td>
<td>5.7x10(^{11}) ± 5.8x10(^{11})</td>
<td>1.9x10(^{11}) (9.9x10(^{10}), 3.8x10(^{10}))</td>
<td>3.1x10(^{11}) (1.5x10(^{11}), 6.1x10(^{11}))</td>
<td>0.33</td>
</tr>
<tr>
<td>Methanogen(^i)</td>
<td>6.6x10(^{9}) ± 1.2x10(^{10})</td>
<td>3.4x10(^{9}) (2.1x10(^{9}), 5.4x10(^{9}))</td>
<td>2.4x10(^{9}) (1.5x10(^{9}), 3.8x10(^{9}))</td>
<td>0.31</td>
</tr>
<tr>
<td>Protozoa(^i)</td>
<td>1.6x10(^{8}) ± 3.5x10(^{8})</td>
<td>1.4x10(^{7}) (5.1x10(^{6}), 3.8x10(^{6}))</td>
<td>1.1x10(^{6}) (4.0x10(^{6}), 2.9x10(^{6}))</td>
<td>0.72</td>
</tr>
<tr>
<td>Fungi(^i)</td>
<td>9.3x10(^{4}) ± 3.0x10(^{5})</td>
<td>3.1x10(^{4}) (1.9x10(^{4}), 5.2x10(^{4}))</td>
<td>2.3x10(^{4}) (1.4x10(^{4}), 3.8x10(^{4}))</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>At slaughter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bacteria(^i)</td>
<td>7.3x10(^{11}) ± 6.2x10(^{11})</td>
<td>4.3x10(^{11}) (2.6x10(^{11}), 6.4x10(^{11}))</td>
<td>7.6x10(^{11}) (5.3x10(^{12}), 1.0x10(^{12}))</td>
<td>0.04</td>
</tr>
<tr>
<td>Methanogen(^i)</td>
<td>7.8x10(^{9}) ± 1.4x10(^{10})</td>
<td>4.9x10(^{9}) (3.0x10(^{9}), 8.0x10(^{9}))</td>
<td>2.3x10(^{9}) (1.4x10(^{9}), 3.8x10(^{9}))</td>
<td>0.04</td>
</tr>
<tr>
<td>Protozoa(^i)</td>
<td>5.8x10(^{9}) ± 2.8x10(^{9})</td>
<td>4.3x10(^{7}) (1.4x10(^{7}), 1.3x10(^{7}))</td>
<td>1.5x10(^{7}) (5.0x10(^{6}), 4.4x10(^{7}))</td>
<td>0.18</td>
</tr>
<tr>
<td>Fungi(^i)</td>
<td>4.2x10(^{4}) ± 1.2x10(^{5})</td>
<td>6.3x10(^{4}) (2.9x10(^{4}), 1.4x10(^{5}))</td>
<td>3.8x10(^{4}) (1.8x10(^{4}), 8.3x10(^{4}))</td>
<td>0.37</td>
</tr>
</tbody>
</table>

\(^i\)Unit = 16s rRNA copy number/ml rumen fluid.
Table 3. Residual feed intake group (inefficient, efficient) means for rumen fluid volatile fatty acids (mean (95% confidence interval)).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean ± SD</th>
<th>Inefficient</th>
<th>Efficient</th>
<th>P-value</th>
<th>Mean ± SD</th>
<th>Inefficient</th>
<th>Efficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-farm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate(^i)</td>
<td>49.4 ± 3.5</td>
<td>49.7 (48.3, 51.2)</td>
<td>49.1 (47.6, 50.5)</td>
<td>0.52</td>
<td>54.5 ± 6.8</td>
<td>54.4 (51.8, 57.1)</td>
<td>54.5 (51.9, 57.1)</td>
<td>0.97</td>
</tr>
<tr>
<td>Propionate(^i)</td>
<td>32.7 ± 5.1</td>
<td>32.4 (30.1, 34.6)</td>
<td>33.1 (30.9, 35.4)</td>
<td>0.63</td>
<td>29.2 ± 7.7</td>
<td>29.0 (25.9, 32.2)</td>
<td>29.3 (26.6, 32.5)</td>
<td>0.89</td>
</tr>
<tr>
<td>Isobutyrate(^i)</td>
<td>1.1 ± 0.3</td>
<td>1.1 (1.0, 1.2)</td>
<td>1.0 (0.9, 1.2)</td>
<td>0.28</td>
<td>1.1 ± 0.3</td>
<td>1.2 (1.0, 1.3)</td>
<td>1.0 (0.9, 1.1)</td>
<td>0.11</td>
</tr>
<tr>
<td>Butyrate(^i)</td>
<td>10.1 ± 2.3</td>
<td>10.2 (9.3, 11.1)</td>
<td>10.0 (9.1, 10.9)</td>
<td>0.76</td>
<td>8.8 ± 2.6</td>
<td>8.9 (7.7, 10.0)</td>
<td>8.6 (7.5, 9.8)</td>
<td>0.75</td>
</tr>
<tr>
<td>Isovalerate(^i)</td>
<td>2.7 ± 1.4</td>
<td>2.5 (2.2, 3.0)</td>
<td>2.4 (2.1, 2.9)</td>
<td>0.71</td>
<td>3.2 ± 1.7</td>
<td>3.0 (2.5, 3.6)</td>
<td>2.7 (2.3, 3.2)</td>
<td>0.41</td>
</tr>
<tr>
<td>Valerate(^i)</td>
<td>3.2 ± 0.97</td>
<td>3.1 (2.7, 3.5)</td>
<td>3.3 (2.9, 3.6)</td>
<td>0.53</td>
<td>2.7 ± 1.7</td>
<td>2.6 (2.1, 3.1)</td>
<td>2.9 (2.4, 3.4)</td>
<td>0.29</td>
</tr>
<tr>
<td>Caproate(^i)</td>
<td>0.7 ± 0.4</td>
<td>0.8 (0.6, 0.9)</td>
<td>0.7 (0.5, 0.9)</td>
<td>0.69</td>
<td>0.6 ± 0.5</td>
<td>0.4 (0.4, 0.5)</td>
<td>0.5 (0.4, 0.6)</td>
<td>0.40</td>
</tr>
<tr>
<td>Total VFA(^ii)</td>
<td>74.4 ± 15.7</td>
<td>73.8 (67.9, 79.9)</td>
<td>74.9 (68.8, 81.0)</td>
<td>0.79</td>
<td>79.2 ± 18.9</td>
<td>78.0 (70.4, 85.6)</td>
<td>80.3 (72.7, 87.9)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

\(^i\)Unit = percent proportion of the total concentration of volatile fatty acids.

\(^ii\)Unit = µmol/ml.
Table 4. Rumen fluid microbial and volatile fatty acid profiles collected on-farm and at slaughter (mean (95% confidence interval)).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean ± SD</th>
<th>Farm</th>
<th>Slaughter</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volatile fatty acid traits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate(^i)</td>
<td>51.9 ± 5.9</td>
<td>49.4 (48.0, 58.0)</td>
<td>54.5 (53.0, 55.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Propionate(^i)</td>
<td>31.0 ± 6.8</td>
<td>32.7 (30.9, 34.6)</td>
<td>29.2 (27.4, 31.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Isobutyrate(^i)</td>
<td>1.1 ± 0.3</td>
<td>1.1 (1.0, 1.2)</td>
<td>1.1 (1.0, 1.2)</td>
<td>0.63</td>
</tr>
<tr>
<td>Butyrate(^i)</td>
<td>9.4 ± 2.6</td>
<td>10.1 (9.4, 10.8)</td>
<td>8.8 (8.0, 9.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Isovalerate(^i)</td>
<td>3.0 ± 1.6</td>
<td>2.5 (2.2, 2.8)</td>
<td>2.9 (2.5, 3.2)</td>
<td>0.11</td>
</tr>
<tr>
<td>Valerate(^i)</td>
<td>3.0 ± 1.1</td>
<td>3.2 (2.9, 3.5)</td>
<td>2.7 (2.4, 3.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Caproate(^i)</td>
<td>0.7 ± 0.5</td>
<td>0.7 (0.6, 0.8)</td>
<td>0.5 (0.4, 0.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total VFA (µmol/ml)</td>
<td>76.8 ± 17.4</td>
<td>74.3 (69.5, 79.2)</td>
<td>79.3 (74.3, 84.0)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Microbiology traits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria(^{ii})</td>
<td>6.5x10(^{11}) ± 6.0x10(^{11})</td>
<td>4.1x10(^{11}) (2.8x10(^{11}), 5.7x10(^{11}))</td>
<td>5.8x10(^{11}) (4.2x10(^{11}), 7.7x10(^{11}))</td>
<td>0.13</td>
</tr>
<tr>
<td>Methanogen(^{ii})</td>
<td>7.2x10(^{9}) ± 1.3x10(^{9})</td>
<td>4.2x10(^{9}) (2.6x10(^{9}), 6.3x10(^{9}))</td>
<td>5.1x10(^{9}) (3.3x10(^{9}), 7.4x10(^{9}))</td>
<td>0.51</td>
</tr>
<tr>
<td>Protozoa(^{ii})</td>
<td>3.7x10(^{9}) ± 2.0x10(^{9})</td>
<td>1.2x10(^{7}) (6.0x10(^{6}), 2.5x10(^{7}))</td>
<td>2.5x10(^{7}) (1.2x10(^{7}), 5.1x10(^{7}))</td>
<td>0.15</td>
</tr>
<tr>
<td>Fungi(^{ii})</td>
<td>2.6x10(^{5}) ± 9.0x10(^{5})</td>
<td>2.7x10(^{4}) (1.7x10(^{4}), 4.2x10(^{4}))</td>
<td>4.9x10(^{4}) (3.2x10(^{4}), 7.7x10(^{4}))</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\(^{i}\)Unit = percent proportion of total VFA.

\(^{ii}\)Unit = 16s rRNA copy number/ml rumen fluid.
Table 5. Residual feed intake group (inefficient, efficient) means for histomorphometric measures of the rumen papillae epithelium (mean (95% confidence interval)).

<table>
<thead>
<tr>
<th>Trait (width; µm)</th>
<th>Papillae area</th>
<th>Mean ± SD</th>
<th>Inefficient</th>
<th>Efficient</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral sac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillae</td>
<td>Base</td>
<td>111.2 ± 12.3</td>
<td>110.1 (104.5, 115.4)</td>
<td>114.4 (108.0, 118.6)</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>121.1 ± 14.6</td>
<td>119.7 (113.8, 125.6)</td>
<td>122.0 (116.0, 128.0)</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Tip</td>
<td>121.5 ± 14.3</td>
<td>122.0 (115.7, 128.2)</td>
<td>120.9 (114.2, 127.6)</td>
<td>0.81</td>
</tr>
<tr>
<td>Stratum corneum</td>
<td>Base</td>
<td>36.0 ± 7.5</td>
<td>35.8 (32.4, 39.2)</td>
<td>36.0 (32.6, 39.4)</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>36.3 ± 11.1</td>
<td>33.5 (37.4, 30.3)</td>
<td>40.0 (37.9, 30.5)</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Tip</td>
<td>40.2 ± 6.7</td>
<td>40.4 (37.6, 43.3)</td>
<td>39.8 (36.8, 42.8)</td>
<td>0.75</td>
</tr>
<tr>
<td>Ventral blind sac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillae</td>
<td>Base</td>
<td>127.2 ± 23.4</td>
<td>110.1 (104.6, 115.4)</td>
<td>113.4 (108.0, 118.6)</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>129.1 ± 27.8</td>
<td>119.5 (107.8, 131.1)</td>
<td>139.4 (127.3, 151.5)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Tip</td>
<td>136.4 ± 25.0</td>
<td>125.0 (115.4, 134.7)</td>
<td>148.4 (138.4, 158.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stratum corneum</td>
<td>Base</td>
<td>40.5 ± 8.1</td>
<td>39.9 (36.6, 43.5)</td>
<td>41.1 (37.4, 44.8)</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>43.2 ± 8.6</td>
<td>41.3 (37.6, 45.0)</td>
<td>45.2 (41.4, 49.1)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Tip</td>
<td>42.9 ± 7.5</td>
<td>41.7 (38.5, 45.0)</td>
<td>44.2 (40.8, 47.6)</td>
<td>0.30</td>
</tr>
<tr>
<td>Dorsal blind sac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillae</td>
<td>Base</td>
<td>124.8 ± 13.7</td>
<td>120.5 (126.1, 115.3)</td>
<td>126.5 (132.5, 120.9)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>133.6 ± 15.7</td>
<td>135.1 (128.1, 142.1)</td>
<td>132.7 (126.3, 139.3)</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Tip</td>
<td>160.4 ± 20.8</td>
<td>154.5 (145.7, 163.3)</td>
<td>165.7 (157.3, 174.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>Stratum corneum</td>
<td>Base</td>
<td>41.3 ± 8.4</td>
<td>39.5 (35.9, 43.1)</td>
<td>43.1 (39.5, 46.6)</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>41.3 ± 7.3</td>
<td>40.4 (36.9, 43.8)</td>
<td>42.0 (38.9, 45.2)</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Tip</td>
<td>43.4 ± 7.7</td>
<td>41.7 (38.2, 45.2)</td>
<td>45.0 (41.7, 48.4)</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Table 6. Residual feed intake group (inefficient, efficient) means for rumen pH and temperature variables (mean (95% confidence interval)).

<table>
<thead>
<tr>
<th>Trait (unit)</th>
<th>Mean ± SD</th>
<th>Inefficient</th>
<th>Efficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RpH range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidotic (pH≤5.6)(^i)</td>
<td>5.5 ± 10.8</td>
<td>1.5 (0.2, 4.1)</td>
<td>4.2 (1.6, 7.9)</td>
<td>0.15</td>
</tr>
<tr>
<td>Optimal (5.6&lt;pH&lt;6.0)(^i)</td>
<td>6.6 ± 5.2</td>
<td>4.4 (2.0, 6.8)</td>
<td>8.5 (6.2, 10.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Suboptimal (6.0&lt;pH&lt;6.4)(^i)</td>
<td>13.2 ± 7.4</td>
<td>11.6 (8.3, 14.8)</td>
<td>14.0 (10.9, 17.1)</td>
<td>0.29</td>
</tr>
<tr>
<td>High (pH≥6.4)(^i)</td>
<td>75.0 ± 18.4</td>
<td>80.3 (71.5, 89.2)</td>
<td>70.5 (62.1, 78.9)</td>
<td>0.11</td>
</tr>
<tr>
<td>RT average during RpH range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidotic (pH≤5.6)(^i)</td>
<td>39.9 ± 0.5</td>
<td>40.1 (39.8, 40.4)</td>
<td>39.8 (39.6, 40.0)</td>
<td>0.16</td>
</tr>
<tr>
<td>Optimal (5.6&lt;pH&lt;6.0)(^ii)</td>
<td>39.7 ± 0.4</td>
<td>39.7 (39.5, 40.0)</td>
<td>39.7 (39.4, 39.9)</td>
<td>0.92</td>
</tr>
<tr>
<td>Suboptimal (6.0&lt;pH&lt;6.4)(^ii)</td>
<td>39.6 ± 0.3</td>
<td>39.6 (39.5, 39.8)</td>
<td>39.7 (39.5, 39.8)</td>
<td>0.69</td>
</tr>
<tr>
<td>High (pH≥6.4)(^ii)</td>
<td>39.6 ± 0.3</td>
<td>39.6 (39.5, 39.8)</td>
<td>39.6 (39.5, 39.8)</td>
<td>0.92</td>
</tr>
<tr>
<td>RpH threshold curve traits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflection point</td>
<td>7.0 ± 0.6</td>
<td>7.1 (6.8, 7.4)</td>
<td>6.9 (6.6, 7.2)</td>
<td>0.43</td>
</tr>
<tr>
<td>Slope</td>
<td>1574.5 ± 139.7</td>
<td>1271.6 (1047.1, 1618.1)</td>
<td>1162.9 (980.4, 1428.6)</td>
<td>0.53</td>
</tr>
</tbody>
</table>

\(^i\)Average % time spent per day in RpH ranges.
\(^ii\)Average temperature during RpH ranges.
Figure 1. Longitudinal rumen papillae micrographs for histomorphometry.

Base, middle, and tip areas (A); Base (B); Middle showing methodology used to perform papillae and stratum corneum width measurements (C); and Tip (D).
Figure 2. Rumen pH and temperature circadian pattern graphs.

Predicted rumen pH (RPH, A) and rumen temperature (RT; °C, B) curves of efficient (solid line) and inefficient (dotted line) cattle and feed intake (DM basis; kg) (broken line) over circadian period (hh:mm). The selected knots used to generate predicted curves are listed within the Figure.
Lines marking RpH and RT curves indicate time points where efficient and inefficient predicted means were compared. Asterisk (*) show significant ($P<0.05$) difference between efficiency group means.