Evaluation of indwelling pH monitoring technologies and growing strategies for beef cattle

by

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B.S Eastern Kentucky University, 2021

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Approved by:

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Abstract

A series of experiments were conducted to evaluate different technologies currently utilized within the cattle industry to continuously and remotely measure ruminal pH, limit-feeding high energy rations on newly received cattle performance and digestion. Subacute ruminal acidosis (SARA) is the predominant digestive disorder affecting feedlot cattle. Despite the prevalence and economic loss, there is still debate about the exact timing, pH range, and time spend under that curve that induces SARA. Historically, measuring ruminal pH has been conducted through digesta samples via cannulation, stomach tube, or aspiration of the ventral sac. A disadvantage to all these methods is the invasiveness and disruption of normal cattle behavior to collect samples. Therefore, in Exp 1. we evaluated two different indwelling monitoring boli that continuously measure ruminal pH. We compared using smaXtec boli (SMAX; smaXtec Inc., Graz, Austria), Moonsyst boli (MOON; Moonsyst International, Kinsale, Republic of Ireland), and a handheld pH meter (METER; PH8500 pH/mV Meter, Apera, Columbus, OH) over 126 d in vivo, ex vivo in known standards, and during an ex vivo acidosis challenge. Eight ruminally cannulated crossbred heifers were utilized in a complete randomized design through 6-consecutive 21-d periods. Ruminal pH measurements obtained from MOON were lesser ($P \leq 0.01$) than METER in every period. As time progressed the difference between the two became larger ~ 84 to 126 d (treatment × period, $P < 0.01$). Conversely, measurements of pH with SMAX were greater ($P < 0.01$) than METER from periods 1 to 3 and 6. In a known pH standard of 7, MOON again yielded measurements lesser than 7 and SMAX yield measurements greater 7 (treatment × period, $P < 0.01$). However, in a standard at lower pH of 4, MOON and SMAX measurements were not different ($P \geq 0.13$) from pH 4 until period 6 (treatment × period, $P = 0.02$). During the acidosis challenge, the degree of change in pH before

and after the addition of vinegar was measured using MOON, SMAX, and METER. The change in pH obtained using METER was considered the true change in pH. MOON yielded measurements similar (P = 0.36) to METER while SMAX measurements were greater (P < 0.01) compared with METER. In Exp 2. we evaluated the effects of increasing dietary energy while restricting intake on growth performance, digestion, and fermentation characteristics in growing beef heifers. In Exp 2.a 70-day growing study was conducted to determine the effects of energy concentration and dry matter intake on growth performance, digestion and fermentation characteristics of newly received growing beef cattle. A total of 392 crossbred heifers (initial bodyweight = 274 ± 26 kg). Heifers were blocked by weight (4) and assigned to 12-13 head pens; pens were randomly assigned to 1 of 4 dietary treatments. Experimental treatments included a high-roughage diet fed for ad libitum intake (AL) or a limit-fed high-energy diet fed at 75% (LIM75), LIM80 (80), or 85% (LIM85) of AL intake within block. Treatments were designed to equalize energy intake between calves assigned to AL and LIM75. In Exp 3, eight ruminally cannulated, crossbred heifers were utilized in a 4 × 4 Latin square to determine digestibility and ruminal fermentation characteristics of the diets from Exp. 2. Heifers were utilized in four consecutive 15-d periods. Final BW and ADG were similar (P = 0.37) between AL and LIM75, and greater ($P \leq 0.05$) in LIM80 and LIM85 compared with AL. By design, dry matter intake was greater ($P \leq 0.01$) in AL heifers compared to all 3 limit fed treatments. As a results feed conversion was improved by 30% ($P \leq 0.01$) in limit fed heifers compared with AL and did not differ ($P \geq 0.20$) among LIM75, LIM80, or LIM85. Dry matter digestibility was 10%, 5%, and 4% greater in among LIM75, LIM80, and LIM85, respectively, compared with AL. Organic matter, neutral fiber, and acid fiber digestibility were also greater in limit-fed treatments compared to AL. A treatment × hour interaction ($P < 0.01$) was observed for ruminal pH. Limit fed calves reached pH nadir earlier compared with AL with LIM75 being lowest, LIM80 intermediate, and LIM85 being greatest. Total ruminal VFA concentrations were unaffected by treatment ($P = 0.34$); however, there was a tendency ($P = 0.08$) for propionate concentrations to be intermediate and greater in LIM80 and LIM85 heifers, respectively, compared with AL and LIM75. Ruminal ammonia concentrations were greater ($P < 0.01$) in AL compared to limit fed treatments and experienced three different peak concentrations 2, 8, and 18 h post feeding compared with limit fed treatments singular peak 2 h post feeding (treatment × hour; $P < 0.01$). Based on our data in Exp 1, there is still variation in pH measured obtained via indwelling pH boli. Both MOON and SMAX experienced drift over time, however, during a simulated acidosis challenge MOON detected changes in pH similar to METER. Conversely, ruminal pH measurements over a 24 h period using SMAX were more closely related to METER. Based on results in EXP 2 and 3, limit feeding higher energy diets that include corn coproducts does not negatively influence growth performance or fermentation products. Subsequently, limit feeding improved total-tract digestibility and feed efficiency compared to a traditional receiving ration.

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Acknowledgements

I would not have been able to complete my Master's thesis without the help of many great people. The decision to pursue graduate school has had plenty of challenge but also has afforded me memories I will keep forever.

I would like to thank Dr. Dale Blasi for allowing me to continue my education with him and being a valued mentor throughout my time at Kansas State. Dr. Evan Titgemeyer has allowed me to visit his office countless times with questions I thought unanswerable. I owe thanks to Bill Hollenbeck for his opinions and expertise approaching research and cattle care.

I owe my committee members, Drs. Tyler Spore, AJ Tarpoff, and Tiruvoor Nagaraja my deepest thanks for their guidance, listening to my countless questions, and knowledge they imparted to me. Thank you to Dr. Zach Duncan for being a great mentor, editor and most importantly a very good friend. Additionally, thank you to Cole Ellis and Macie Weigand for always lending a helping hand and the friendship they have given me over these last two years.

Finally, I would like to thank my family and Hailey Weaver, without their encouragement and love I would not have even made it to graduate school. I am grateful for my time at Kansas State University and will hold the memories and knowledge I obtained here with me forever.

Chapter 1 - Review of Literature

The productivity and health of ruminants are significantly mediated by their digestive physiology; the microbial population within the rumen plays a critical role in the host's ability to convert feedstuffs into metabolizable nutrients for the animal. In cattle, disruption of the microbial population through digestive disorders such as sub-acute ruminal acidosis (SARA) can reduce dry matter intake which leads to reduced growth performance, decreased milk production, or death (Hernandez et al., 2014, McCann et al., 2016, Nagaraja and Titgemeyer 2007). Subacute ruminal acidosis is the primary contributor to digestive deaths in feedlots and accounts for 30 to 40% of digestive deaths (Galyean and Rivera, 2003). Additionally, SARA is a large economic concern and costs the dairy industry $500 million to $1 billion annually (Donovan, 1997). In the feedlot industry, a calf that experiences an sub-acute acidotic event takes on average 4 days to recover; costing cattle feeders an extra money in feed, treatment, labor, and in the event of death (Malafaia et al., 2016). Understanding the exact timing, causative agents, and physiological impacts associated with SARA are critical for accurately diagnosing and preventing SARA (Villot et al., 2018). The need for such understanding has led to the development of technologies that continuously monitor ruminal pH; however, direct comparisons of these technologies *in vivo* have not been evaluated. Understanding how ruminal pH measurements are determined using these technologies compared to each other could potentially help diagnose and prevent SARA during potential acidotic events.

Additionally, management of newly received lightweight calves in backgrounding/stocker operations often poses challenges for feeders and nutritionist. Cattle can be shipped up to 6 times in their lives (Cernicchiaro et al., 2012). The stress caused by transportation, co-mingling, pathogen exposure, dehydration and starvation generally results in low dry matter intake on arrival, increased morbidity, and reduced performance. One strategy producers can use to combat low dry matter intake upon arrival is to limit feed a high-energy diet. Limit-feeding can be particularly useful in feeder operations where newly received, lightweight cattle consume inadequate amounts of dry matter to meet nutritional demands, exacerbating metabolic/pathogen diseases caused by the marketing and shipping stress complex. However, with limit-feeding, a large concern lies with higher rates of fermentable carbohydrates; primarily corn leading to an increase in metabolic and respiratory diseases in newly received cattle (Lofgreen et al., 1975; Richeson et. al., 2019 & Riveria et al., 2005). The addition of corn coproducts such as wet corn gluten feed and distillers grains could reduce the risk of ruminal acidosis that results from feeding large amounts of starch because these co-products are low in starch but high in fermentable fiber (Spore et al., 2019; Montgomery et. al., 2003).

The purpose of this review is to explore the role ruminal pH has on the rumen environment and function. Additionally, to discuss techniques and technologies that could improve how we measure ruminal pH as well as discuss the impacts of limit-feeding growing cattle on performance, digestion and health.

Regulatory effects of ruminal pH

Ruminant animals' ability to convert feeds into energy and protein relies predominantly on anaerobic fermentation within the ruminoreticulum. The microbial population within, including bacteria, protozoa, fungi and archaea, possess the ability to convert structural and non-structural carbohydrates found in plant materials into usable energy and protein sources other animals cannot (Perez et al., 2024). Many factors help regulate the rumen microbiome but perhaps one of the largest regulatory factors is ruminal pH. Ruminal pH is affected by diet, intake, environment, and the animal's intrinsic buffering capabilities and varies from 5.6 to 7.2

under normal physiological conditions (Nagaraja and Lechtenberg, 2007). Variations in ruminal pH can lead to shifts in the microbiome, digestive physiology, and metabolism products.

Microbial changes at lower pH

The rumen is an ideal habitat to host microbial populations because of the constant temperature, anaerobic environment, access to substrate and relatively constant pH. However, many microbial populations are susceptible to changes in ruminal pH and may have increased cell lysis at lower pH. The most susceptible microbial groups in the rumen to low pH are fibrolytic bacteria, lactic acid-utilizing bacteria, protozoa and methanogens.

Fibrolytic bacteria include those that digest cellulose, hemicellulose, and pectin. Cellulolytic bacteria are an important component involved in fiber digestion because cellulose constitutes 35 to 55% of the lignocellulose biomass (Benatti and Polizeli, 2023). The primary cellulolytic bacteria in the rumen include *Fibrobacter succinogenes, Ruminococcus albus, and Ruminococcus flavefaciens* (Hua et al., 2022). At lower pH volatile fatty acids (VFAs) become more dissociated and can move freely across the cell membrane. To counteract this, acid-tolerant bacteria lower their intracellular pH to maintain a small pH gradient and prevent intracellular accumulation of VFAs. However, cellulolytic bacteria are more susceptible to lower pH because they cannot regulate their own intracellular pH and the increased pH gradient leads to anion toxicity (Russell and Wilson, 1996).

Stewart (1977) observed changes in cellulolytic bacterial population and subsequent disappearance of rolled cotton fiber (RC) *in vitro* utilizing rumen contents from forage fed sheep. Three treatments were applied to measure the cellulolytic activity of bacteria; 100 ml of RC with pH of the culture maintained at 6.6, 100 ml of RC with 2.5 g of added barley with pH maintained at 6.6, and 100 ml RC + 2.5 g barley without pH control.

When compared to RC in pH controlled; RC+ barley in the pH-controlled culture reduced RC disappearance by 4%, this reduction could be attributed to the inhibitory effects displayed by saccharolytic bacteria on cellulolytic bacteria through nutrient competition and enzyme production. In the culture where pH was not controlled, pH fell to 5.2 and the disappearance of RC decreased from 31.8% to 0.5% compared to pH-controlled RC. Cellulolytic bacterial concentrations decreased from 10^7 to 10^2 but total anaerobes in the rumen increased from 8.2 to 8.6 (log, 10 CFU/ml). Comparable results were observed by Hoover (1986) where cellulose digestion was reduced linearly as pH decreased from 6.5 to 5.0 *in vitro*. Both studies indicated that cellulose digestion ceases completely once pH of contents had reached 5.3 or less.

A quantitative analysis of cellulose degradation and activity in ruminants conducted by Russell et al. (2008) also determined that cellulose digestion can occur as long as pH remains above 5.4; but is subsequently reduced and the rate of reduction is dependent on pH, bacterial growth rate, and ruminal flow rate.

Bacterial populations are not the only microbial populations highly affected by ruminal pH. Ciliated protozoa account for 40 – 60% of the microbial cell mass in the rumen and account for 40 – 60% of total VFA production. Protozoa are more beneficial in grain fed diets compared to forage fed diets because they slow down starch fermentation through starch encapsulation and bacterial predation (Franzolin and Dehority, 2010). However, ciliated protozoa are sensitive to changes in ruminal pH and cannot survive at the lower pH which often occurs in grain fed animals. Complete defaunation in cattle has been reported when fed diets contained ≥ 60% concentrate inclusion (Dehority, 2005; Dehority and Orpin, 1997).

Hook et al. (2011) reported changes in ciliated protozoa concentrations in dairy cows rapidly transitioned from a hay-based diet to a high-grain diet. Average ruminal pH was 6.4 for cows on the hay-based diet; whereas ruminal pH averaged < 5.6 for 4.6 h and reached a minimum of 5.25 for cattle transitioned to the grain-based diet. Subacute ruminal acidosis occurs when ruminal pH ranges from 5.5 – 5.0 for 5 hours or more, suggesting the cows used in the study nearly reached sub-acute acidotic levels. After one-week, ruminal pH stabilized when the high-grain diet was fed, suggesting cattle can adapt to lower pH in the rumen along with microbial populations. When fed the hay diet, protozoal concentrations averaged 3.88×10^6, subsequently, when cows were transitioned to the high-grain diet, protozoal concentrations decreased to 1.77×10^7. However, by the third week of the grain fed diet, protozoal concentrations returned to similar concentrations during the hay diet suggesting protozoa may be able to adapt to lower pH more effectively than cellulolytic bacteria.

In contrast, Franzolin and Dehority (2010) observed lower protozoa populations in 4 cannulated steers in diets containing 50%, 65%, and 100% corn inclusion. The steers were fed a 50% corn diet for 6 weeks, transitioned to a 65% corn diet for 4 weeks, and fed a 100% corn diet for 6 weeks. Populations of ciliated protozoa on average decreased by 70% to completely defaunated in all 4 steers and populations never recovered to week 1 concentrations.

When pH is reduced in the rumen, even though fibrolytic bacterial and protozoal populations decrease, the total microbial population in the rumen typically increases. More acid-tolerant, saccharolytic bacteria such as *Megasphera elsdenii, Streptococcus bovis, and Selenomonas ruminantium begin* to dominate the ecosystem. It is evident that there are other factors affecting microbial populations at lower pH and there is high variation from animal to animal. However, pH has been demonstrated to be one of the largest regulatory factors that affect microbial populations and can shift the products created in the rumen.

Effects of pH on digestion and fermentation products

Ruminal pH has profound effects on the microbial population in the rumen and thus is associated with changes in digestion and fermentation products. Fermentation of carbohydrates produces organic acids that readily decrease pH if not absorbed or metabolized, conversely, fermentation of protein results in ammonia that can increase pH. Depressed ruminal pH for prolonged periods of time has been shown to negatively affect apparent digestibility, feed intake, and shift ratios of VFAs produced (Dijkstra et al., 2012).

The rumen environment experiences a diurnal pattern in pH and fermentation products following meal consumption. This pattern is due to the production and absorption of organic acids produced through microbial fermentation. This is affected by intake, diet composition, and individual animal variation. Typically, as meal frequency decreases, fluctuations in VFA production become larger which leads to larger fluctuations in pH (Robinson, 1987). As more feed is offered, cattle meal behavior becomes less aggressive, and meal size is reduced but more constant.

Rumsey et al. (1989) observed changes in VFA concentrations and pH based on diet and intake. A high-roughage diet or a high-concentrate diet was fed at 0.5, 1.0, 1.5, or 2.0 % of BW (DM basis) to Holstein steers. As intake increased in both diets, pH decreased. Minimum ruminal pH for both diets occurred when feed was offered at 2.0% BW; however, minimal pH was greater for the high roughage diet (6.0) compared with the high grain diet (5.6). However, the diurnal variation in pH was more variable in the lower intake treatments, reaching minimum values between 4 – 6 h post-feeding. Subsequently, the greatest VFA concentrations occurring 4 – 6 h post-feeding as well. An inverse relationship was observed for pH and VFA concentrations; as pH decreased in both diets, VFA concentrations subsequently increased. As pH decreases in the rumen, VFAs become dissociated and can move across the rumen epithelium without needing a proton exchange which leads to greater VFA absorption.

This increase in VFA concentrations in Rumsey et al. (1986) at lower pH contradicts findings from Erfle et al. (1982) who evaluated the effects of pH on fermentation and protein degradation in a continuous culture. A difference in the studies is the degree of pH change. When pH is reduced between 5.6 to 5.1, VFA concentrations are higher due to the increase in pH resistant saccharolytic bacteria that rapidly ferment carbohydrates into VFAs. However, when pH declines further most bacteria are lysed leaving primarily lactic acid producers behind which causes lactate to accumulate in the rumen (Nagaraja et al., 1998). Erfle et al. (1982) observed as pH decreased from 7 to 5, total VFA concentration decreased from 80 mmoles/d to 50 mmoles/d. The reduction in VFA concentrations was driven by a decreased production in both acetate and butyrate. Acetate and butyrate are primarily produced by fibrolytic bacteria which are more susceptible to changes in pH. A subsequent increase in propionate and valerate was observed as pH decreased. Saccharolytic bacteria such as *Megasphera elsdenii* and *Streptococcus bovis* are more active at lower pH which likely contributed to increased concentrations of ruminal propionate and valerate. Increased lactate concentration was observed as well, most likely because lactate producing bacteria survive at lower pH while lactate utilizing bacteria are more pH susceptible (Russell and Dombrowski, 1980). Ammonia concentrations decreased from 5.9 mM to 0.2 mM as pH decreased from 7.0 to 5.0. Reduction in protein fermentation was a result of a 90% and 80% reduction in deaminase and protease as pH decreased, respectively. Proteolytic bacterial counts were reduced as pH reached 5.0 contributing to the reduced protein degradation and ammonia production as well.

The reduction in VFAs and ammonia concentrations is often the result of impaired diet digestibility at lower pH. It is well established that depressed pH causes concurrent reductions in fiber digestibility due to reduced fibrolytic bacteria populations and competition with saccharolytic bacteria (Franzolin and Dehority, 2010; Hoover, 1986). Shriver et al. (1986) observed that as pH decreased from 7.0 to 5.8 total organic matter, neutral detergent fiber, and nitrogen digestibility was reduced. Similarly to Erfle et al. (1982), acetate and ammonia reduced linearly as pH decreased with a subsequent increase in propionate, lactate, and valerate.

The diurnal variation in pH can be volatile in cattle, and likely does not impair diet digestibility or ruminal characteristics so long as time spent at pH under 5.6 remains less than 5-6 h/d (Nagaraja and Lechtenberg, 2007). Cattle can adapt to lower ruminal pH overtime without severe consequences, but a transition period must be established to avoid impaired rumen performance or digestive upset.

Ruminal Acidosis in Feedlot Cattle

Ruminal pH is not only a regulatory factor for digestion products and fermentation characteristics but can be a causative agent for numerous metabolic/digestive disorders when values fall outside physiological ranges. Digestive disorders are the second leading cause of death in feedlots, understanding the causative ranges and causes of depressed ruminal pH in these disorders is paramount for clinicians, feeders, and nutritionist. Ruminal acidosis is the most common digestive disorder in feedlots and is defined by the accumulation of organic acids in the rumen beyond physiological ranges (Nagaraja and Lechtenberg, 2007). The severity of acidosis is categorized into acute and subacute. Acute acidosis results from the accumulation of lactic acid in the rumen and depresses ruminal pH <5.0 and is also referred to as lactic acidosis. Subacute ruminal acidosis results from accumulations of VFAs in the rumen depressing pH to

<5.6 – 5.0 and is more commonly observed in feedlots and dairies (Nagaraja and Titgemeyer, 2007).

Acidosis is generally caused by over-feeding or inadequate adaptation of fermentable carbohydrates. Additionally, feedlot cattle are at increased risk of acidosis because of inadequate secretion of saliva resulting in subsequent bicarbonate reduction, reducing buffering capacity of the rumen. Acute acidosis is accompanied by reduced dry matter intake, dehydration, weight loss, and death. Additionally, there is correlation that acidosis is metabolic catalyst for additional disorders in cattle such as laminitis, polioencephalomalcia, and liver abscesses (Kleen et al., 2003). However, clinical signs are often not observed in SARA but harmful systemic changes in the rumen epithelium and microbial population are observed (McCann et al., 2016). Often at the onset of an acidotic event, VFA concentrations increase because as pH decreases VFAs become more pronated. Linder et al. (2024) observed greater total VFA concentrations 12 to 32 h post-feeding in finishing steers facing an acidotic challenge compared to non-acidotic control steers. The advantage of increased energy from free absorption of VFAs can be quickly diluted by shifts in the microbial population towards lactate production further lowering pH and the increased fluid secretion into the rumen to counteract the increased osmolality (Huber, 1976).

Brown et al. (2000) observed a reduction in dry matter intake in steers that were experimentally induced with acute and subacute acidosis. Dry matter intake recovered in subacute acidotic steers 3 days after the challenge; however, acute acidotic cattle did not return to previous intake until 10 days after the challenge. The reduction in intake represents not only a health concern for the animals but a larger economic loss associated with extended days on feed when animals have an acidotic event.

One acidotic event is enough to catalyze systemic changes in the rumen that alter absorptive capabilities and protective barriers. Steele et al. (2011) observed when cows were rapidly transitioned to a high-grain diet, rumen papillae stratum basale, spinosum, and granulosum layers and epithelial depth were reduced within the first week. Papillae in the rumen are the primary organelle to increase surface area and absorptive capacity of VFAs and ammonia; when they are damaged, absorptive capacity can be permanently damaged which can result in reduced performance. The epithelium acts as protective barrier shielding the microbial population in the rumen from escaping into the bloodstream into the rest of the body. When epithelium is damaged by acidotic events the integrity weakens catalyzing disorders such as rumenitis, liver abscess, and laminitis.

Both lactic and subacute ruminal acidosis can cause harmful systemic changes to the rumen which may inhibit future rumen function. Understanding the minimum ruminal pH, time spent under that value, clinical signs and treatments/preventions can help lower the risk for acidosis in finishing cattle. The economic loss represented by acidosis is not well defined in beef cattle but can be estimated by increased days on feed, treatment costs, and deaths. By utilizing management techniques like forage or byproduct inclusion, bunk management, and introducing monitoring technologies, producers can reduce the economic risk associated with SARA

Methods for measuring ruminal pH

Experimentally evaluating ruminal pH requires adequate methods to collect ruminal digesta. Different techniques and technologies have been developed for sampling ruminal contents in both intact and fistulated animals. Each method of ruminal digesta collection comes with advantages and disadvantages; the three primary collection methods are via ruminal cannulation, stomach tube passed into the ventral sac, and rumenocentesis. Variation in pH and fermentation products has been noted when utilizing different methods. In commercial settings, rumen collections are often used as diagnostic criterion for acidosis. Historically, collections via stomach tube or rumenocentesis are utilized because of the ability to collect contents from an intact animal. Experimental approaches to observed digestion, fermentation characteristics, and pH often utilized ruminal cannulation. More recently, indwelling pH monitoring technologies have been developed to monitor rumen pH continuously in both commercial and experimental settings.

Pathak et al. (2024) compared pH of rumen fluid collections between cannulated animals and a stomach tube. When contents were collected via stomach tube, the first 200 ml of contents were discarded to minimize contamination of saliva from the esophagus. Contents were strained through cheesecloth and pH was measured using a handheld meter, pH on average was 0.63 units greater in stomach tube contents compared with cannulated collections. Generally, the higher pH in stomach tube collects contents is attributed to degrees of saliva contamination ranging from 2 to 30% (Dirksen and Smith, 1987); however, another possibility is a stomach tube may not be able to penetrate past compacted feedstuff beyond the cranial/dorsal sacs. Conversely, collection via cannula can be collected from every region in the rumen, possibly providing a more representative sample.

Unfortunately, cannulation is not realistic in commercial settings. Rumenocentesis is another method used to measure ruminal pH in commercial settings. To accomplish this, ruminal contents are aspirated from the ventral sac. The first portion of contents are typically discarded due to possible saliva contamination. This method offers advantages over stomach tubes and cannulation because it is inexpensive, quick, less invasive, and requires few instruments. Nordlund and Garrett (1994) compared pH measurements using a guided stomach tube and rumenocentesis collections. Ruminal pH of contents collected via stomach tube averaged 1.1 units greater than rumenocentesis contents (6.66 vs 5.56, respectively). Variation within different rumen sacs exist and contents towards the ventral side of the animal often have lower pH than dorsal sections (Soltis et al., 2023).

Because of the variation in pH measurements and workload associated with rumen content collections, indwelling probes have been developed to monitor rumen pH continuously. These boli are administered with a balling gun into the reticulum and continuously monitor pH for 90 to 120 days and are widely used in the dairy industry. Typically, digestive disorders in beef cattle such as ruminal acidosis occur during the receiving and transition period because cattle are being adapted to high-concentrate diets. The ability to measure pH continuously during these periods could provide real time diagnosis and prevention of ruminal acidosis. However, there is variation in pH measurements determined using these indwelling technologies similar to other collection methods.

Klevenhusen et al. (2014) observed variation in pH measurements determined by using indwelling boli (BOLI), free rumen liquid (FRL), and particle associated rumen liquid (PARL). FRL was collected via rumenocentesis of the ventral sac and PARL was collected from the hay mat in the rumen via cannula. Ruminal pH was greatest in FRL, intermediate in BOLI, and least in PARL. Analysis of the concordance correlation coefficient indicated high agreement between FRL and BOLI but low agreement between PARL and BOLI. This suggests a low precision in measurements from BOLI when predicting PARL measurements. While FRL is often used more for diagnostics and measurements, the PARL contains most of the solid feed within the rumen and contains the primary site for fiber digestion. Therefore, monitoring the pH of the PARL is an important characteristic in measuring the extent of fiber digestion because fiber digestion is heavily dependent on pH.

To further explore the variation in pH measurements, Sato et al. (2012) compared indwelling boli measurements to hand grab samples collected from the same location in the rumen were boli were located. There was a high correlation between the boli, and hand grab samples and mean pH values only differed by 0.14 (6.36 hand grab vs 6.22 boli). Cows used in that study were subjected to acidotic challenge where pH values reached a minimum of 5.5. Measurements from the boli and hand grab samples at lower pH still maintained a high degree of accuracy suggesting the boli could be used to measure pH in animals fed different diets and at different rates.

The variation associated with different sampling techniques in collecting ruminal pH can create challenges for clinicians, researchers, and feeders in diagnosing, preventing, and studying cattle’s digestive physiology. However, the continued improvement of technologies that can continuously and accurately measure ruminal pH could improve future methods. There still is variation in the effectiveness and accuracy of each sampling method and technology.

Newly Received Growing Cattle Diets

Lightweight receiving cattle have been purchased by producers for decades as an economic choice to maximize profit of cattle before entering the feedlot. The receiving calf can pose risk to producers and the management of health and efficiency of these animals is essential when developing a sound production model. Typically, receiving cattle face stressors in the form of transportation, dehydration/starvation, pathogen exposure, weaning, and diet adaptation (Nagaraja et al., 1998; Hoerlein and Marsh, 1957; Galyean et al., 1981). Receiving calves travel on average 698 km, with a maximum of 3087 km (Cernicchiaro et al., 2012). These cattle vary in source, management, age, and weaning status making health management challenging following transport. As a result, dry matter intake (DMI) upon arrival is typically low. Hutcheson and Cole (1986) reported that average DMI for a 240 kg steer transported Texas, ranged from 0.5 to 1.5% of body weight during the first 7 days of the feeding period. The stress encountered during the marketing process combined with the low feed intake often results in calves entering a negative energy balance early in the feeding period. This can make formulating rations that meet animals' nutritional requirements for maintenance and growth difficult when intakes are below 1.5% and sporadic eating occurs. Therefore, metabolic and respiratory challenges arise when cattle that are already immunologically challenged from transportation do not meet their nutritional demands and cannot use the appropriate amount of energy to mount an immune response. A strategy in receiving diets to mitigate low feed intake early in the feeding period is to increase the energy density of the diet by replacing roughage with cereal grains and co-products.

Effect of forage and energy concentrations on health and performance

In typical receiving diets, forage is often used to help mitigate digestive disorders and promote intakes without over feeding energy (Lofgreen et al., 1975). Predominantly, in younger calves with developing rumens, forage stimulates rumen papillae which increases blood flow and surface area and promotes absorption of fermentation end-products (NASEM, 2016). A survey of consulting nutritionists reported that receiving diets typically contain 30% or more roughage and 30 to 40% processed grains (DM basis; Samuelson et al., 2016). One challenge associated with feeding a diet that contains 30% or more roughage to receiving cattle is that feed intake upon arrival is often low. Low feed intake combined with feeding a low-energy diet may create situations where receiving cattle do not consume enough feed to meet their energy and protein requirements. An additional challenge associated with feeding high-roughage diets is the cost of roughage. The season-average farm price for hay in 2022 increased 16% over the past 9 years; additionally, 46% of hay growing acres experienced drought (USDA, 2022). While feed reports may differ geographically and annually, the trends for feed forage price and availability are similar, making it difficult for producers to have a steady, cost-effective supply of forage.

A meta-analysis conducted by Rivera et. al. (2005) used 6 studies in a regression analysis to determine the relationship between dietary energy density and DMI, average daily gain (ADG), and BRD morbidity. For every 20% increase in dietary roughage, BRD morbidity was reduced by 1.35%; however, ADG was reduced by 0.18 kg. Subsequently, as concentrates increased in rations, DMI and ADG increased. Similarly, Lofgreen et al. (1975) conducted a series of experiments to evaluate the effects of shipping/marketing stress and energy density in diets on receiving calf performance and health. One hundred seven calves were transported 1989 km from Southeast Texas to El Centro, California. Calves were fed 1 of 4 diets formulated to provide 1.01, 1.10 or 1.20 Mcal NEg/kg DM with levels of concentrate increasing across the diets from 55, 72, and 90% by replacing portions of alfalfa with rolled barley. Average daily gain increased as the energy density of the diet increased from 1.01 to 1.10 Mcal NEg/kg; however, ADG was similar for calves fed diets formulated to contain 1.10 or 1.20 Mcal NEg/kg. Additionally, BRD morbidity was 14% and 10% greater in calves receiving the 1.20 Mcal NEg/kg compared with the 1.01 and 1.10 Mcal NEg/kg treatments, respectively. Overall, Lofgreen et al. (1975) concluded that even though feed conversion was slightly improved for cattle consuming the 1.20 Mcal NEg/kg DM ration compared to the 1.10 Mcal NEg/kg ration, the reduction in morbidity and similar ADG observed in the 1.10 ration made 1.10 Mcal/NEg/kg DM the more favorable energy concentration and concluded diets with concentrates as the primary energy source should not exceed 1.10 Mcal NEg/kg in receiving diets.

Conversely, Spore et al. (2019) conducted a trial that evaluated the effects of programmed feeding high-energy diets on health and performance of growing cattle. Three hundred fifty-four crossbred heifers (214 ± 4 kg) were purchased from auction markets throughout the Southeast U.S and transported 1,086 km to Manhattan, Kansas. In that study, four diets with increasing dietary energy (0.99, 1.10, 1.21 or 1.32 Mcal NE_g/kg DM) were offered to provide an estimated gain of 1 kg/d. To achieve similar weight gains, DMI for the 1.10, 1.21, and 1.32 treatments were fed at 95%, 90%, and 85% of the 0.99 treatment DMI, respectively. By design, DMI decreased linearly as the energy density of the diet increased. Because ADG did not differ among treatments, gain: feed increased linearly from 0.142 to 0.174 which resulted in a 22% improvement in feed conversion. Additionally, respiratory morbidity ranged from 11% to 13% for heifers fed the 0.99 and 1.32 Mcal NEg/kg DM rations but did not differ among treatments.

However, Spore et al. (2019) energy intake across diets was not fully equalized. Net energy intake above maintenance was 3%, 9.5% and 18% greater in the limit-fed treatments, respectively, compared to the ad libitum treatment. Because gains were targeted producing similar bodyweights the efficiency of the limit fed treatment was greatly improved. However, further studies utilizing equalized net energy intake is warranted.

Scilacci et al. (2024) compared growth performance and behavior of cattle fed two rations; a high-roughage diet (0.99 Mcal NEg/kg DM) for ad libitum intake or a high-energy diet (1.32 Mcal NEg/kg DM) limit fed at 85% of the ad libitum cattle's intake. The authors utilized 409 crossbred heifers (279 kg ± 24 kg) for an 84-day feeding period. ADG was 15% greater and DMI was 22% lower compared to the ad libitum cattle resulting in a 47% improvement in gain: feed in limit fed cattle. This contrasts with findings from Spore et al. (2019), however, by design

ADG was programmed to be similar in that study whereas the current study had 14% greater intake of dietary of NEg. Net energy intake above maintenance was 1.1% greater in limit-fed calves compared to ad libitum, which likely contributed to the improved gain. Additionally, the ad libitum diet yielded 0.68 Mcal NEg/kg DM, and the limit-fed diet yielded 1.01 Mcal NEg/kg DM based on calf performance. These values were lower than the formulated 0.99 and 1.32 Mcal NEg/kg DM, respectively and was greater in the limit-fed diet. The authors noted the study was conducted during a hot and wet summer in Kansas and thus environmental factors could have affected how the heifer's metabolized energy. This compounded with the increase dietary energy intake in the limit fed calves could explain the differences in gain and efficiency. Finally, heifers experienced low morbidity and did not differ between treatments with 0.5% and 1.0% of cattle being treated once for respiratory illness in the ad libitum and limit fed treatments, respectively. Differences in morbidity from the three studies possibly could have stemmed from distance traveled with Lofgreen et al. (1975) calves traveling nearly twice the distance than heifers from Spore et al. (2019) or Scilacci et al. (2024). Additionally, digestive disorders like ruminal acidosis are often linked as the primary etiological factor in increase morbidity when feeding higher amounts of carbohydrates (Nagaraja and Lechtenberg, 2007). It is possible Lofgreen et. al. (1975) and other early research evaluating the effects of dietary energy concentrations fed to receiving cattle on respiratory morbidity could have misdiagnosed ruminal acidosis because the clinical signs are both similar. In more recent studies, diet composition has shifted into utilizing diets with high-fiber, low-starch coproducts that help reduce the risk of digestive upset. Lofgreen et. al. (1975) rations utilized rolled barely as the primary energy source while Spore et al. (2019) and Scilacci et al. (2024) utilized diets that contained 40% wet corn gluten feed to have comparable energy contributions from digestible fiber similar to starch from corn.

Balancing forage and energy concentrations with health and performance is complex paradigm for nutritionists. The use of corn co-products and improved feeding strategies has reduced the risk associated with higher-energy diets fed to lightweight receiving cattle. By balancing intake with energy concentrations, morbidity can be reduced without sacrificing performance of newly received growing cattle. The addition of energy sources that primarily consist of fermentable fiber have lowered the incidences of digestive upset associated with high grain feeding and possibly lowered the misdiagnoses of respiratory disease for digestive disorders.

Energy Sources

Typical cattle total mixed rations rely on concentrates as the primary energy source because of the high-energy density and total digestible nutrients they provide. The most common concentrates are cereal grains due to the constant supply chain and readily fermentable starch content. Cereal grains are high in starch which is readily fermented by microbial digestion in the rumen and produce strong organic acids and VFAs. Cereal grains range in starch content from 57-77% with wheat being the greatest, whole corn intermediate, and oats the least (Huntington et al., 2006). Over-feeding rapidly fermentable carbohydrates can lead to digestive disorders such as ruminal acidosis and rumenitis. Balancing their inclusion to minimize digestive risk while maximizing animal efficiency is paramount for nutritionists and feeders.

The U.S. produces 15.36 billion gallons of ethanol annually (RFA, 2022). Because of increased ethanol production, corn co-products have become a popular choice for energy and protein sources in cattle rations because of the fermentable fiber and cost competitiveness with cereal grains. Of coproducts, distillers' grains and wet corn gluten feed account for 83% of coproducts used in beef rations (Samuelson et al., 2016). In ethanol production, corn is combined with yeast and fermented to produce alcohol and CO_2. Once the alcohol is separated, the solid corn mash remains as whole stillage. The whole stillage is then centrifuged creating distiller's grain (DG) and solubles. The grain can be sold wet as wet distiller's grain (WDG) or dried into dried distiller's grain (DDG). Dried distillers allow for a more shelf stable feedstuff that can be trucked further distances without spoilage. The solubles can be added back to the distiller's grain to create distillers grain plus solubles (DGS; Stock et al., 2000). Due to corn's high starch content, the removal of starch during the distilling process increases protein, fat, phosphorus, and neutral detergent fiber approximately 3-fold (Stock et al., 2000). Because most of the protein in corn is undegradable in the rumen, the protein content in DG can escape rumen microbes and be utilized by the host later in the G.I. tract. The increase in protein, fat and digestible fiber represents an energy value to be 120% to 174% the value of dry-rolled corn for finishing cattle (Klopfenstein, 1996; Farlin, 1981).

Corn gluten feed (CGF) is obtained via the wet corn milling industry that produces oil and syrups from corn (Weigel et al., 2008). The wet milling process separate starch, oil, protein and bran from the kernel through soaking in sulfurous acid. The steep liquor from the process contains protein, vitamins, and minerals from the corn kernel. The swollen kernel is then ground, and the starch is separated from the bran (fiber). The resulting steep liquor and bran are mixed creating wet corn gluten feed (WCGF) and can be dried similar to DDG creating dry corn gluten feed (DCGF). Corn gluten feed has increased protein, fiber, phosphorus, and sulfur content compared to corn grain, but the exact nutrient concentrations can have wide variation (Weiss et al., 1989). The feeding value of CGF is greater in high-roughage diets because it supplies energy and protein to the diet without compromising fiber digestibility reported in diets where corn provides a majority of energy (Blasi et al., 2001). Incorporating WCGF at 30% of DM in rations can help reduce the incidences of subacute acidosis while still allowing for a reduction in roughages (Krehbiel et al., 1995). Associative effects could elevate the response observed in performance and health when the coproducts are fed together in a ration (Loza et al., 2010).

In Kansas, the average price of DDG and WCGF is $173/ton and $24/ton, respectively. In contrast average price of whole corn and soybean meal is $177/ton and $350/ton, respectively (USDA, 2024). The cost competitiveness of corn by-products compared with traditional energy and protein sources makes co-products a viable option for feeders to supplement protein/energy without increased feed cost. Replacing portions of roughage or corn with coproducts so that contributions from protein and fiber to energy are similar to those of starch from corn can increase animal profitability and productivity.

Diets utilizing coproducts

Feeders utilize different corn processing methods to improve efficiency of starch utilization by rumen microbes from the corn kernel. Methods exist to alter corn through heat, moisture, time, and reduction in particle size (Huntington, 1997). Additional corn processing may increase the risk for metabolic upset if processed grains are over consumed by cattle. Pairing extensively processed corn rations with coproducts could improve the feeding value of coproducts while additionally reducing the risk of acidosis.

Scott et al. (2003) observed changes in feedlot steer performance in diets containing wet corn gluten feed (WCGF) and corn processed using different methods. Five dietary treatments were formulated; dry rolled corn (DRC) with or without WCGF, steam flaked corn (STC) with or without WCGF, or whole corn (WC) with WCGF. Diets containing WCGF included WCGF at 32% of diet DM and diets without WCGF included soybean meal to ensure diets were isonitrogenous and provided similar amounts of RDP. Dry matter intake was 14% greater in WC

+ WCGF steers compared to the average of all other treatments. Additionally, DMI was on average 9% greater in DRC + WCGF steers compared with the other treatments aside from WC + WCGF. Gains were greatest among steers fed DRC + WCGF, STC + WCGF, and WC + WCGF, intermediate in STC, and least in steers fed only DRC. As a result, G: F was least in WC + WCGF compared to other treatments and greatest feed conversion in STC + WCGF and STC. Dietary net energy for gain concentrations determined based on growth performance was 12% lesser in WC + WCGF compared to the average of other treatments and greatest in DRC, STC + WCGF, and STC. Incorporating WCGF into finishing diets increased passage rate in finishing steers (Montgomery et al., 2004). Thus, by including WCGF diets that contain less processed corn would have less time spent in the rumen and less ruminal starch digestion, leading to decreased feed conversion and limited hindgut digestion of starch by the animal.

Loza et al. (2010) evaluated the effects of WDGS and WCGF fed individually or together in finishing diets on growth performance and carcass characteristics. Five dietary treatments were created utilizing dry-rolled and high-moisture corn as the primary energy sources. Treatments included a control diet with 0% WDGS or WCGF (CON), 30% WGCF inclusion (30WGCF), 30% WDGS inclusion (30WDGS), 15% WDGS and 15% WCGF blend (30BLEND), or a 30% WDGS and 30% WCGF blend (60BLEND) DM basis. Average daily gain was greatest in 30WDGS and 30BLEND, intermediate for 30WCGF, and least in CON and 60BLEND. Intakes were 9%, 5%, and 6% greater in 30WCGF, 30WDGS, and 30BLEND compared with CON, respectively. Dry matter intake was similar between CON and 60BLEND. Each ration was formulated to meet NRC requirements for energy and protein, therefore, additional protein provided by the coproducts may have been used towards energy. Protein provides 36% more energy than carbohydrates (Huntington and Archibeque, 1999).

Montgomery et al. (2003) conducted experiments to evaluate the effects of alfalfa hay and WCGF combinations on growing steer performance in limit-fed diets. Two hundred twenty crossbred steers (262 kg) were used in a complete randomized block design to evaluate optimum concentrations of alfalfa hay (AH) in limit-fed diets containing steam-flaked corn (STC) and WCGF. The control diet included 0% WCGF, 20% AH, and 65% STC (DM basis). The remaining three diets included 40% WCGF with increasing concentrations of AH (0,10,20%) with AH replacing portions of STC. Growth performance and feed conversion decreased linearly AH in the diet increased. Net energy for gain decreased as roughage concentrations increased which contributed to the differences in growth performance. Additionally, net energy and growth performance were greater in steers fed 0% AH compared with the control diet which contained no WCGF. This suggests that the energy value and fiber content of WCGF alone in a corn-based ration improved performance over any combinations with AH.

These results agree with the findings from Scott et al. (2003) where diets containing >30% of WCGF and STC as the primary contributions to energy increased performance in growing cattle. Optimum inclusion of coproducts based on cattle performance is between 30 to 40% DM (Vander Pol et al., 2006; Klopfenstein et al., 2008). Including co-products can help improve cattle performance, mitigate digestive upset, and reduce feed cost when fed at modest inclusion demonstrated by the research above. However, when inclusions increase above 40% diminishing returns can be seen and feeding co-products in tandem may not always be beneficial.

Limit-feeding

Historically, feeding cattle for ad libitum intake is thought to improve performance by allowing the animal to eat to its full potential (Rossi et al. (2001). However, maximum efficiency has been observed lower than ad libitum intake and could have associated economic and performance advantages (Clark et al., 2007; Knoblich et al., 1997; and Sainz et al., 1995) Limit feeding could provide an opportunity for increased efficiency and help start calves in the receiving period transition to the growing/finishing phase more readily. Limit-feeding or programmed feeding involves restricting an animal's intake based on net energy calculations to target a specific rate of gain or based on a percentage of the animal's bodyweight (Galyean et al., 1999). Improvements in energy utilization, reduced feed and manure waste, and subsequent compensatory gains when entering the feedlot have been observed in cattle that were limit-fed compared with those fed for ad libitum intake (Loerch and Fluharty, 1998; Scilacci et al. 2024; Schoonmaker et al., 2004; Wagner and Mader, 1990). However, reductions in subsequent carcass merit, increased morbidity, and rate of gain have also been noted (Felix et al., 2011).

Performance

Schoonmaker et al. (2003) conducted experiments to analyze the effects of energy source, intake, and grain concentrations on weaned feedlot steers (~119 d of age) performance and carcass characteristics. Four experimental diets were formulated to include a high-fiber diet fed for ad libitum intake (ALFIBER), a 70% concentrate diet fed for ad libitum intake (ALCONC), or a 70% concentrate diet programmed fed at 0.8 kg/d (0.8CONC) or 1.2 kg/d (1.2CONC). Experimental diets were fed from d 119 to 217 of the growing period. Following the growing period, steers were transitioned to a common finishing diet until harvest.

At the end of the growing phase, DMI was least for steers fed 0.8CONC and 1.2CONC compared with steers fed ALCONC and ALFIBER, whose intakes did not differ. Because ADG was targeted at specific rates, BW was greater for steers fed for ad libitum intake compared with steers fed 0.8CONC and 1.2CONC. In addition, BW was greater in ALCONC compared with ALFIBER. As a result, feed conversion was greater for 1.2CONC and 0.8CONC compared with ALCONC and ALFIBER. Average daily gain during the finishing phase was similar among treatments, however, steers that were limit-fed during the growing period averaged 30.5 more days on feed compared with those fed for ad libitum intake because steers were finished to identical fat thickness.

During the finishing phase, DMI did not differ among treatments suggesting limit feeding during the growing period did not negatively influence DMI during the finishing phase. The cost savings associated with improvements in feed conversion that were observed for steers that were limit fed during the receiving period were likely diluted by the extended days on feed. At harvest, no differences were observed in marbling, quality grade distribution, or yield grade distributions between ad libitum and limit-fed steers.

This agrees with findings in a second experiment conducted by Schoonmaker et al. (2003) where steers were fed identical diets throughout the growing and finishing phase similar to the previous experiment. However, instead of finishing steers based on fat thickness, steers were fed 273 days so no difference in days on feed were observed. Average yield grade was slightly improved for steers fed for ad libitum intake compared with limit-fed steers (2.8 vs 2.6, respectively). However, yield grade distribution, marbling, dressing percentage, and quality grade were all similar between steers fed for ad libitum intake and limit-fed steers. The increased performance from limit-fed steers was likely due compensatory growth experienced during the finishing period.

Wagner and Mader (1990) (a) evaluated the effects of limit-fed high energy diets on the growth performance of growing feedlot steers. One hundred ninety steers (251 kg) were arranged in a 2 x 2 factorial that evaluated feeding strategy (ad libitum high-roughage diet vs. limit-fed high energy diet) and roughage source (corn silage vs. alfalfa hay). This created four dietary treatments; limit fed with corn silage (LIMCS), limit fed with alfalfa (LIMA), ad libitum with corn silage (ALCS), or ad libitum with alfalfa (ALA). Limit-fed steers were programmed fed to achieve a gain of 1 kg/d and the diet was formulated to provide 1.24 Mcal NEg/kg DM. Diets offered for ad libitum intake were formulated to provide 1.04 Mcal NEg/kg DM to support daily gains of 1 kg/d. Average daily gains were greater in steers fed for ad libitum intake compared with limit-fed steers. By design, DMI was greater for steers fed for ad libitum steers; however, G: F was greater in limit-fed steers compared with steers fed for ad libitum intake. Average daily gain greater than 1 kg/d in both feeding strategies which likely resulted from higher DMI than anticipated in ad libitum steers. For limit-fed steers, daily feed allowance was increased in cases of extreme wind chill to ensure nutrient requirements were met for growth; however, these increases may have been too large. Improvement in feed conversion likely resulted from an 11% increase in the efficiency metabolizable energy (ME) utilization in limit-fed steers compared with ad libitum steers.

A similar study was conducted by Wagner and Mader (1990) (b) who evaluated the effects of energy concentration and intake on growth performance and carcass characteristics. Two diets were formulated to be limit-fed (71% concentrate) or fed for ad libitum intake (23% concentrate). Limit-fed steers were restricted to 80% of the ad libitum steer’s intake and were fed for a 77-d growing phase, with a subsequent 28 d step-up and 45 d finishing phase. During the first 12 d of the step-up period, limit-fed cattle were maintained on the same diet and intakes were increased until ad libitum intake was reached, additionally, steers on the high-roughage diet were stepped-up to the higher-energy ration. Once adequate intakes were reached for previously restricted steers, all animals were stepped up to a common finishing ration (84% concentrate) and fed for ad libitum intake for the remainder of the finishing phase.

At the end of the growing period, steers fed for ad libitum intake had greater ADG and BW compared with limit-fed steers. However, following the step-up period and 16-d common diet adaptation, BW for limit-fed steers were greater compared with steers fed for ad libitum intake. Differences in gut fill were likely reduced following the adaptation period; therefore, the increase in limit-fed steer BW was likely due to compensatory gains seen in previously restricted cattle once entering the feedlot also seen in Schoonmaker et al. (2003). By design, DMI during the growing phase was greater in ad libitum steers compared with limit-fed steers leading to an 11% increase in feed conversion in limit-fed steers. During the step-up phase, cattle previously fed low-energy diets for ad libitum intake compensate by increasing DMI. Because the ad libitum cattle were adapted to consuming more feed to meet requirements this is likely why increased DMI carried over during the step-up phase until cattle were adapted to the higher energy ration. During the rest of the finishing phase, growth performance and intake were unaffected by previous feeding program. These data are also in agreement with Loerch and Fluharty (1998) and Schoonmaker et al. (2003) who observed that restricting intake during the growing phase had no negative impact on growth performance during the finishing phase. Dressing percentage, quality grade, yield grade and marbling were similar between ad libitum and limit-fed steers similar findings reported by Schoonmaker et al. (2003).

Diet Digestibility

Restricting intake of more energetically dense diets requires increases in dietary protein and nutrients in order to meet daily requirements. Changes in energy and protein utilization can affect nutrient requirements and change how animals digest and metabolize nutrients (Glimp et al., 1989; Murphy et al., 1994). As previously discussed, feed efficiency is often greater for limit fed cattle compared with cattle fed for ad libitum intake. It has been hypothesized improvements in feed efficiency that are observed with limit feeding may result from improvements in diet digestibility (Galyean et al., 1979). Improvements in diet digestibility may be associated with reduced passage rate in restricted intake diets compared with diets fed for ad libitum intake and diet composition.

Galyean et al. (1979) evaluated changes in the extent and site of digestion in cannulated steers fed increasing intakes. Steers were fed at maintenance, 1.33, 1.67- and 2-times maintenance. As DMI increased from maintenance to 2 times maintenance, total tract dry matter digestibility (DMD) and ruminal starch digestibility linearly declined across treatments. Ruminal fermentation of starch is accompanied by losses in heat and gas (Hungate, 1966). Starch digestion into glucose in the small intestine (S.I.) provides up to 40% more energy to cattle than digestion into VFAs by ruminal microbes (Owens et al., 1986). Increased starch escaping to the S.I. is correlated to a decrease in α-amylase (pancreatic enzyme that hydrolyzes starch in S.I.) thus limiting the starch digestibility (Harmon et al., 2004).

In the study by Galyean et al. (1979), greater starch escape from the rumen that occurred when intake was increased from maintenance to 2-time maintenance was correlated with a linear increase in liquid dilution rate and fecal starch concentrations. Agreeably, Murphy et al. (1994) reported that liquid dilution rate was lesser in steers fed a high-energy diet at 70% of ad libitum intake compared with steers fed the same diet for ad libitum intake. Subsequently, dry matter, organic matter, and nitrogen digestion were greater in the limit-fed steers compared with steers fed for ad libitum intake.

Clark et al. (2007) evaluated the effects of restricted DMI while maintaining net energy (NE) and metabolizable protein (MP) on diet digestion and energy balance in crossbred steers. Two dietary treatments were formulated to be fed for ad libitum intake (AL) or restricted to 80% of AL intake (IR80). Diets were program fed to achieve a gain of 1.6 kg/d and provided equal amounts of NE, ruminally degradable protein (RDP), MP, Ca, and P. Fecal output was reduced 40% in IR80 steers compared with steers fed AL. The decrease in fecal output by IR80 was accompanied by an 8% increase in DM digestibility compared with AL steers. Energy loss through feces was greater in AL steers compared with IR80 but energy losses through urine were greater in IR80 compared with AL. Increases in water consumption and subsequent fluid losses have been observed in limit-fed cattle and may be a compensative behavior when less feed is available Clark et al. (2007). Though urinary losses were greater in IR80, protein losses through the urine and feces were similar between AL and IR80 which was by design because protein intake was held constant across both diets.

By design, metabolizable energy (ME) density of AL was lesser compared with IR80; however, ME intake was similar among both treatments. This agrees with Schmidt et al. (2005) who reported that performance and feed efficiency were improved in limit-fed steers when ME intake was held constant in two diets fed ad libitum and 80% restricted. The improvements in growth performance that were observed by Schmidt et al. (2005) were attributed to improved diet digestibility which was also reported by Clark et al. (2007) and Galyean et al. (1979).

Schmidt et al. (2005) and Galyean et al. (1979) suggested improvements in diet digestibility that were observed for limit-fed cattle resulted from slower dilution rates compared with diets fed for ad libitum intake; however, Clark et al. (2005) reported that dilution rates were similar between AL and IR80. Instead, increased efficiency in limit-fed cattle could stem from a reduction in visceral organ mass which could lower maintenance requirements, thus resulting in more energy available for growth (Johnson et al., 1990).

Fermentation Products

Diet composition and modulating intake can alter total concentrations and diurnal production patterns of VFAs and ammonia. Scilacci et al. (2024) conducted a trial to evaluate changes in ruminal fermentation characteristics and products in a high-energy limit-fed diet compared with a high-roughage diet offered for ad libitum intake. Diets were formulated to provide 0.99 Mcal NEg/kg DM fed for ad libitum intake (AL) or 1.32 Mcal NEg/kg DM and fed at 85% of ad libitum intake (LIM85). Portions of prairie hay and alfalfa hay were replaced with dry-rolled corn to increase the energy density of the diet.

Ruminal ammonia concentrations were greater in AL heifers compared with LIM85 heifers. The AL diet included 22.5% alfalfa, 22.5% prairie hay, and 8.6% dry-rolled corn (DM basis) compared with 6.5% alfalfa, 6.5% prairie hay, and 38.8% dry-rolled corn (DM basis) in the LIM85 diet. Alfalfa contains 20% crude protein with 75% being rumen degradable protein (RDP), while corn contains 7% crude protein with 35% being RDP (NASEM, 2016). Crude protein concentrations were 15.9% and 15.1% (DM basis) in the AL and LIM85 rations, respectively and likely contributions from rumen degradable protein were greater in AL from the increased alfalfa leading to greater ruminal ammonia concentrations. Additionally, total VFA concentrations were greater in AL heifers largely due to a 33% and 35% increase in acetate and butyrate production in AL heifers compared with LIM85 heifers, respectively. No differences in propionate or valerate production were observed.

Clark et al. (2007) equalized protein and RDP in limit-fed steers compared with steers fed for ad libitum intake and noted within the first 4 h post feeding ruminal ammonia was greater in limit-fed steers; however, ruminal ammonia concentrations averaged across the 24 h sampling period did not differ among treatments. Clark et al. (2007) and Scilacci et al (2024) are in contradiction with others work that have found ruminal ammonia concentrations are typically lowered when intake is restricted (Murphy et al., 1994; Montgomery et al., 2004; Spore et al., 2019). Additionally, Clark et al. (2007) noted total VFA concentrations were 20% greater in steers fed for ad libitum intake with a larger molar percentage of acetate compared with limit-fed steers. The increase in acetate agrees findings from Scilacci et al (2024). Conversely, molar proportions of butyrate were similar while molar proportions of propionate were greater in limit-fed steers compared with steers fed for ad libitum intake (Clarck et al., (2007). High-grain, low-fiber diets increase propionate production with subsequent decreases in acetate production (Bauman et al., 1971). Differences in VFA concentrations between the studies can be linked to diet composition, feeding time, and range of dry matter restriction.

Modulating intake in cattle changes the fermentation products and digestibility through different pathways. Changes in liquid dilution rate, organ mass, and diet composition all contribute to this change along with other possible unidentified pathways.

Conclusions

Managing newly received growing cattle comes with multiple challenges regarding health and performance and imposes elevated risks on producers and nutritionists. Diseases associated with respiratory infections and digestive upset are common in lightweight cattle entering the yard because of the stressors associated with shipping and procurement. As a result, calves entering the yard are often already immunologically challenged and do not consume enough dry matter to meet nutrient requirements, further exacerbating morbidity. Utilizing feeding strategies targeted for calves to achieve similar caloric intake while consuming less feed is a viable option for producers. With the addition of high fiber, low starch corn coproducts the risk for digestive upset has been reduced in higher energy rations without negatively affecting growth performance. Additionally, understanding how pH regulates the physiology of the rumen and utilizing technologies like wireless pH monitoring bolus can provide producers with an advantage in predicting, diagnosing, and preventing metabolic upset associated with high-grain diets.

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Chapter 2 - Evaluation of indwelling monitoring technologies for measuring reticulorumen pH in cattle

Abstract

We compared reticulorumen pH measurements determined using smaXtec boli (SMAX; smaXtec Inc., Graz, Austria), Moonsyst boli (MOON; Moonsyst International, Kinsale, Republic of Ireland), and a handheld pH meter (METER; PH8500 pH/mV Meter, Apera, Columbus, OH) over time and during an *ex vivo* acidosis challenge. Eight ruminally cannulated heifers were used for 6 consecutive 21-d periods. On d 0, SMAX and MOON were placed in the reticulum of each heifer to continuously measure ruminal pH. On d 20 of each period, pH of ruminal digesta samples collected from four locations within the rumen were measured with METER prior to feeding and 2, 4, 6, 8, 12, 18, and 24 h post feeding. Over the 126-d, ruminal pH was lesser for MOON than METER in every period ($P \leq 0.01$) with the difference becoming larger in periods 4 through 6 (treatment × period, $P < 0.01$). Measurements of pH with SMAX were greater ($P < 0.01$) than METER from periods 1 to 3 and period 6. On d 21 of each period, boli were removed and placed sequentially in pH 4 and 7 solutions, each for 3 h. In pH 4 solution, a treatment × period interaction ($P = 0.02$) was observed where MOON and SMAX yielded measurements that were not different from pH 4 ($P \geq 0.13$), except in period 6. Similarly, in pH 7, a treatment × period interaction ($P < 0.01$) was observed where MOON yielded measurements less than pH 7 ($P \leq 0.05$) in periods 4 to 6 whereas pH measurements with SMAX were greater than 7 ($P \leq 0.05$) in periods 1 to 3. Boli were then placed in a container of strained ruminal fluid, and pH was measured using SMAX, MOON, and METER. Subsequently, vinegar was added to the ruminal fluid to simulate an acidosis challenge and pH was measured. The change in pH in response to

vinegar addition as measured by METER was considered the true response. During the acidosis challenge, there were no treatment × period interactions ($P = 0.34$), pH measurements from MOON were similar ($P = 0.36$) to pH measurements from METER, and pH measurements from SMAX were greater ($P < 0.01$) than those from METER. Overall, ruminal pH measurements determined using SMAX and MOON drifted over time. During an acidosis challenge, MOON detected changes in pH similar to METER which may suggest that MOON could be capable in detecting acidotic events. Conversely, indwelling ruminal pH measurements determined using SMAX were more closely related with METER throughout the experiment.

Key words: Moonsyst, pH, ruminal acidosis, smaXtec

Introduction

Cattle in the feedlot often consume diets rich in cereal grains that contain rapidly fermentable carbohydrates necessary to meet the targeted growth performance. Over-feeding cereal grains in diets containing low inclusions of roughage (physically effective fiber) can disrupt the physiological conditions within the animal's rumen. Digestive disorders account for ~25% to 30% of feedlot deaths and impact the efficiency of production (Galyean and Rivera, 2003). Among nutritional disorders in feedlot cattle, subacute ruminal acidosis (SARA) is most common (Nagaraja and Titgemeyer, 2007). Subacute ruminal acidosis results from the accumulation of organic acids and insufficient buffering in the rumen which often results from cattle consuming high concentrate-based diets. As a result, ruminal pH declines below normal physiological ranges which can be harmful to ruminal microflora, damage the ruminal epithelium, and catalyze health and welfare concerns (Nagaraja and Lechtenberg, 2007).

Additionally, ruminal acidosis is an economic concern with extra cost for finishing acidotic animals from extended days on feed, treatment cost, and deaths (Malafaia et al., 2016). Despite the impact of SARA in feedlots, there is still much debate on the ruminal pH threshold and time spent under that threshold that define SARA and contribute to harmful systemic changes in the rumen (Villot et al., 2018). Accurately measuring rumen pH is essential in diagnosing and preventing SARA. The need for accurate ruminal pH measurements has led to the development of wireless pH monitoring systems. Preliminary research evaluating ruminal pH variation and organic acid concentrations began in the 1950s (Lampila et al., 1955) and advancements have led to improvements over the last 70 years; however, literature directly comparing these pH measuring technologies *in vivo* is lacking. The objective of this experiment was to compare ruminal pH measurements determined using smaXtec boli (SMAX; smaXtec, Graz, Austria), Moonsyst boli (MOON; Moonsyst International, Kinsale, Republic of Ireland), and a handheld pH meter (METER; PH8500 pH/mV Meter, Apera Instruments, Columbus, OH) over time and during an *ex vivo* acidosis challenge.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee reviewed and approved all animal handling and animal care practices used in our experiment. All animal procedures were conducted in accordance with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2020).

Eight ruminally cannulated crossbred heifers (initial body weight = 500 ± 41.6 kg) were arranged in a randomized block design that utilized 6 consecutive 21-d periods. On d 0, each heifer was assigned a MOON and SMAX pH monitoring bolus. Prior to placement in each heifer, each bolus was calibrated individually in a pH 7 solution (VWR Chemicals, Radnor PA) for 3 h at 40 °C, rinsed with deionized water, and placed in pH 4 solution (VWR Chemicals, Radnor PA) and incubated for 3 h at 40 °C. Subsequently, boli were placed in the reticulum of the assigned heifer via ruminal cannula. Heifers were fed a corn-based concentrate diet (Table 1) in a self-feeder and had ad libitum access to ground warm-season grass hay delivered once daily to a bunk at 0700 h.

Boli and Hand-held Systems

The three pH-monitoring systems used in our experiment utilized different techniques for the conversion of mV to pH. The Moonsyst pH monitoring bolus utilizes a glass head pH electrode specifically tailored for the rumen environment and has four junctions to create an electrical contact between the digesta and the internal reference system within the bolus. Additionally, MOON does not utilize any corrections for the raw data entering the system. Instead, the raw mV readings are translated to pH according to the calibration values registered in the system during the initial calibration process using standards of pH 4 and pH 7. Temperature corrections are not used by MOON because temperature within the reticulorumen is stable. SMAX pH monitoring boli utilize a proprietary equation to convert mV to pH values and average the data by 10-minute intervals. Additionally, SMAX utilizes temperature compensation to correct values. The hand-held meter was a standard pH meter designed for use in general aqueous solutions with a lithium glass membrane that ensures relative accuracy of pH measurements within ±0.01 pH units (APERA instruments, 2024). METER does not utilize corrections for converting mV to pH nor temperature compensation. Both MOON and SMAX are designed to maintain a relative accuracy of ±0.02 pH units up to 90 d after activation.

Rumen Sampling

On d 20 of each period, ruminal digesta samples were collected prior to feeding and again 2, 4, 6, 8, 12, 18, and 24 h post feeding. Digesta samples were collected from four locations within the rumen, mixed, and strained through 4 layers of cheesecloth. Subsequently, ruminal pH was measured using METER. Prior to each sampling, METER was calibrated according to manufacturer's instructions in pH 7 and pH 4 solutions. Ruminal pH measurements determined using the SMAX system were recorded every 10 min, whereas ruminal pH measurements determined using the MOON system recorded pH measurements every 1 min. Measurements from each boli were averaged using measurements from 10 minutes before to 10 minutes after the sampling point. Because of the differences in measurement frequency from the two boli systems this resulted in a differing number of measurements used in the average. For SMAX, 3 measurements were used to develop an average pH, whereas 20 measurements were used to develop an average pH for MOON.

Location of Boli

On d 21 of each period, boli were removed from each heifer and the location where each bolus was located was recorded (i.e., rumen or reticulum). The pH thresholds of SARA have been developed measuring the pH of liquor from the ventral sac of the rumen (Zebeli et al., 2012), but boli are applied with a balling gun in production settings and may be retained in the reticulum. To determine the difference in pH between the reticulum and rumen, we compared pH measurements determined using SMAX and MOON from periods 1 to 3. Data from only the first three periods were analyzed because 1) all boli were found in the rumen in period 4 making comparisons between reticular and ruminal pH in that period unachievable and 2) drift in pH readings during the final 2 periods was deemed likely to yield spurious conclusions. All pH measurements determined using METER during the 24-h sampling periods were determined using ruminal digesta samples, so measurements with METER could not be used to compare pH in the rumen and reticulum.

Bolus Debris Index

Manufacturers of SMAX and MOON have different designs for their devices which could impact how each bolus accumulates organic matter and debris. On d 21 following the 24-h sampling, boli were removed from each heifer and photographed to evaluate feed/debris compaction on the sensor. Photographs were then assigned a debris score (Fig. 1) based on the degree of compaction and percentage of sensor visibility (Table 2). A score of 1 indicated that the sensor was 100% visible with no feedstuff or debris present, whereas a score of 5 indicated that 0% of the sensor was visible with heavy feedstuff and debris compaction. After photographing, boli were cleaned to remove any retained debris and ensure the sensor was completely visible. Subsequently, boli were rinsed with deionized water and dried before further testing.

pH Solutions

Following debris evaluation and cleaning, boli were individually placed in a standing pH 7 solution (VWR Chemicals, Radnor, PA) for 3 h at 40 °C. Subsequently, boli were removed from pH 7 solution, rinsed with deionized water, dried, and placed in a standing pH 4 solution (VWR Chemicals, Radnor PA) for 3 h at 40 °C. Boli were allowed a stabilization period to ensure no erroneous measurements were included in the analysis. Subsequently, pH measurements recorded after the stabilization period were averaged. Differences in measurements from a pH of 7 or 4 were used to determine pH measurement drift of MOON and SMAX over time.

Ex Vivo Acidosis Challenge

On d 21 of each period, following pH measurement of pH 7 and 4 solutions, boli were rinsed with deionized water, dried, and placed in 6.5 L of standing, strained rumen fluid for 1 h at 40 °C. Measurements from METER were obtained before and after the 1 h and averaged. Boli were allowed the first 3 min of each challenge to stabilize pH readings before measurements were averaged over the hour. After 1 h, 500 mL of vinegar (5% acetic acid vol/vol) was added and mixed to the ruminal fluid to simulate an acidosis challenge and incubated for 1 h at 40 °C. Measurements of pH were collected at the beginning and end of the challenge using METER and measurements of pH from MOON and SMAX were collected over the hour. Differences in pH determined using METER before and after acidification with vinegar were considered the true pH change in response to the acidosis challenge.

Statistical analyses

Data were analyzed as a randomized block design with each heifer serving as a block. All *in vivo* and *ex vivo* pH measurements were analyzed using the MIXED procedure of SAS (SAS 9.4, SAS Inst. Inc, Cary, NC). The model for *in vivo* pH measurements from d 20 of each period included fixed effects of treatment, period, hour, and all interactions. Heifer and bolus location were included as random effects. Hour was a repeated measure with the subject being heifer × period. The covariance structure was compound symmetry as determined by Akaike Information Criterion fit statistics.

To evaluate how pH measurements drifted over time, all boli were placed in pH 7 and pH 4 solution, the difference in the obtained measurements from the known standards was calculated (i.e., pH standard – observed pH), and the data were analyzed as repeated measures. The model included fixed effects of treatment, period, and treatment × period and a random effect of heifer.

Period served as the repeated measure with the subject of heifer; the covariance structure was compound symmetry as determined by Akaike Information Criterion fit statistics.

For the ex vivo acidosis challenge, average pH measurements of strained ruminal fluid pH before and after the addition of vinegar, measured by SMAX, MOON, and METER, were analyzed using a model that included treatment, period, and treatment x period as fixed effects and heifers as a random effect. Subsequently, the reduction in pH that resulted from the ex vivo acidosis challenge was evaluated with the same statistical model.

All pH measurements were analyzed for the presence of outliers. The SGPLOT procedure in SAS was used to create a boxplot of the Studentized residuals against the predicted values. Parameters were applied where measurements with absolute values of the Studentized residuals greater than 3 were considered as outliers. However, no residuals were greater than 3; thus, no outliers were identified in the in vivo or ex vivo pH measurements.

For debris ranking data, the FREQ procedure in SAS generated frequency tables of treatment × rank. The frequencies of each rank were analyzed using the GLIMIMIX procedure in SAS with a model including treatment, period, and treatment × period as fixed effects and heifer as a random effect. To determine the difference between reticular and ruminal pH, pH measurements from periods 1 to 3 were evaluated. The model included treatment, hour, period, location, and all interactions as fixed effects and heifer and heifer × period × hour as random effects. Hour was a repeated measure with the subject being animal × period and the covariance structure being compound symmetry. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 \leq P < 0.10$.

Results and Discussion

Ruminal pH Measurements

One potential limitation to our data is that pH measurements obtained using SMAX, MOON, and METER were not always determined from the same location within the reticulorumen. At the start of each period, SMAX and MOON boli were placed in the reticulum of each heifer; however, when boli were removed at the end of each period, boli were located in the reticulum or the rumen. Differences in pH among regions of the reticulorumen have been well documented (Bryant, 1964; Falk et al., 2016; Han-Kim et al., 2018). Neither SMAX nor MOON were consistently located in the reticulum or the rumen, and we collected digesta samples from four locations within the rumen, composited them, and measured pH of the composited sample using METER.

Ruminal pH measurements from d 20 are presented in Fig. 2. A treatment × period interaction was observed ($P < 0.01$) where ruminal pH measurements from SMAX were greater ($P < 0.01$) than METER from periods 1 to 3, similar to METER ($P \geq 0.12$) in periods 4 and 5, and greater than METER ($P < 0.01$) in period 6. Although SMAX pH measurements were greater (0.20 to 0.39 pH units) than METER during 4 of the 6 periods, both SMAX and METER followed similar trends. Ruminal pH measurements determine using MOON were less ($P \leq 0.01$) than METER in every period. During periods 1, 2, and 3, pH measurements determined using MOON were respectively 0.29, 0.70, and 0.35 pH units less ($P < 0.01$) than METER. Beginning in period 4 (d 84), differences in pH measurements between MOON and METER began to increase. During periods 4, 5, and 6, pH measurements determined using MOON were respectively 0.80, 0.91, and 1.07 pH units less ($P < 0.01$) than METER.

Data from the 24-h sampling period on d 20 of each period are presented in Fig. 3. No treatment × hour interactions ($P \geq 0.25$) nor treatment × period × hour interactions ($P \geq 0.99$) were observed. Throughout the 24-h sampling, pH measurements from MOON (average pH of 5.35) were lower ($P < 0.01$) compared with METER (average pH of 6.03). Conversely, SMAX measurements (average pH of 6.25) were greater ($P < 0.01$) compared with METER. A diurnal pattern in ruminal pH has been observed in many studies and is affected by diet and timing of feed intake. Rumsey et al. (1970) fed steers twice daily either a high-concentrate or high-roughage diet at 1% or 2% of body weight daily (dry matter basis). Regardless of treatment, ruminal pH decreased over the initial 4 to 6 h post-feeding with the greatest change occurring in the first 2 h. In our experiment, ruminal pH declined after feeding; however, based on data from METER, the change in ruminal pH was only 0.14 pH units at 6 h after feeding. Heifers were allowed ad libitum access to a concentrate-based diet via a self-feeder and provided warm-season grass hay once daily for ad libitum intake. Rustomo et al. (2006) reported that diets fed for ad libitum intake with low acidogenic value concentrates (oats and wheat bran) and coarse forage (1.3 and 1.9 cm length) experienced less drastic changes in ruminal pH than those with high acidogenic value concentrates (barley and wheat) and finely processed forage (19.0 to 8.0 mm). In that experiment, authors attributed the low daily variation in ruminal pH to the intrinsic buffering capacity in the cattle fed coarse forage. Providing heifers with ad libitum access to warm-season grass hay in our experiment may have improved buffering capacity and contributed to the minimal variation in ruminal pH over the day.

Ex vivo pH measurements

Data from measurements of the pH 7 and pH 4 solutions are presented in Fig. 4 and Fig. 5, respectively. Values of pH 7 and 4 were considered the true pH of the respective solutions, and pH measurements from the boli were compared to these values. Normal ruminal pH in feedlot cattle ranges from 5.6 to 6.5 (Nagaraja and Lechtenberg, 2007). Therefore, a pH of 7 is closer to physiologically normal ruminal fluid than pH 4, although both boli are designed to measure pH from a range of 3 to 9.

For data from measurements of the pH 7 solution on d 21 of each period, a treatment × period interaction ($P < 0.01$; Fig. 4) was observed. MOON yielded measurements that were not different ($P \geq 0.25$) from pH 7 during periods 1 through 3 but were less ($P < 0.05$) than pH 7 from periods 4 to 6. In period 5 and 6, pH measurements in the pH 7 solution determined using MOON were 5.26 and 6.08, respectively. Measurements determined using SMAX were greater ($P \leq 0.05$) than pH 7 for periods 1 to 3 and periods 5 to 6 but were similar ($P = 0.28$) to pH 7 in period 4. Both boli drifted further from pH 7 solution over time, particularly for measurements at 105 or 126 d for MOON and at 126 d for SMAX.

A treatment × period interaction ($P = 0.02$; Fig. 5) was observed for data from boli placed in the pH 4 solution. Both MOON and SMAX yielded pH measurements that were not different ($P \geq 0.13$) from pH 4 until period 6. During period 6, SMAX pH measurements were greater ($P < 0.01$) than pH 4, whereas MOON measurements were less ($P < 0.01$) than pH 4. Using averaged pH readings from solutions of pH 4 and 7, Schori et al. (2022) reported smaXtec boli measurements varied -0.29 pH units from solutions of pH 4 and 7 over 154 d. In that study, researchers utilized a pH solution challenge at pH 4 and 7 similar to the current study. In our study, SMAX averaged +0.42 and +0.33 pH units relative to pH solutions of 7 and 4, respectively, over the 126 d. Conversely, MOON averaged -0.60 and -0.10 pH units relative to pH solution of 7 and 4, respectively.

The smaXtec pH monitoring bolus is designed to maintain an accuracy of ± 0.2 pH units of the true value for 90 d and remain within ± 0.4 pH units for 150 d within a pH range of 3 to 9 (smaXtec, 2024). When in pH 7 solution, SMAX yielded measurements within 0.2 of pH 7 in period 4 but deviated from 0.33 to 0.83 units in other periods. Moonsyst pH monitoring boli are designed to maintain an accuracy of ± 0.2 pH units up to 90 d within a pH range of 3 to 9, validated by Konyves (2017) at University of Veterinary Medicine - Budapest. When MOON was placed in a pH 7 solution in our experiment, pH measurements were within 0.2 pH units of 7 for periods 1 and 2 (42 d), but measurements with MOON deviated from 7 by 0.33 to 1.74 units in periods 3 to 6. While in a pH 4 solution, MOON remained within ± 0.2 units of pH 4 from periods 1 to 5 (105 d). In pH 4 solution, SMAX remained within 0.2 units of pH 4 in period 4 and yielded greater values in all other periods. Data from our experiment suggest that both MOON and SMAX pH measurements drifted from a pH 7 solution sooner than a pH 4 solution.

Results from the *ex vivo* acidosis challenge are presented in Fig. 6, 7, and 8. The average pH of the strained ruminal fluid determined by METER, which was considered the true value, was 5.45. In strained ruminal fluid, a treatment × period interaction ($P = 0.02$; Fig. 6) was observed. The pH measurements determined using SMAX were not different ($P \geq 0.11$) from those determined using METER and remained within 0.31 pH units from periods 1 through 5. In period 6, pH measurements determined using SMAX were greater (0.71 pH units, $P < 0.01$) compared with those determined using METER. Similarly, in strained rumen fluid, MOON yielded pH measurements that were not different ($P \geq 0.41$) compared with METER and remained within ± 0.14 pH units until period 6. During period 6, pH measurements determined using MOON were 0.91 units less ($P < 0.01$) than pH measurements determined using METER.

In period 6 (126 days), SMAX overestimated ruminal fluid pH whereas MOON underestimated ruminal fluid pH when compared with METER.

The pH of ruminal fluid following the vinegar-induced acidosis challenge indicated no treatment × period interactions ($P = 0.34$; Fig. 7). Average pH determined from METER after the addition of vinegar was 4.12. Measurements from MOON were similar (-0.12 pH unit deviation, $P = 0.36$) to those from METER, whereas measurements from SMAX were 0.81 units greater ($P < 0.01$) than METER.

Values for pH determined by METER, SMAX, and MOON in strained rumen fluid before and after the acid addition were used to calculate the change in pH as a result of the acidification. The average pH of strained ruminal fluid measured by METER was 5.45. After the addition of acid, the average pH of the ruminal fluid was 4.12, which resulted in a 1.33-unit decrease in pH. A treatment × period interaction ($P < 0.01$; Fig. 8) was observed for the change in ruminal pH detected by each bolus. MOON detected changes in pH similar to METER ($P \geq 0.30$) from periods 1 to 3; however, the change in pH detected by MOON was lesser than METER ($P = 0.05$) in period 4, greater than METER ($P < 0.01$) in period 5, and tended ($P = 0.07$) to be lesser than METER in period 6. The change in pH detected by SMAX was less than ($P \leq 0.01$) the change detected by METER from periods 1 to 5 but was similar ($P = 0.19$) between SMAX and METER in period 6. Overall, MOON detected changes in pH similar to METER more often than SMAX, which may suggest that MOON would be more capable of accurately detecting ruminal acidosis in cattle, but *in vivo* confirmation is needed.

Our data suggests that both MOON and SMAX experienced variability in pH measurements based on the experimental criteria measured. During the *in vivo* measurements, SMAX followed more closely to METER in all periods compared with MOON. Over the 6 periods, SMAX yielded greater measurements in 4 periods compared with METER with the largest difference in period 3 (0.20 pH units greater than METER). Additionally, during the *in vivo* experiments, measurements from SMAX appeared to follow fluctuations in ruminal pH that were measured by METER more closely than MOON. Conversely, measurements from MOON were lesser in every period compared with METER with the greatest differences in period 6 (1.07 pH units lesser than METER). This was driven by a progressive reduction in pH measurements by MOON beginning after more than 85 d in the heifer. However, in the experiments where pH was purposefully lowered (pH 4 solution and acidosis challenge), measurements by MOON were closer to METER than they were during *in vivo* measurements and only differed from METER at 126 d after activation.

Bolus Location

All boli were placed in the reticulum of each heifer at the beginning of each period, but 6 MOON boli were found in the reticulum and 18 MOON boli were found in the rumen at the end of the first 3 periods. For SMAX boli, 15 were located in the reticulum and 9 were located in the rumen. Based on these data, it appears that boli that are placed in the reticulum may not remain there.

On average, reticular pH was 0.27 units greater (treatment effect: $P < 0.01$; Fig. 9) than ruminal pH. A tendency for a treatment × location interaction ($P = 0.06$; Fig. 9) was also observed where SMAX reticular and ruminal pH tended to be greatest, MOON reticular pH intermediate, and MOON ruminal pH the least. These observations are consistent with observations from 1) the full d-20 data set where pH measurements from SMAX were greater than those from MOON (Fig. 2) and 2) as pH declined, measured decreases in pH were greater for MOON than for SMAX (Fig. 8); these congruencies suggest the data set from periods 1 through 3 that were used to evaluate location effects was reflective of the entire data set. In previous experiments, the magnitude of pH differences between the reticulum vs. the rumen has been variable among researchers. Using indwelling devices, Sato et al. (2012) and Falk et al. (2016) reported that reticular pH was 0.17 and 0.24 pH units greater than ruminal pH, respectively. Similarly, Neubauer et al. (2017) indicated that reticular pH measured using an indwelling bolus was 0.19 pH units greater than ruminal fluid collected from the ventral sac and measured using a portable pH meter. Because reticular and ruminal pH differ, pH measurements determined using indwelling boli may be difficult to interpret if the location of the bolus is unknown.

Debris Ranking

Results from debris scores are presented in Table 3. Debris scores were based on a 1 to 5 scale with a greater score indicating more debris or compaction. The two boli had different designs, but no differences ($P = 0.12$) in debris scores were observed between MOON and SMAX. Overall, both boli experienced high levels of compaction and debris accumulation with 69% of the MOON boli and 60% of SMAX boli scored as 5. *Ex vivo* pH measurements determined using cleaned SMAX and MOON boli were more closely related to METER (Fig. 6) compared with *in vivo* measurements of non-cleaned boli (Fig. 3). Nocek et al. (2002) reported that placing a protective shield around the pH sensor resulted in a slower rate of pH reduction after feeding for shielded probes compared with non-shielded probes. In that experiment, pH nadir was 0.3 units greater for shielded probes compared with non-shielded probes. Authors reported that adding a shield to the probe increased compaction of ruminal digesta which may have contributed to their observed differences in pH.

Conclusions

Achieving accurate *in vivo* measurements of ruminal pH is a challenging task. Indwelling boli technologies provide continuous data with minimal labor input. However, results from this experiment suggest that there is variability associated with these technologies and limitations to the lifespan of the equipment. Specifically, Moonsyst pH monitoring boli were able to detect abrupt changes in pH (*ex vivo*), although measurements drifted over time when placed in pH standards more than 85 d after activation. Compared to Moonsyst boli, smaXtec pH monitoring boli yielded in vivo pH measurements that were closer to values determined using a handheld pH meter when measured over time, but measurements of pH from smaXtec still differed from those determined with the handheld pH meter. Understanding the timing and variation in ruminal pH will continue to be an important aspect when diagnosing metabolic disorders, understanding fermentation and absorption, and studying the microbiome within the rumen. Technologies like these boli could provide the opportunity to learn more about the constantly changing ruminal environment, but more studies are warranted to further confirm their accuracy, precision, and specific roles in research and production.

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Figures

Figure 2.1 Photographs of boli debris ranking. No score 1 or 3 for MOON was observed.

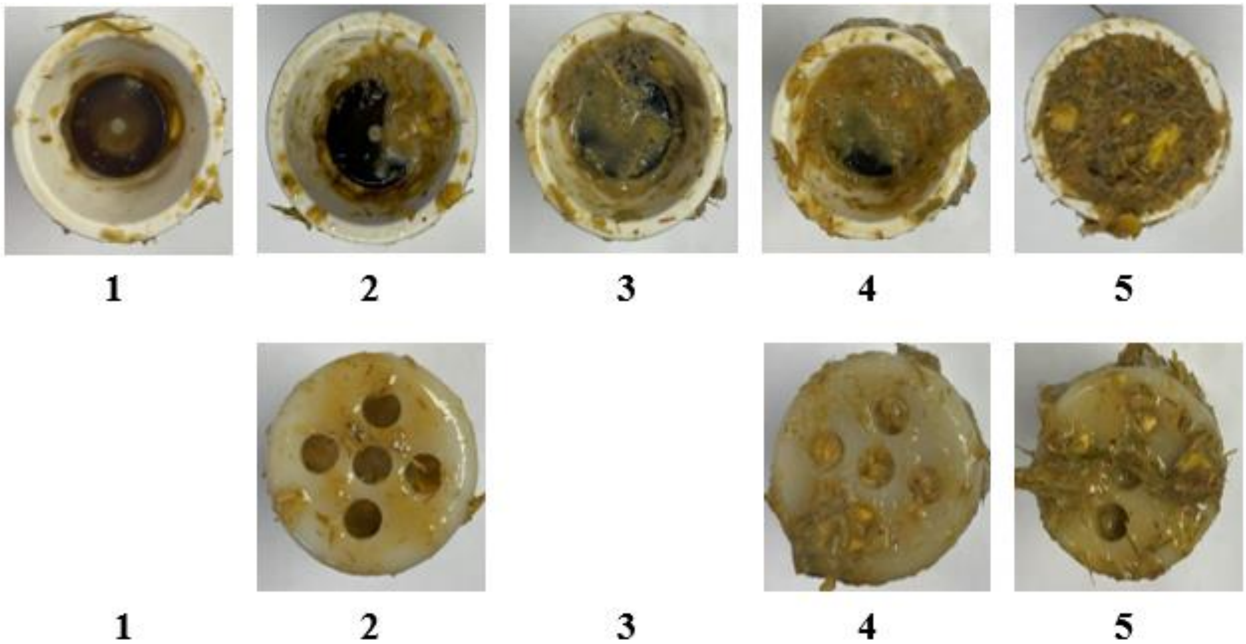


Figure 2.2 Comparison of pH measurements determined using smaXtec boli (SMAX), Moonsyst boli (MOON), and a handheld pH meter (METER) over 126 d. Each period was 21 d, with pH measurements collected on d 20 at feeding and 2, 4, 6, 8, 12, 18, and 24 h after feeding. Treatment: *P* < 0.01; Period: *P* < 0.01; Treatment × period: *P* < 0.01; SEM = 0.09. Within period, means with uncommon superscripts differ (*P* < 0.05).

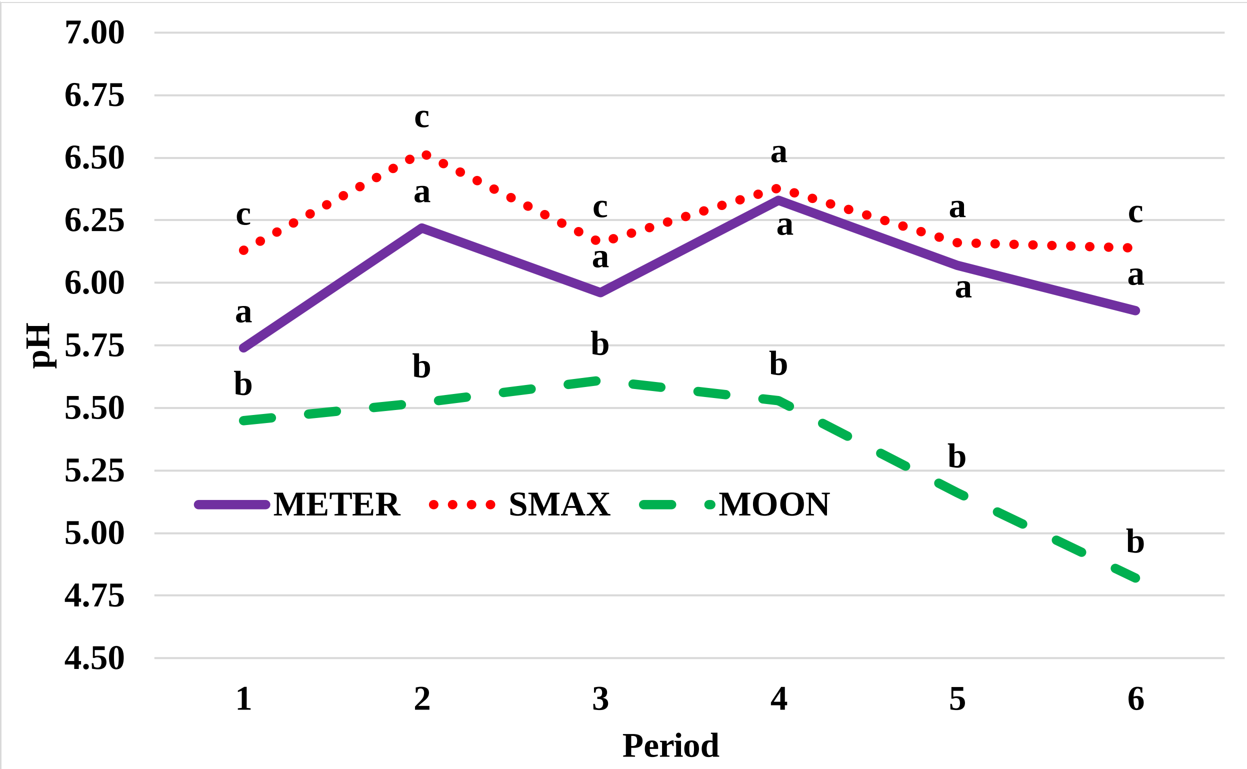


Figure 2.3 Comparison of pH measurements determined using smaXtec boli (SMAX), Moonsyst boli (MOON), and a handheld pH meter (METER) over a 24-h sampling period on d 20 of each of six consecutive 21-d periods. Treatment: P < 0.01; Hour: P < 0.01; Treatment × hour: *P* = 0.25. SEM = 0.10.

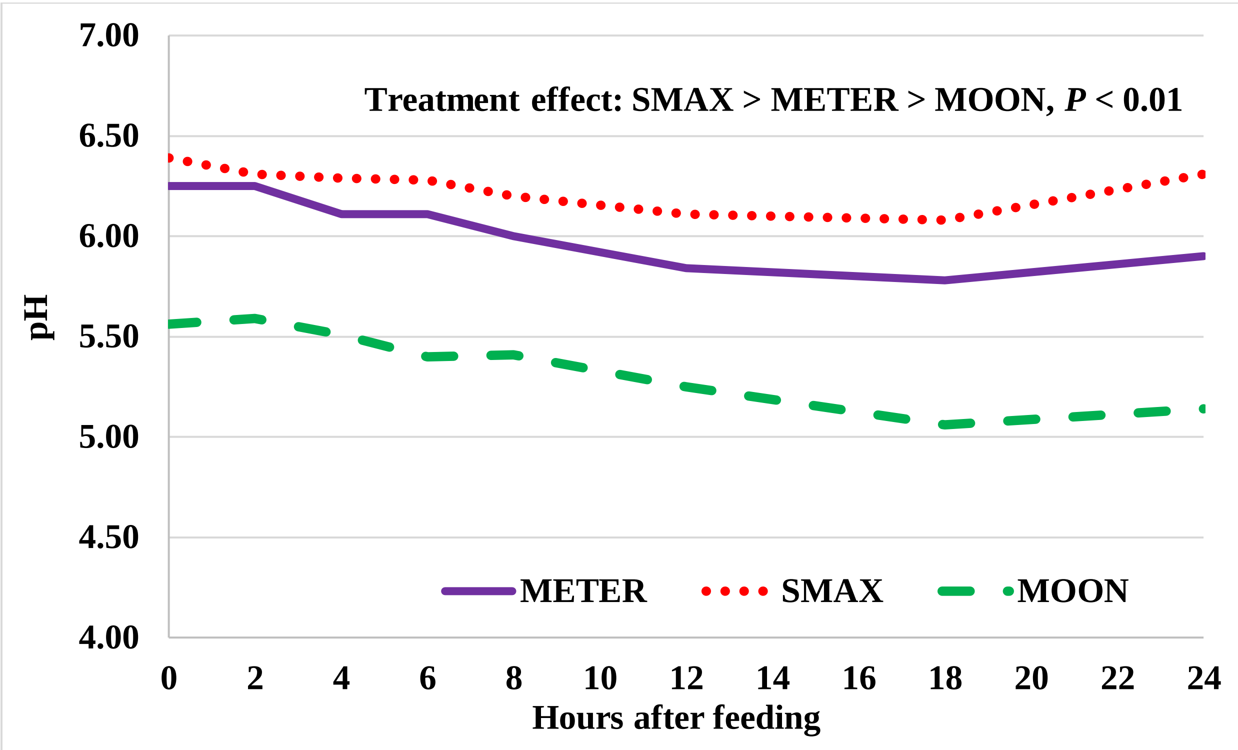


Figure 2.4 Comparison of pH measurements determined using smaXtec (SMAX) and Moonsyst (MOON) boli when placed in a pH 7 standard solution on d 21 of each six consecutive 21-d periods. Treatment: P < 0.01; Period: P < 0.01; Treatment × period: P < 0.01. SEM = 0.20. Within treatment, means with * or + differ from pH 7 (*P* < 0.05)

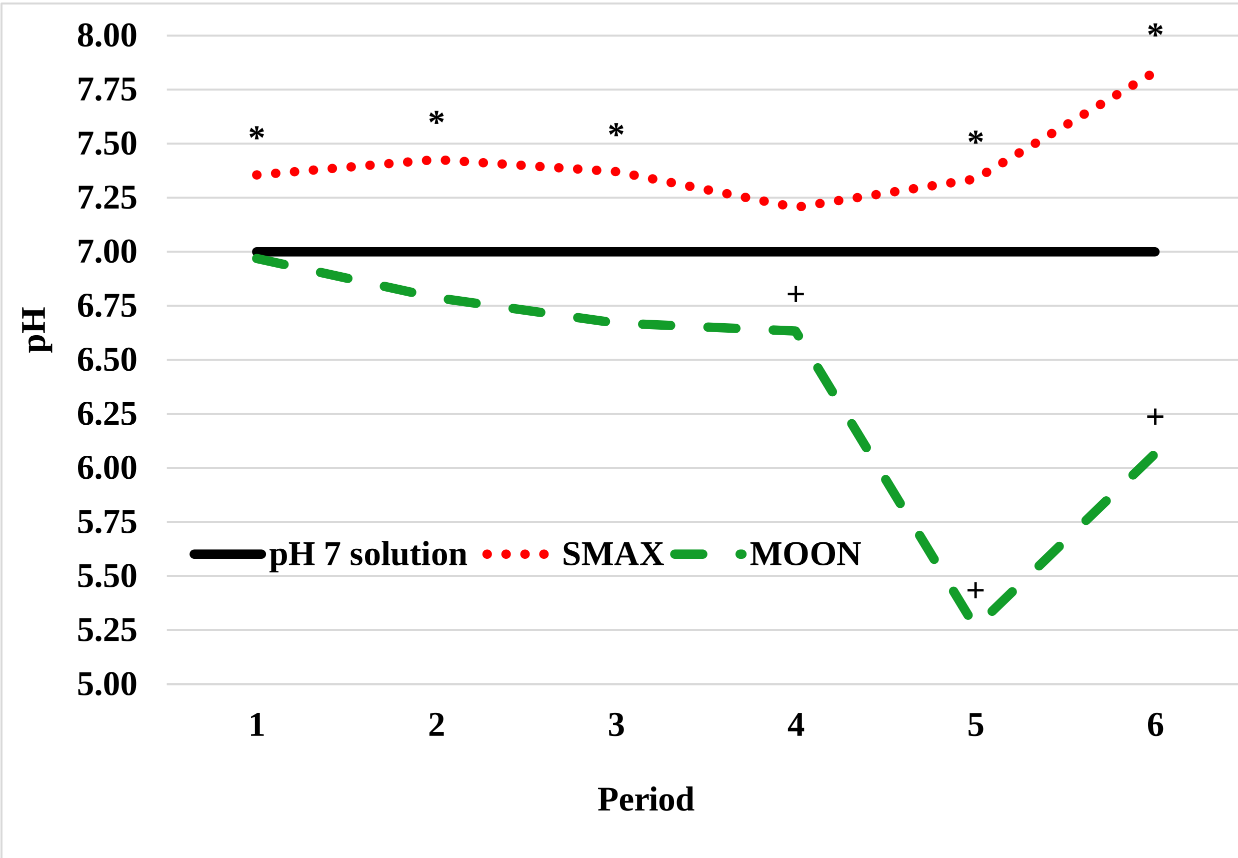


Figure 2.5 Comparison of pH measurements drift over time of smaXtec (SMAX) and Moonsyst (MOON) boli when compared to pH 4 standard solution (STAND). Treatment: P < 0.001; Period: P = 0.56; Treatment × period: P = 0.02. SEM = 0.15. Within treatment, means with a * or + differ from pH 4 (*P* < 0.05)

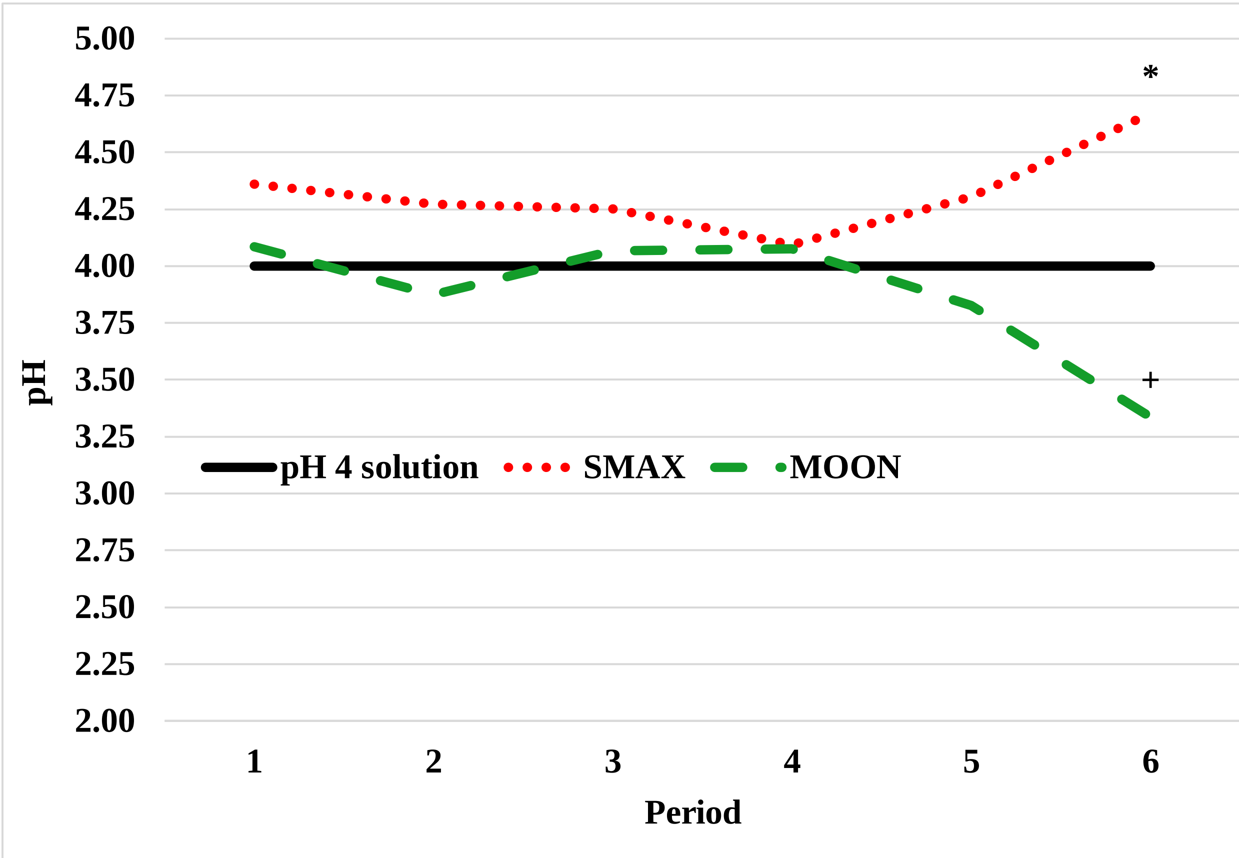


Figure 2.6 A comparison of strained ruminal fluid ex vivo pH measurements determined using Moonsyst boli (MOON), smaXtec boli (SMAX), or a handheld pH meter (METER). Treatment: P < 0.01; Period: P = 0.30; Treatment × period: P = 0.02; SEM = 0.20. Within period, means with uncommon superscript letters differ (*P* < 0.05).

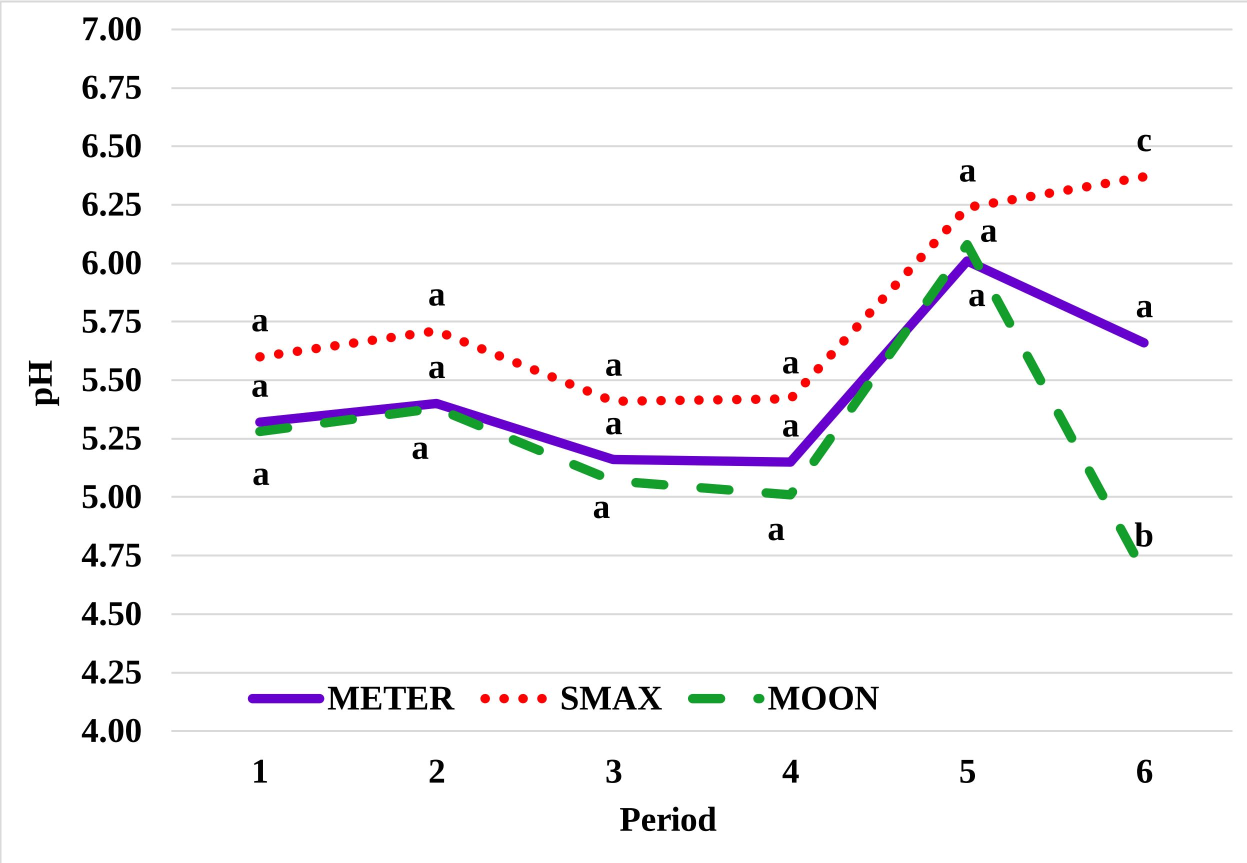


Figure 2.7 A comparison of ruminal fluid pH measurements after addition of vinegar to create an ex vivo acidosis challenge using a Moonsyst bolus (MOON) or smaXtec bolus (SMAX) or a handheld pH meter (METER). Treatment: $P < 0.01$; Period: $P = 0.07$; Treatment × period: $P = 0.34$; SEM = 0.25.

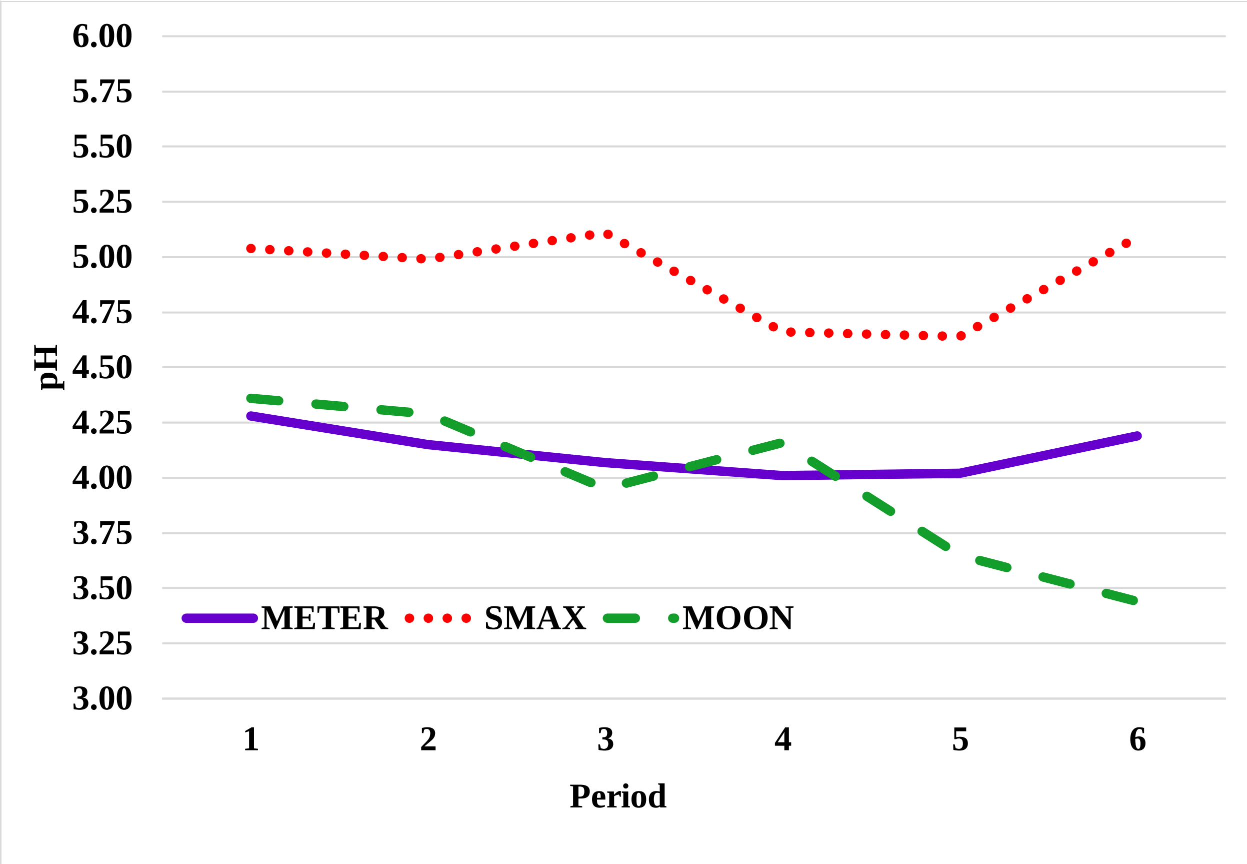


Figure 2.8 A comparison of the decrease in pH detected during an ex vivo acidosis challenge measured by a Moonsyst bolus (MOON), smaXtec bolus (SMAX), or a handheld pH meter (METER). Treatment: P < 0.01; Period: P < 0.01 Treatment × period: P < 0.01; SEM = 0.16. Within period, means with uncommon superscript letters tend to differ (*P* < 0.06).

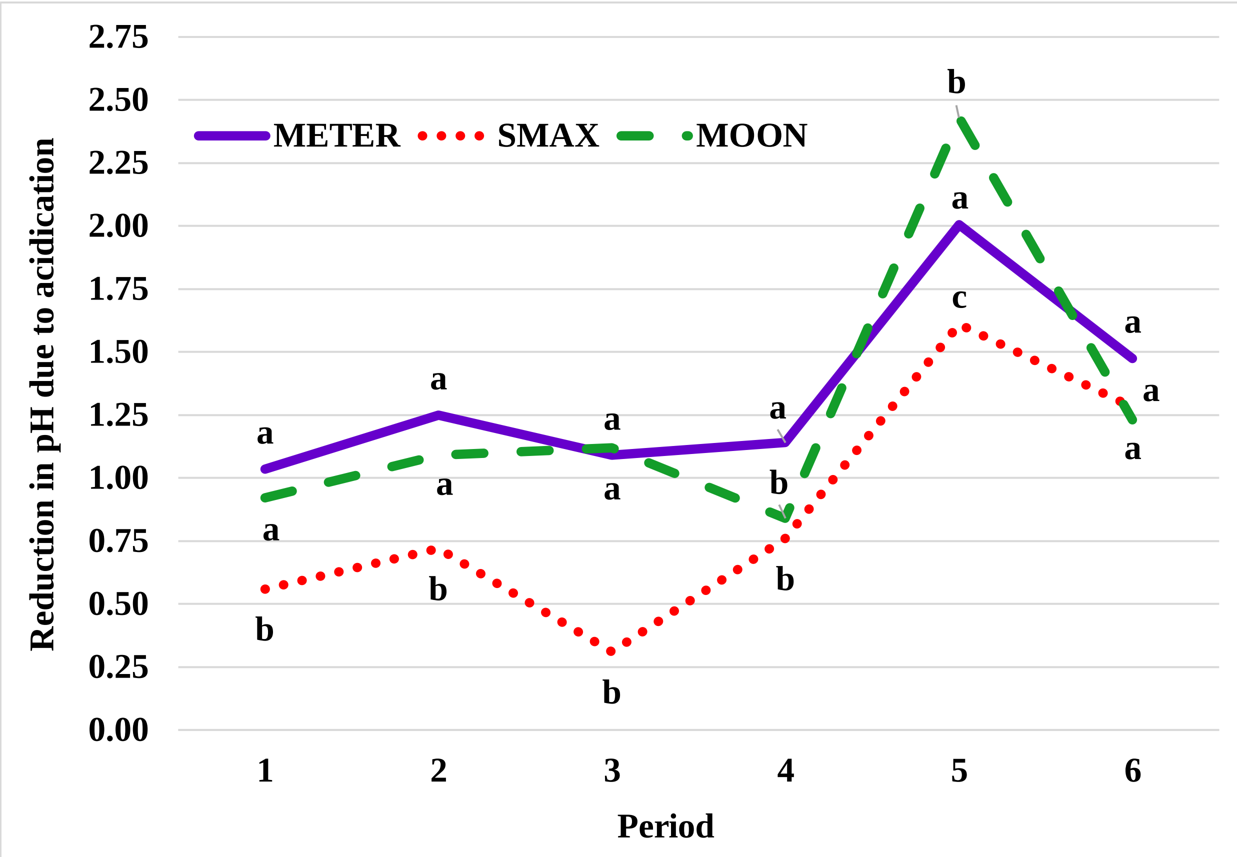
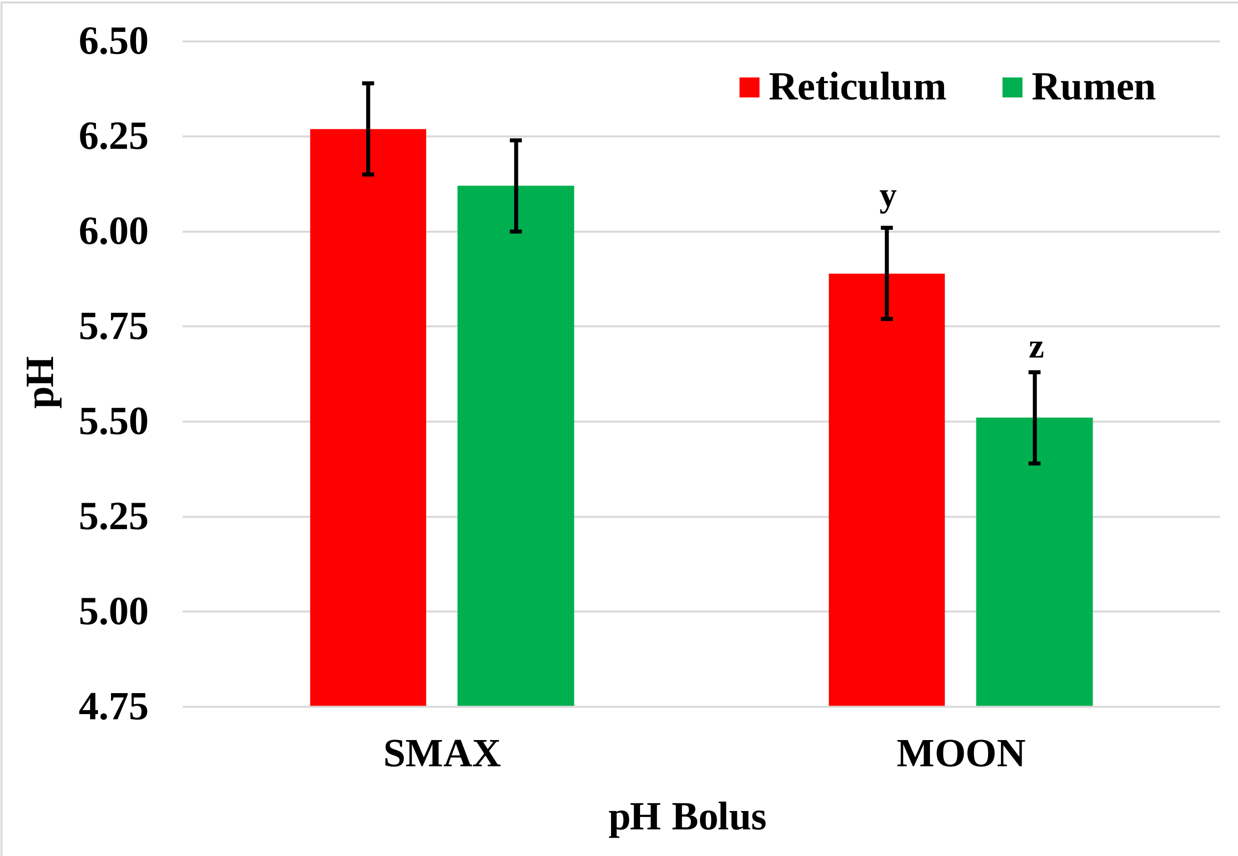


Figure 2.9 Effects of location on pH measurements in the first three periods determined by smaXtec bolus (SMAX) and Moonsyst bolus (MOON). Treatment: P < 0.01; Location: P < 0.01; Treatment × Location: P = 0.06; SEM = 0.12. Within period, means with uncommon superscript letters tend to differ (*P* < 0.06).



Tables

Table 2.1 Concentrate mix provided to heifers via self-feeder

Ingredient	**% DM**
Dry-rolled corn	76.48
36% crude protein pellet[1]	10.00
Dried distillers grains	10.00
Liquid mill product	2.00
Calcium carbonate	1.50
Rumensin[2]	0.02

[1] Protein supplement, 36% crude protein, pellet: Stkrmkr 36-0 pellet (Countryside Feeds LLC, Hillsboro, KS).

[2] Provided 44.4 mg monensin/kg concentrate mix DM (Elanco, Greenfield, IN).

Table 2.2 Description of bolus debris scores

Score	DESCRIPTION
1	Sensor 100% visible with no compaction of feedstuff or debris noticeable.
2	Sensor 75% visible with light compaction of feedstuff around sensor.
3	Sensor 50% visible with moderate debris and compaction on bolus or sensor.
4	Sensor 25% visible with moderate to heavy debris and compaction over all of bolus.
5	Sensor 0% visible with heavy compaction completely blocking sensor and bolus.

Table 2.3 Frequency of debris scores for smaXtec (SMAX) and Moonsyst (MOON) boli on d 21 of each period after removal from each heifer but before rinsing.

Score[1]	MOON	SMAX
	Frequency, % [2]	
1	0	6
2	2	8
3	0	14
4	29	12
5	69	60

[1] Debris scores are described in Table 2.
[2] Treatment main effect: $P = 0.12$; SEM = 0.42.

Chapter 3 - Effects of dietary energy concentration and dry matter intake on growth performance, digestion, and ruminal fermentation characteristics of newly received growing cattle

Abstract

A 70-day growing study was conducted to determine the effects of feeding a high-roughage diet for ad libitum intake or a high-energy limit-fed diet at equal or increasing energy intakes on growth performance, digestion and fermentation characteristics of newly received growing beef cattle. In experiment 1, a total of 392 crossbred heifers (initial bodyweight = 274 ± 26 kg) were blocked by weight (4), stratified by individual arrival weight within block, and allocated to 1 of 8 pens. Pens were randomly assigned to 1 of 4 dietary treatments. Experimental diets included a high-roughage diet fed for ad libitum intake (AL) or a limit-fed high-energy diet fed at 75% (LIM75), LIM80 (80), or 85% (LIM85) of ad libitum intake. Treatments were designed to equalize energy intake between calves assigned to AL and LIM75. In experiment 2, eight ruminally cannulated, crossbred heifers were utilized in a 4 × 4 Latin square to determine digestibility and ruminal fermentation characteristics of the diets from Exp. 1. Heifers were utilized in four consecutive 15-d periods. Final BW and ADG were similar ($P = 0.37$) between AL and LIM75, and greater ($P \leq 0.05$) in LIM80 and LIM85 compared with AL. By design, dry matter intake was greater ($P \leq 0.01$) in AL heifers compared to all 3 limit fed treatments. As a results feed conversion was improved by 30% ($P \leq 0.01$) in limit fed heifers compared with AL and did not differ ($P \geq 0.20$) among LIM75, LIM80, or LIM85. Dry matter digestibility was 10%, 5%, and 4% greater in among LIM75, LIM80, and LIM85, respectively, compared with AL. Organic matter, neutral fiber, and acid fiber digestibility were also greater in limit-fed treatments compared to AL. A treatment × hour interaction ($P < 0.01$) was observed for ruminal pH. Limit fed calves reached pH nadir earlier compared with AL with LIM75 being lowest, LIM80 intermediate, and LIM85 being greatest. Total ruminal VFA concentrations were unaffected by treatment ($P = 0.34$); however, there was a tendency ($P = 0.08$) for propionate concentrations to be intermediate and greater in LIM80 and LIM85 heifers, respectively, compared with AL and LIM75. Ruminal ammonia concentrations were greater ($P < 0.01$) in AL compared to limit fed treatments and experienced three different peak concentrations 2, 8, and 18 h post feeding compared with limit fed treatments singular peak 2 h post feeding (treatment × hour; $P < 0.01$). Based on our data limit feeding high concentrations to newly receiving growing cattle does not negatively affect performance and fermentation characteristics.

Key words: dietary energy, limit-feeding, receiving period

Introduction

The receiving period is a critical time in respect to cattle health and performance, because of the economic impact during this period different growing strategies have been debated (Rivera et al., 2005). Generally, performance of growing and finishing cattle is maximized when cattle are fed for ad libitum intake (Murphy and Loerch, 1994; Rossi et al., 2001). However, newly received growing cattle are subjected to numerous stressors such as marketing, transportation, co-mingling, and pathogen exposure. Stress can produce or aggravate nutrient deficiencies in cattle often observed through insufficient dry matter intakes in calves fed traditional high-roughage rations on arrival (Hutcheson and Cole, 1986). Instead, increasing the amount of dietary energy available in receiving diets by replacing portions of roughage with cereal grains allows for more energy density within the ration. However, the addition of more rapidly fermentable carbohydrates in the form of cereal grains can catalyze metabolic disorders leading to increased morbidity, specifically respiratory diseases, and acidosis if overconsumed (Lofgreen et al., 1975; Richeson et al., 2019). Limit-feeding high-energy rations has been shown to decrease the risk of morbidity without reducing performance and allows cattle feeders to target specific weight gains (Schoomaker et al., 2003; Spore et al., 2018; Wagner et al., 1990). Replacing portions of cereal grains with corn by-products that are high in fermentable fiber such that energy contributions from fiber are equal to starch can reduce the incidence of morbidity in growing cattle (Watson et al., 2015). Spore et al. (2019) reported that limit-feeding growing cattle a diet that contained 40% wet corn gluten feed (Sweet Bran; Cargill Animal Nutrition, Blair, NE) formulated to provide 1.32 Mcal net energy for gain (NEg)/kg DM did not negatively effect on morbidity, mortality, or growth performance in growing heifers when fed at 85% of intake from a high-roughage ration formulated to provide 0.99 Mcal NE_g/kg DM and offered for ad libitum intake. In that study, net energy intake above maintenance was 18% greater for limit-fed heifers compared with heifers fed for ad libitum intake suggesting that energy intake was not fully equalized between treatments (Galyean et al., 2022). Therefore, the objective of the current experiment was to determine if providing equal amounts of energy from a high-roughage diet fed for ad libitum intake or a high-energy limit-fed diet influenced growth performance of growing beef cattle.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee reviewed and approved all animal handling and animal care practices used in our experiment. All animal procedures were conducted in accordance with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2020).

Experiment 1. Receiving Study

A total of 392 crossbred heifers (initial bodyweight = 274 ± 26 kg) were purchased from a single source ranch in Deming, New Mexico and transported to the Kansas State Beef Stocker Unit on Friday May 26, 2023. On arrival, heifers were randomly sorted into groups containing 12 to 14 head, weighed on a pen scale (Rice Lake Weighing Systems; Rice Lake, WI), randomly assigned to 1 of 32 pens, and limit-fed a 1.32 Mcal NEg/kg DM diet at 2% of bodyweight until May 30, 2023.

Heifers were arranged in a randomized complete block design to evaluate the effects of dietary energy concentrations on performance and intake of newly received growing cattle. Heifers were blocked into 4 weight groups; light, medium-light, medium-heavy, and heavy, stratified by individual weight within block, and assigned to 1 of 8 pens that contained 12 to 13 head. Within block, pens were randomly assigned to one of four experimental treatments which provided 8 pens per treatment for a total of 32 pens. Pens were soil surfaced (9.1 × 15.2 m) with concrete bunks (9.1 m) that were attached to a concrete apron (3.6 m). Experimental diets (Table 1) included a high-roughage diet formulated to provide 0.99 Mcal NE_g/kg DM fed for ad libitum intake (AL) or a high-energy diet formulated to provide 1.32 Mcal NE_g/kg DM and limit fed at 75% (LIM75), 80% (LIM80), 85% (LIM85) of the AL intake. All diets contained 40% wet corn gluten feed (Sweet Bran; Cargill Animal Nutrition, Blair, NE; dry matter [DM] basis). To increase the energy density of the diet, dry-rolled corn replaced portions of warm-season grass hay. In addition, as feed delivery was increased from 75% to 85% of AL intake, dry-rolled corn replaced portions of supplement so that monensin intake was similar across treatments.

Prior to the start of the experiment (d -1) heifers were individually weighed using a hydraulic squeeze chute, given a visual identification number ear tag, and assessed for lameness and disease. The following morning (d 0), heifers were weighed individually, tagged with a pen number, and treated for internal and external parasites with a 10% albendazole drench (Valbazen; Zoetis, Kalamazoo, MI), and pour-on diflubenzuron (Clean Up 2; Elanco Animal Health, Greenfield, IN). Prior to arrival, heifers had completed a Vac-45 vaccination protocol; therefore, no additional vaccines were administered.

Heifers were fed once daily beginning at 0700 h using a Roto-Mix feed wagon (Model #414-14B; Roto-Mix, Dodge City, KS). On d 0, AL heifers were fed at 2.2% of BW; thereafter, feed refusals were collected at 0600 h and weighed. Feed deliveries for AL were then adjusted to target 10% of feed remaining in the bunk at 0600 h the following morning. To determine feed delivery for limit-fed heifers, feed intake of the two AL pens within each block were averaged.

Feed offered to limit-fed heifers was then calculated within each weight block by multiplying average ad libitum intake by 75, 80, or 85%.

Pen weights were measured beginning on d 14 then alternate weeks until the conclusion of the study and adjustments to the AL treatments bodyweights were made to adjust feed calls. Performance results were observed from d 0 and d 70.

Heifers were checked twice a day for morbidity and mortality. If signs of morbidity were observed animals were pulled from the pen and restrained in the chute. Rectal temperature and clinical illness score was recorded and if animals presented with clinical signs and a rectal temperature ≥ 40° C they were treated following the KSU Beef Stocker Unit protocol. Animals were treated once and every three days if needed. If animals were treated for signs of morbidity thrice, they were considered chronic and removed from the trial. Any heifers that were assessed for lameness were assessed, treated, and removed from the trial on the first treatment.

Individual ingredient samples were collected weekly and composited for analysis (Table 2) by a commercial laboratory (SDK Laboratories, Hutchinson, KS).

Net Energy Calculations

Data collected from the performance study was used to determine net energy for maintenance and gain provided by the three diets using equations from NRC (1996) and Galyean (2019). A 4% shrink was applied to weights and finish weight of 650 kg was used in the analysis

Experiment 2. Digestibility Trial

Eight ruminally cannulated crossbred heifers (initial BW = 197 ± 24 kg) were arranged in a replicated 4 × 4 Latin square to determine apparent diet digestibility and ruminal fermentation characteristics of the diets used in Exp. 1. Data from one heifer was removed from all analysis due to poor health and low dry matter intake. Heifers were fed once daily at 1000 h. When feeding occurred for Exp. 1, the amount needed for Exp 2. was removed from the feed wagon prior to feeding for Exp. 1. Heifers were housed in individual, outdoor pens with ad libitum access to water. During the experiment, orts for AL were targeted at 10% of feed delivered the previous day and were collected at 0900 prior to feeding. Intakes for the AL heifers were averaged and used to calculate feed delivery for limit-fed heifers identical to Exp 1.

The experiment consisted of four consecutive 15-d periods. Each period included 10 d of diet adaptation, 4 d of fecal sampling, and 1 d of ruminal digesta sampling. On d 4 to 14 of each period, ten grams of chromic oxide (Cr_2O_3) was dosed into the rumen via cannula of each heifer in a dissolving gelatin capsule (TORPAC, Leedstone; Melrose, MN). Fecal samples were collected from d 10 to d 14. Samples were collected from the rectum of each heifer every 8 h. Sampling times advanced 2 h each day so that each 2 h interval over a 24 h sampling period was represented. After each collection, samples were composited for every heifer within each period. From d 10 to 14 individual ingredient samples were collected and composited within period for analysis. At the completion of the experiment, individual feed ingredient and fecal samples were sent to a commercial laboratory for analysis of dry matter, organic matter, neutral detergent fiber, and acid detergent fiber (SDK Laboratories, Hutchinson, KS).

On d 15 ruminal digesta samples were collected from the cannula of each heifer prior to feeding. Immediately following digesta collection, heifers were dosed with cobalt-EDTA (3 g) dissolved into 200 mL of DI water. Ruminal digesta samples were then collected again 2, 4, 6, 8, 12, 18, and 24 h post-feeding. Following each collection, digesta samples were mixed and immediately strained through 8-layer cheesecloth. Strained rumen fluid (1 mL) was pipetted into microcentrifuge tubes containing 250 µL of m-phosphoric acid (25% wt/vol). Beginning 2 h post-feeding, an additional 15 mL of strained rumen fluid was collected in scintillation vials to measure cobalt concentrations and estimate liquid passage rate. Additionally, pH of strained ruminal fluid was measured using a handheld pH meter (PH8500 pH/mV Meter, Apera Instruments, Columbus, OH). All samples were immediately frozen after collection at -20° C for future analysis.

Laboratory Analysis

Rumen fluid samples collected to determine volatile fatty acid (VFA), and ruminal ammonia concentrations were thawed and centrifuged for 30 min at 17,000 × *g* at 4° C. Concentrations of VFA were analyzed by gas-liquid chromatography according to Vanzant and Cochran (1994). Ruminal ammonia concentrations were analyzed using methods described by Broderick and Kang (1980).

Dried fecal samples were ground in a 1-mm screen and ashed in a muffle oven at 400° C for 6 h to measure apparent diet digestibility. Chromium and ruminal cobalt concentrations were determined by atomic absorption spectrophotometry described by Williams et al. (1962). Chromium concentrations were used to determine total fecal output and diet digestibility as described by Cochran and Galyean (1994). Using the nonlinear procedure in SAS, liquid passage rate was determined by regressing the natural logarithm of cobalt in the 2 to 18 h samples for each heifer within period against time with passage rate identified as the negative slope of regression.

Statistical Analysis

Performance data from Exp. 1 were analyzed in SAS (v9.4, SAS Institute Ince., Cary, NC.) using the MIXED procedure. Data were analyzed as a complete randomized block design with pen as the experimental unit. The model included treatment and block as fixed effects. In Exp 2. apparent diet digestibility and ruminal parameters were analyzed as a replicated Latin square using the MIXED procedure of SAS with fixed effects of treatment and period and heifer as a random effect. VFA and ammonia concentrations and pH measurements were analyzed as repeated measures. The model included treatment, hour, and period as fixed effects and random effect of heifer. Hour served as the repeated measure with a subject of animal × period. The covariance structure was autoregressive. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 \leq P < 0.10$.

Results and Discussion

Experiment 1. Receiving Trial

Growth performance and intake data are presented in Table 3. Among limit-fed heifers, final BW and ADG were intermediate to not different ($P \geq 0.13$) in LIM80 heifers compared with LIM75 and LIM85; however, final BW and ADG were greater ($P < 0.01$) in LIM85 compared with LIM75. By design, DMI was greater ($P < 0.01$) in LIM80 and LIM85 compared with LIM75. Dry matter intake did not differ ($P = 0.39$) between LIM80 and LIM85. Observed DMI for LIM85 was 3% lesser (82%) than expected. Other research has demonstrated similar results when attempting to limit feed growing cattle at 85% of ad libitum intake of a diet formulated to provide 1.32 Mcal NEg/kg DM in growing cattle (Scilacci et al., 2024). In that study, cattle were limit fed a similar diet to ours at 85% of ad libitum intake; however, cattle only consumed 78% of ad libitum intake. One possibility is that based on energy intake of the LIM85 treatment, heifers were nearing their capacity to consume excess energy (Grovum, 1986).

Feed efficiency was 30% greater ($P < 0.01$) in LIM75 heifers compared with AL heifers. The increase in feed conversion resulted from a 25% reduction ($P < 0.01$) in DMI which was by design, because final BW and ADG were similar between the two treatments. Similarly, G:F was greater ($P \leq 0.03$) in LIM80 and LIM85 compared with AL. A 10% and 16% increase ($P < 0.01$) in ADG in addition to a 20% and 18% reduction ($P < 0.01$) in DMI in LIM80 and LIM85, respectively, compared with AL heifers led to the greater conversion. The increase in feed efficiency of limit fed calves agrees with Schoonmaker et al. (2004) who observed a 25% increase in feed efficiency in steers limit fed a high-energy diet compared to a high-forage diet fed for ad libitum intake.

Final BW and ADG were similar ($P \geq 0.38$) in LIM75 and greater ($P \leq 0.02$) in LIM80 and LIM85 compared with AL. These data agree with Loerch (1990) who reported that ADG was similar in steers fed high-forage diets fed for ad libitum intake compared with steers fed high-concentrate diets restricted to 70% of ad libitum consumption. A 40% increase in feed: gain was observed and is 10% greater than our LIM75 heifers likely due to the extent of restriction and the study lasting an extra 15 d compared with our experiment. This is in agreement with findings from Scilacci et al. (2024) observed a 47% increase in feed efficiency in heifers limit-fed at 85% of ad libitum intake compared with ad libitum counterparts. The increase in feed efficiency was attributed to a 15% increase in ADG and 22% decrease in DMI in limit fed heifers.

Spore et al. (2019) program fed either a high-roughage diet (0.99 Mcal NEg/kg DM) or a high-energy (1.32 NEg/kg DM) diet to growing heifers targeted at similar ADG of 1 kg/d. Heifers on the high-energy diet exceeded the targeted gain (ADG 1.1 kg/d) but still achieved similar final bodyweights compared with the high-roughage heifers. However, DMI was reduced

1 kg/d in the high-energy heifers leading to a greater feed efficiency compared with the high-roughage heifers. However, in that study net energy intake above maintenance was 18% greater in heifers fed the high-energy diet compared with the high-roughage heifers. In our study we attempted to equalize net energy intake between the AL and LIM75 treatments. A difference in both Spore et al. (2019) and Scilacci et al. (2024) and our study is a gut fill equalization. Spore et al. (2019) fed a common diet for 14 d to attempt to equalize differences in gut fill while Scilacci et al. (2024) withheld feed 12 h prior to weighing. There was no gut fill equalization in our study. In a series of 18 experiments, Watson et al. (2013) determined cattle that were limit fed had less variation in final bodyweights compared to ad libitum fed cattle. Differences in gut fill in our study may have contributed to the differences in calculated net energy intake based on performance between the AL heifers compared to limit-fed heifers.

However, without a gut fill equalization period in our study, AL and LIM75 heifers performed similarly even though energy intake was greater in AL heifers. This could outline the physiological differences that could improve efficiency such as visceral organ mass reduction (Johnson et al., 1990), reduction of energy losses as gas or heat (Clark et al., 2007), and improved digestibility (Galyean et al., 1979) observed in limit-fed cattle.

Experiment 2. Digestibility Trial

Intake and total-tract digestibility data is presented in Table 4. Ruminal VFA and ammonia concentrations, liquid passage rate, rumen liquid volume and ruminal pH data is presented in Table 5. By design, DMI was lesser ($P \leq 0.01$) for LIM75, LIM80, and LIM85 compared with AL. Subsequently, intake of organic matter, neutral detergent fiber, and acid detergent fiber was lesser ($P \leq 0.01$) in LIM75, LIM80, and LIM85 compared with AL heifers.

Apparent total-tract digestibility of dry matter (DMD), organic matter (OMD), neutral detergent fiber (NDFD), or acid detergent fiber (ADFD) did not differ ($P \geq 0.22$). However numerically, DMD was 7.5%, 3.8%, and 3.1% greater in LIM75, LIM80, and LIM85 heifers compared with AL heifers. Additionally, OMD, NDFD, and ADFD were numerically greater in LIM75, LIM80, and LIM85 heifers compared with AL. The increase in fiber digestibility in LIM75, LIM80, and LIM85 could be related to greater proportion of fiber in the diet coming from wet corn gluten feed compared to the AL heifers. Fiber in wet corn gluten feed is more digestible than that of traditional roughages (NASEM, 2016). This combined with the lower total NDF and ADF intake in limit-fed treatments could explain the increase in fiber digestibility in limit-fed heifers.

Clark et al. (2007) determined that when intake was restricted to 80% of ad libitum intake with net energy and metabolizable protein equal steers had 8% increase in DM digestibility compared to steers fed ad libitum. The work agrees with Galyean et al. (1979) who observed a reduction in DM and OM digestibility when intake increased from 1 to 2 times maintenance. The authors hypothesized that the increase in digestibility was associated with a decrease in liquid dilution rate as intake decreased; however, Clark et al. (2007) observed no differences in liquid dilution rate between steers restricted to 80% DMI and those fed for ad libitum.

In our experiment, liquid dilution rate did not differ ($P = 0.63$) among all four treatments. Instead, the improved diet digestibility is a product of diet composition. Replacing 35% of warm-season grass hay in the AL diets with dry-rolled corn in the limit-fed rations likely contributed to a more digestible diet.

Ruminal total VFA concentrations did not differ ($P = 0.34$) among the four treatments. However, ruminal propionate concentrations tended to be greatest ($P =0.08$) for LIM85, intermediate for LIM80 and least for LIM75 and AL. The greater proportion of dry-rolled corn consumed by LIM80 and LIM85 likely contributed the increase in propionate production because of the increase in starch entering the rumen.

A treatment × hour interaction ($P < 0.01$) was observed for ruminal pH. Among limit-fed treatments, ruminal pH reached nadir 2 to 4 h post feedings with LIM75 being lowest (5.5), LIM80 intermediate (5.7), and LIM85 greatest (5.8). However, AL heifers reached nadir (5.8) 4 and 12 h post feeding. Duncan et al. (2022) limit-fed growing steers at 2.0% BW (DM basis) and reported that steers consumed all feed or only left feed residue within 4 to 6 h of feeding. Because heifers in our study likely consumed feed faster, as intake was further restricted fermentation peaked early and the accumulation of VFAs decreased pH. However, average ruminal pH over the entire 24 h sampling did not differ ($P = 0.52$) pH among AL, LIM75, LIM80, and LIM85 treatments Our diets contained 40% wet corn gluten feed (WCGF), because WCGF contributes energy from fiber fermentation rather than starch the fermentation is slower and can provide buffering contributing to the similar pH seen across treatments (Sullivan et al., 2012).

Additionally, meal eating behavior reflects the differences in ruminal ammonia concentrations. Ruminal ammonia concentrations were greater ($P < 0.01$) in AL heifers compared with LIM75, LIM80, and LIM85. A treatment × hour ($P < 0.01$) interaction was also observed where AL heifers peaked 2, 8 and again 12 h post feeding and was greater than the limit fed treatments. Limit-fed treatments peaked once 2 to 4 h post feeding but concentrations were still lesser than AL (Figure 3). Our results contradict with those from Murphy et al. (1994) who observed as intake was restricted, ammonia concentrations in the rumen increased compared to ad libitum cattle. However, in that study dietary energy and diet composition were not changed as intake was restricted unlike our study. Changes in ruminal ammonia concentrations in limit fed cattle has been attributed to the decrease in liquid passage rate often seen (Montgomery et al., 2004 and Spore et al., 2019). As passage rate increases in cattle fed for ad libitum consumption, there may be more dilution of ammonia and less microbial protein synthesis. Our work agrees with Scilacci et al. (2024) where ruminal ammonia concentrations were greater in cattle fed ad libitum compared to those limit-fed. Additionally, in that study they observed similar peaks in ammonia concentrations compared with our findings

Implications

Our results indicate that limit-feeding high-energy diets with the inclusion of wet corn gluten feed (Cargill Milling, Blair NE) did not negatively affect growth performance or fermentation characteristics in growing beef heifers. Limit-feeding a high energy diet improved feed efficiency and total tract digestibility without comprising metabolic health. Therefore, this strategy could be beneficial to producers because it could help minimize the risk of energetic deficiencies on arrival when DMI is often at its lowest and improve productivity in receiving phase.

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Figures

Figure 3.1 Effects of dietary energy concentration and intake on ruminal volatile fatty acid concentrations in ruminally cannulated beef heifers. AL: 0.99 Mcal NEg/kg DM fed ad libitum access; LIM-75: 1.32 Mcal NEg/kg DM limit-fed at 75% of AL intake; LIM-80: 1.32 Mcal NE_g/kg DM limit-fed at 80% of AL intake; LIM-85: 1.32 Mcal NE_g/kg DM limit-fed at 85% of AL intake. Acetate: treatment (P = 0.65), treatment × hour (P = 0.99), hour (P < 0.01), SEM = 1.63. Propionate: treatment (P = 0.08), treatment × hour (P = 0.98), hour (P < 0.01), SEM = 1.66. Butyrate: treatment (P < 0.88), treatment × hour (P= 0.99), hour (P = 0.07), SEM = 0.70. Valerate: treatment (P = 0.75), treatment × hour (P = 0.99), hour (P < 0.01), SEM = 0.28. Isobutyrate: treatment (P = 0.37), treatment × hour (P = 0.94), hour (P < 0.01), SEM = 0.06. Isovalerate: treatment (P = 0.90), treatment × hour (P = 0.99), hour (P < 0.01), SEM = 0.14.

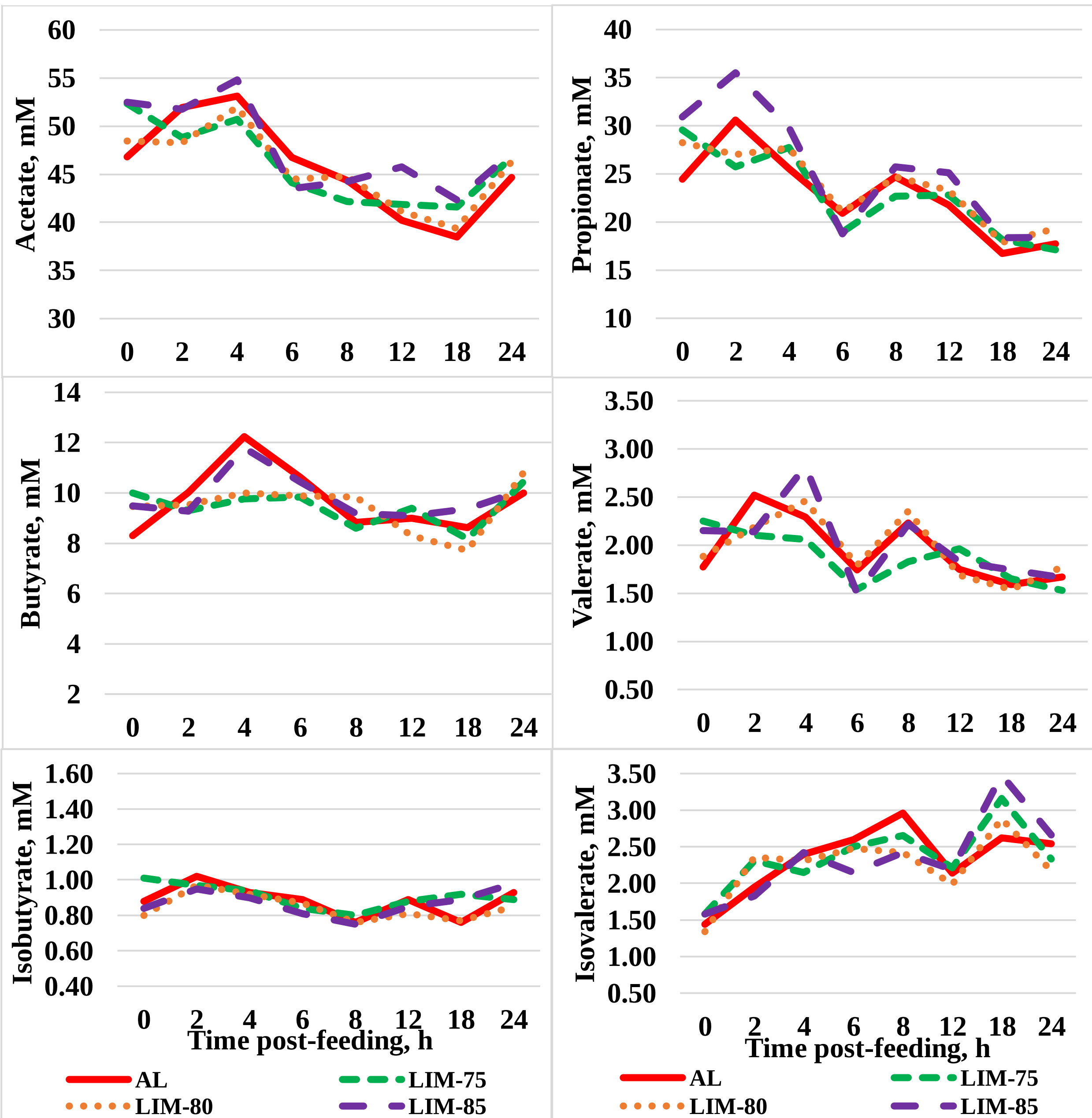


Figure 3.2 Effects of dietary energy concentration and intake on ruminal pH in ruminally cannulated beef heifers. AL: 0.99 Mcal NEg/kg DM fed ad libitum access; LIM-75: 1.32 Mcal NEg/kg DM limit-fed at 75% of AL intake; LIM-80: 1.32 Mcal NEg/kg DM limit-fed at 80% of AL intake; LIM-85: 1.32 Mcal NE_g/kg DM limit-fed at 85% of AL intake. Treatment (*P* = 0.52); hour (*P* < 0.01); treatment × hour (*P* < 0.01); SEM = 0.10.

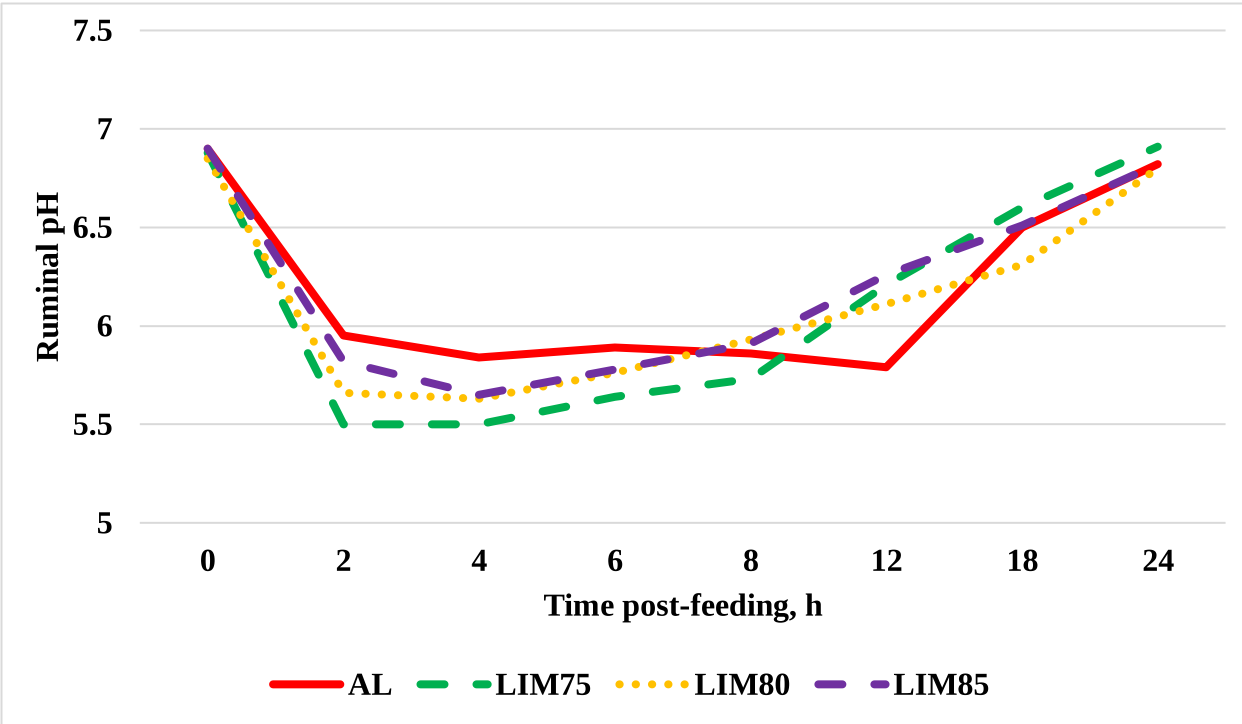
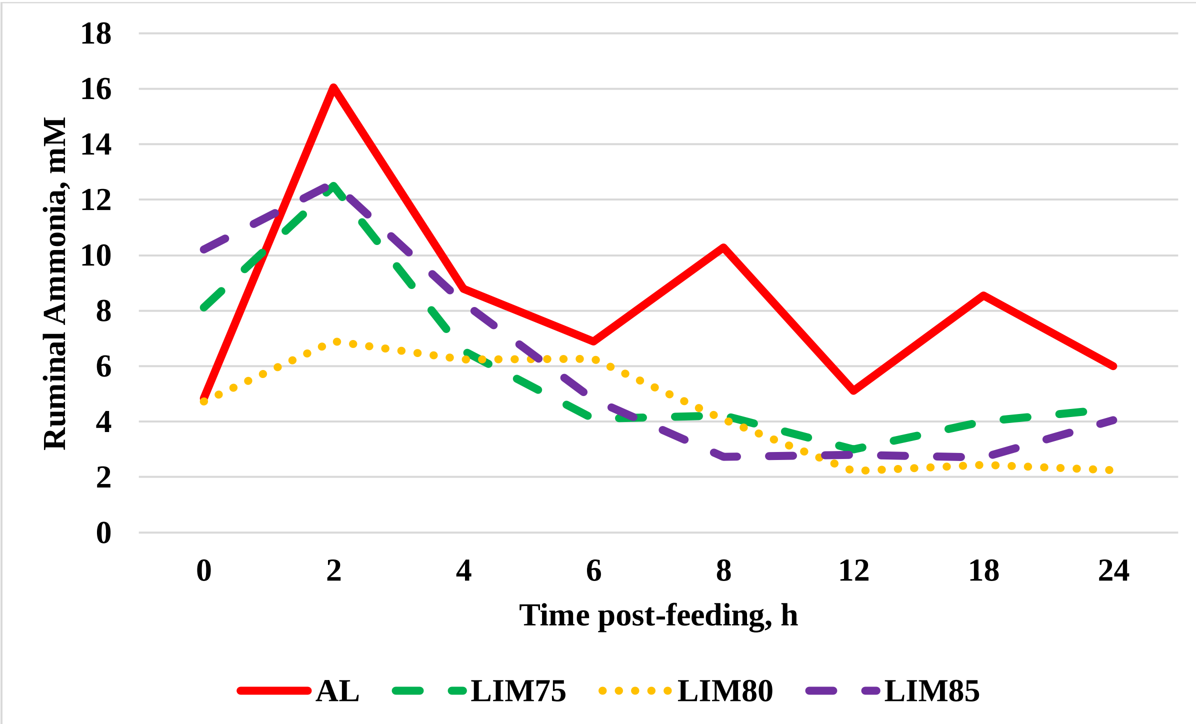


Figure 3.3 Effects of dietary energy concentration and intake on ruminal ammonia concentrations in ruminally cannulated beef heifers. AL: 0.99 Mcal NEg/kg DM fed ad libitum access; LIM-75: 1.32 Mcal NEg/kg DM limit-fed at 75% of AL intake; LIM-80: 1.32 Mcal NEg/kg DM limit-fed at 80% of AL intake; LIM-85: 1.32 Mcal NE_g/kg DM limit-fed at 85% of AL intake. Treatment (*P* < 0.01); hour (*P* < 0.01); treatment × hour (*P* < 0.01); SEM = 0.22.



Tables

Table 3.1 Experimental diets

	Treatments[1]			
Ingredient, % DM	**AL**	**LIM-75**	**LIM-80**	**LIM-85**
Dry Rolled Corn	14.38	40.50	40.97	41.38
Supplement[2]	5.63	7.50	7.03	6.62
Prairie Hay	40.00	12.00	12.00	12.00
Sweet Bran[3]	40.00	40.00	40.00	40.00

[1]AL: ad libitum, 0.99 Mcal NE_g/kg DM; LIM-75: 75% of AL intake, 1.32 Mcal NE_g/kg DM, LIM-80: 80% of AL intake, 1.32 Mcal NE_g/kg DM; LIM-85: of AL intake, 1.32 Mcal NE_g/kg

[2]Pelleted supplement formulated to include (DM); 11.5% crude protein, 0.60% phosphorus, 4.7% salt, 0.80% potassium, 2.5% fat, and 307.2 g/ton monensin (Rumensin; Elanco, Greenfield, IN)

[3] Cargill Animal Nutrition (Blair, NE)

Table 3.2 Nutrient composition of experimental diets

Item,	**Treatments**			
Nutrient composition[1], % DM	**AL**	**LIM-75**	**LIM-80**	**LIM-85**
Dry matter	77.9	77.1	77.1	77.0
Crude protein	17.7	19.3	18.7	18.6
Organic matter	93.9	98.4	95.4	95.1
Neutral detergent fiber	41.1	26.5	25.4	25.2
Acid detergent fiber	19.1	9.5	9.2	9.2
Calculated Composition[2], Mcal/kg				
NE_m	1.63	1.97	1.97	1.97
NE_g	0.99	1.32	1.32	1.32

[1] Nutrient analysis conducted by SDK Laboratories (Hutchinson, KS)

[2] `Net energy of maintenance (NEm) and net energy of gain (NEg) were calculated using NASEM (2016) values of diet ingredients.

Table 3.3 Effects of dietary energy concentrations and dry matter intake of corn co-product diets on newly received growing beef heifers

	Treatment[1]					
Measurement	**AL**	**LIM-75**	**LIM-80**	**LIM-85**	**SEM[2]**	***P*-value[3]**
Body Weight, kg						
Day 0	274	274	274	274	0.12	0.34
Day 70	365[a]	369[ab]	375[bc]	381[c]	2.88	< 0.01
Average Daily Gain, kg/d	1.30[a]	1.35[ab]	1.44[bc]	1.53[c]	0.04	< 0.01
Dry Matter Intake, kg/d	10.72[a]	8.00[b]	8.58[c]	8.81[c]	0.19	< 0.01
Gain: Feed, kg/kg	0.12[a]	0.17[b]	0.16[bc]	0.17[bc]	0.01	0.03
Diet NE_m[4], Mcal/kg DM	1.24[a]	1.59[b]	1.56[b]	1.58[b]	0.03	< 0.01
Diet NE_g[5], Mcal/kg DM	0.68[a]	0.99[b]	0.96[b]	0.98[b]	0.03	< 0.01

[1] AL: ad libitum, 0.99 Mcal NE_g/kg DM; LIM-75: 75% of AL intake, 1.32 Mcal NE_g/kg DM, LIM-80: 80% of AL intake, 1.32 Mcal NE_g/kg DM; LIM-85: of AL intake, 1.32 Mcal NE_g/kg DM

[2] Standard error of the mean

[3] Treatment main effect

[4] Net energy for maintenance, as described by Galyean (2019) using values from NRC (1996)

[5] Net energy for gain, as described by Galyean (2019) using values from NRC (1996)

[a, b, c] Within row, means withs with uncommon superscript differ ($P \leq 0.05$)

Table 3.4 Effects of dietary energy concentrations and intake on intake and apparent total-tract digestibility in ruminally cannulated beef heifers

	Treatment[1]					
Item	**AL**	**LIM-75**	**LIM-80**	**LIM-85**	**SEM[2]**	***P*-value[3]**
Number of observations	8	8	8	8		
Intake, kg/d[4]						
DM	6.21^{a}	4.70^{b}	4.96^{b}	5.20^{b}	0.20	< 0.01
OM	5.80^{a}	4.45^{bx}	4.70^{bc}	4.91^{cy}	0.18	< 0.01
NDF	2.49^{a}	1.15^{b}	1.20^{b}	1.25^{b}	0.08	< 0.01
ADF	1.11^{a}	0.40^{b}	0.43^{b}	0.44^{b}	0.03	< 0.01
Digestibility, %[4,5]						
DM	70.6	78.1	74.4	73.7	3.48	0.53
OM	70.2	80.6	76.9	76.4	3.36	0.22
NDF	65.9	75.6	68.8	68.4	3.83	0.35
ADF	60.7	71.5	63.6	61.3	4.81	0.39

[1] AL: ad libitum, 0.99 Mcal NE_g/kg DM; LIM-75: 75% of AL intake, 1.32 Mcal NE_g/kg DM; LIM-80: 80% of AL intake, 1.32 Mcal NE_g/kg DM; LIM-85: 85% of AL intake, 1.32 Mcal NE_g/kg DM

[2] Standard error of the mean

[3] Treatment main effect

[4] DM = Dry matter, OM = Organic Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

[5] Apparent total-tract digestibility

[a,b,c] Within row, means with uncommon superscripts differ ($P \leq 0.05$)

[x,y,z] Within row, means with uncommon superscripts tend to differ ($0.05 \leq P$ 0.10)

Table 3.5 Effects of dietary energy concentrations and intake on ruminal fermentation characteristics in ruminally cannulated beef heifers

	Treatment[1]					
Item	**AL**	**LIM-75**	**LIM-80**	**LIM-85**	**SEM[2]**	***P*-value[3]**
Volatile fatty acids[4], m*M*						
Total	83.49	83.46	83.77	88.14	3.80	0.34
Acetate	45.78	46.05	45.60	47.78	1.63	0.65
Propionate	22.81[x]	22.85[x]	23.65[xy]	25.35[y]	1.66	0.08
Butyrate	9.71	9.44	9.44	9.85	0.70	0.88
Valerate	1.95	1.87	1.96	2.01	0.29	0.75
Isobutyrate	0.88	0.91	0.85	0.87	0.06	0.37
Isovalerate	2.34	2.36	2.24	2.34	0.14	0.91
Acetate:Propionate	2.24	2.18	2.19	2.12	0.12	0.41
Ammonia[4], m*M*	8.7[a]	6.04[b]	4.52[c]	6.28[b]	0.22	< 0.01
Liquid passage rate[5], %/h	9.4	6.6	9.5	8.1	0.02	0.63
Ruminal liquid volume[6], L	45.5	43.8	41.3	40.4	6.78	0.90
Ruminal pH[4]	6.21	6.13	6.13	6.21	0.10	0.52

[1] AL: ad libitum, 0.99 Mcal NE_g/kg DM; LIM-75: 75% of AL intake, 1.32 Mcal NE_g/kg DM; LIM-80: 80% of AL intake, 1.32 Mcal NE_g/kg DM; LIM-85: 85% of AL intake, 1.32 Mcal NE_g/kg DM

[2] Standard error of the mean

[3] Treatment main effect

[4] Average of values collected at 0, 2, 4, 6, 8, 12, 18, and 24 h post feeding

[5] Calculated from rumen samples collected at 2, 4, 6, 8, 12, and 18 h post feeding

[a,b,c] Within row, means with uncommon superscripts differ ($P \leq 0.05$)

[x,y,z] Within row, means with uncommon superscripts tend to differ ($0.05 \leq P < 0.10$)