IMPACT OF DIFFERENT BUFFERS ON MEASURES OF POST-RUMINAL FERMENTATION

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of a Bachelor's of Science Honors Degree in Pre-Veterinary Medicine and Animal Biosciences with Distinction

Spring 2017

IMPACT OF DIFFERENT BUFFERS ON MEASURES OF POST-RUMINAL ACIDOSIS

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ACKNOWLEDGMENTS

I would like to thank my thesis director, Dr. Tanya Gressley, for all of the opportunities she has given me as well as her guidance and encouragement over the past four years. I would not have been able to do this project without the help of Kassandra Moyer, Alexis Trench, Katherine Pacer, and Michael Palilo who helped with my trial throughout the summer and waking up at all hours of the night to help with sampling. I would also like to thank Elizabeth Hellings, Shane Cronin, Sofia Bialkowski and Ashley Taylor for helping me prepare my many samples for analysis. Also, the guidance and assistance I received from the graduate students Amanda Barnard, Stephanie Polukis, and Becca Savage was invaluable in completing this project. A special thank you to Amanda Barnard for helping me through the entire process and being a mentor to me throughout my experiences with undergraduate research. A big thank you to Mr. Ron Gouge, Mr. Richard Morris, and Mr. Mark Baker for their help with the care of dairy cows throughout my trial. Also, thanks to the Undergraduate Research Program who have provided guidance and support in writing my thesis. Lastly, I would like to thank all of my friends and family for their encouragement and support throughout this process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

With an emphasis on increased production, dairy cow rations have shifted to higher energy feeds with higher concentrations of starch. High starch rations are rapidly digested within the rumen, causing pH levels to decline and ruminal acidosis. However, there is also increased passage of fermentable carbohydrates to the intestines which can lead to intestinal acidosis. The purpose of this study was to evaluate the effects of different buffers on the severity of hindgut acidosis. In a 5 x 5 Latin square design, ten multiparous cows were randomly assigned to treatments of a high starch diet as the control (CON), the control diet supplemented with sodium bicarbonate (FSB), calcium carbonate (FCC), or calcium carbonate and magnesium oxide (FCCM), or the control diet with abomasally infused encapsulated sodium bicarbonate (ISB). It was hypothesized that the ISB, FCC, and FCCM treatments would be effective in ameliorating hindgut acidosis. Cows were housed in a tie-stall barn and were fed twice a day for ad libitum intake. Cows were infused twice daily with corn starch suspended in 1.5 L of tap water. Rumen fluid and fecal samples were collected on day 7 of each period at 4-hr intervals beginning at 6:30 am and ending at 2:30 am on the following day for measurement of pH, volatile fatty acids (VFA), and lipopolysaccharide (LPS). Feed samples were collected on day 7 of each period after the 6:30 am sampling. Milk samples were collected on day 7 during the morning and afternoon. Treatment did not affect rumen pH, but fecal pH was higher in the FCCM group (pH 6.64; $P < 0.001$) than in the CON group (pH 6.47; $P<0.001$). Time affected total rumen VFA, but not rumen lactate, acetate, propionate, isobutyrate, valerate, isovalerate, or total VFA ($P > 0.10$). There were no effects of treatment on fecal

lactate, butyrate, isobutyrate, valerate, or isovalerate ($P > 0.10$); however treatment affected acetate ($P = 0.04$) and propionate ($P= 0.03$) and tended to affect total VFA ($P = 0.07$). Total VFA were greater for FCC and FCCM compared to CON ($P = 0.03$ and 0.007, respectively). Similarly, acetate was greater for FCC and FCCM compared to CON ($P = 0.02$ and 0.003, respectively), and propionate was greater for FCC and FCCM compared to CON ($P = 0.01$ and 0.005, respectively). In addition, fecal acetate was lower in FSB compared to FCCM ($P = 0.05$). The contrast of CON vs. (ISB + FCC + FCCM) was also significant for total VFA, acetate, and propionate, due to lower VFA for CON vs. the proposed post-ruminal buffers. Fecal dry matter was affected by time ($P < 0.001$), due to the lowest dry matter at 0 h (13.0%), intermediate dry matter at 4, 12, and 16 h (13.4 to 13.8%), and greatest dry matter at 8 and 20 h (14.4 and 14.5%, respectively, however treatment did not affect fecal dry matter. The data suggest that FCC and FCCM have postruminal buffering capability, but data on LPS levels and digestibility (pairing of feed and fecal composition data) will be needed to support this conclusion.

Chapter 1

LITERATURE REVIEW

1.1 Bovine Digestions

The ruminant digestive system varies anatomically from that of monogastric animals as it evolved to enable the utilization of a forage-based diet. Feed in ruminants is channeled through three distinct diverticula (located towards the end of the esophagus) before entering the glandular stomach. The three diverticula are referred to as the forestomach and are lined with non-glandular stratified squamous epithelium. Within these three compartments, digestion is primarily done by microorganisms which ferment feed materials. The largest of the diverticula are the first and second compartments which are the rumen and reticulum, respectively. The two compartments are collectively referred to as the ruminoreticulum because of their functional and anatomical similarities. In the beginning stages, digesta is able to move between the shared space located cranially between the two compartments. The lower part of these compartments are separated by the muscular ruminoreticular fold (Frandson et al., 2009). The last of the three diverticula is the omasum, a spherical organ containing muscular laminae. From the omasum, digesta travels into the glandular stomach known as the abomasum. The abomasum contains two glandular regions, anatomically similar to the fundic gland region and the pyloric gland region

of the monogastric stomach. These two glandular regions secrete water, hydrochloric acid, mucus, and pepsin, collectively known as gastric juices, to begin the chemical portion of digestion. Some of the protein in the digesta is hydrolyzed by pepsin, but further proteolysis as well as hydrolysis of carbohydrates and lipids takes place in the small intestines. The intestinal epithelial cells absorb the nutrients released by this breakdown and the remainder of the digesta continues to the large intestine. The large intestine is mainly responsible for water reabsorption and further fermentation of carbohydrates (Frandson et al. 2009).

1.2 The Rumen

The rumen acts as a fermentation chamber to begin the digestion of the forage based diet consumed by foregut fermenters such as cattle. Carbohydrates that are commonly seen in the plant based diet of ruminants are soluble fibers, hemicellulose, starches, and cellulose. The latter cannot be digested by mammals as they do not possess enzymes that can cleave the presences of β-linkages between the glucose molecules within cellulose (Pond et al., 2005). Therefore, due to the presence of cellulose in the cell walls of plants, cattle have adapted numerous techniques to get the most out of the feed they ingest, two of which are rumination and a mutualistic relationship with the bacteria that make up their gut microbiome. The process of rumination, in which the animal regurgitates larger pieces of food to be further broken down mechanically before re-swallowing, results in increased surface area available

for bacteria to aid in digestion. The delay in digestion allows for further solubilizing of the fibrous materials of the cell wall in forages (Mackie, 2002). A wide array of microorganisms that reside in the rumen are responsible for the fermentation of carbohydrates found within forages, some of which the animal would not be able to digest on its own. The microbiome of the rumen is dominated by bacteria that ferment starch and perform cellulolytic digestions, releasing volatile fatty acids (VFA) that can be absorbed directly through the rumen wall, but ciliate and flagellate protozoa, bacteriophages, anaerobic fungi are also present (Jewel et al., 2015).

1.3 Small and Large Intestines

After passing through the abomasum, partially digested feed and rumen microbes enter the small intestines as digesta. Enzymes break down proteins, lipids, and some carbohydrates, and the monomers (amino acids, fatty acids and monosaccharides) are absorbed through the intestinal epithelium. Any feed or microbes that are not digested and absorbed by the small intestines are then passed through to the large intestine where microbiota are present that further breakdown previously undigested carbohydrates, a process referred to as hindgut fermentation. This fermentation produces VFAs, which are absorbed through the epithelial wall of the large intestines. Anything that is not degraded and absorbed by the end of the large intestines is excreted as feces. On average 41% of organic matter is fermented within the rumen, 26% is absorbed within the small intestine, and 4% is fermented within the

large intestine. The remaining 29% of organic matter is passed as feces (Gressley et al., 2012).

1.4 Rumen Acidosis

In an effort to keep up with the energy needs of high producing dairy cattle, total mixed rations (TMR) for dairy cattle are high in rapidly fermentable carbohydrates, primarily starch. Rumen acidosis is characterized by the decrease of ruminal pH leading to an increase in VFA and propionate production, increases in microbial endotoxins, decreased milk production, and reduced dry matter intake. Rumen acidosis also reduces rumen motility, thereby reducing rumen efficiency, and salivary production, which provides buffers and acts as a lubricant to protect the walls of digestive organs (Slyter, 1976). Buffers like sodium bicarbonate and magnesium carbonate can also be used to increase rumen pH immediately after feeding and maintain a less acidic pH (Schaefer et al., 1982). The proportion of cellulolytic bacteria is decreased by a drop in pH, making digestion of a forage-based diet more challenging. In addition to decreased efficiency and tissue damage, acidosis also increases endotoxin levels within the rumen. After only 24 hours of feeding high concentrate diets, lactobacilli become the most numerous microbe in the rumen. Microbial endotoxin-producing coliforms and *C. perfringens* increase as well. Under acidosis conditions in the large intestines, microbial endotoxins levels increase in the cecum (Slyter, 1976) Endotoxin is a term used to describe toxins released by gram-

negative bacteria, such as lipopolysaccharides (LPS), during periods of growth or during lysis of these bacteria. As populations of lactobacilli grow and produce lactic acid, many bacteria are lysed because of their inability to survive in the low pH environment; while other forms of bacteria that can be considered acidophilic thrive in the low pH environment (Mao et al., 2013).

In a study completed by Steele et al. (2011), four mature non-lactating dairy cows were fed high forage (HF) diets, then transitioned to a high grain (HG) diet for a three-week period. After completion of the three-week period, cows were transitioned back to the HF diet and fed this diet for another three weeks. Rumen pH was measured weekly throughout the study and biopsies of rumen papillae were taken during the first and last week of each feeding period. Rumen pH dropped during the first week of the HG diet, indicating subacute ruminal acidosis (SARA). During this time, microscopic examination of the papillae showed a decrease in stratum basal, spinosum, and granulosum layers as well as a reduction in the total depth of the rumen epithelium. The most dramatic differences in the papillae were seen during the decrease in rumen pH when SARA was diagnosed (Steele et al. 2011). These results demonstrated that when damage occurs in the rumen from SARA, the junctions between cells loosen. Potentially allowing endotoxins into the peripheral blood stream, leading to localized and systemic inflammation.

1.5 Post-Ruminal Acidosis

The drop in pH caused by increased fermentation of carbohydrates within the rumen during ruminal acidosis continues to have a negative effect on ruminant digestion as fermented carbohydrates and VFAs travel past the rumen and into the small and large intestines. Lowered pH in the intestines not only damages the intestinal epithelium, it also decreases intestinal absorption of nutrients. A pH below 6.9 inhibits pancreatic alpha amylase which breaks down starch to release glucose for absorption by the small intestine (Wheeler et al. 1980). In a study done by Wheeler et al. (1980), fecal pH and fecal starch levels proved to be a good indicator of intestinal pH. Cattle fed high energy diets had low fecal pH and large amounts of starch in feces, corresponding to low pH and high starch concentrations within the intestines. Ruminants evolved in environments with forage that contained only very small amounts of starch. Therefore they produce less pancreatic alpha amylase than monogastric animals. The activity of the already low amounts of pancreatic alpha amylase is reduced by the lowered pH and therefore, the small intestines are unable to utilize a large amount of the starch in high energy feeds. The starch is then passed directly into the large intestine and partially fermented into VFA before moving into the feces. In addition to decreased utilization of starch, lowered pH also increases the populations of endotoxin releasing bacteria. Lactic acid producing bacteria such as lactobacilli have been found in the intestines of cattle when ruminal pH becomes less than 5.5 to 5.0 (Slyter, 1976). When Bissell (2002), infused 5 grams of starch/kg of body weight per day over a three-day period in order to analyze post-ruminal acidosis, cows receiving the infusion showed a decrease in fecal pH and the appearance of

mucus, tissue segments, and mucin casts in the feces, providing evidence of epithelial damage and hindgut acidosis.

In a study by Tao et al. (2014a), eight goats with rumen cannulas were divided into two groups. One was fed a high concentrate diet (65% concentrate of DM) and the other a low concentrate diet (35% concentrate of DM) for six consecutive weeks. Throughout the six weeks, rumen fluid, plasma, and hindgut mucosa tissue was collected. The feeding of the high concentrate (HC) diets led to a significant decrease in ruminal pH and obvious damage to the mucosal epithelium of the hindgut. Tight junctions between the cells of the hindgut epithelium were compromised in HC goats but not low concentrate (LC) goats. This caused the hindgut to become "leaky" since there is only a single layer of epithelial cells in the large intestine (Tao et al. 2014a). In a similar study by Tao et al. (2014b), twelve mid-lactation goats were randomly assigned to either a HC or LC diet for a 10-week period. Goats fed the HC diet showed a significant increase in VFA and of starch contents within their colonic digesta as compared to those of the LC goats. HC goats also showed severe changes in the structure of their colonic epithelium and a loosening of the tight junctions between colonic cells, leading to an inflammatory response and apoptosis of the colonic epithelial layers (Tao et al. 2014b). Together, these studies demonstrate that high grain diets not only compromise the rumen but can also lead to inflammation and epithelial damage in the hindgut.

1.6 Release of Endotoxins in the Gastrointestinal Tract

As explained by Nagaraja et al. (1978), the increased presence of carbohydrates from high concentrate diets causes a change in the microbial population of both the rumen and cecum. These observed changes are characterized by the destruction of protozoa and cellulolytic bacteria and an increase in gram positive cocci. The destruction of gram negative bacteria leads to the release of lipopolysaccharides (LPS), a type of endotoxin. The release of LPS causes damage to rumen epithelium barrier function and inflammation in the rumen wall (Naragaja et al. 1978). In a study by Gohzo (2005), the relationship between SARA inflammatory response increase in LPS endotoxins was analyzed. Free endotoxins were able to travel into the peripheral bloodstream and trigger an inflammatory response throughout circulation in cows fed a high concentrate diet, likely due to damage of epithelial cells (Gozho et al. 2005). Due to the lowered pH during SARA, the concentration of free LPS endotoxins increases by about five-fold (Emmanuel et al. 2007). This significant increase in endotoxins in combination with the ability for these endotoxins to enter the peripheral blood stream allows for infiltration of pathogens to surrounding organs, causing localized and systemic inflammation (Khafipour et al. 2009).

In a study by Li et al. (2012), six non-lactating Holstein cows with cannulas in the rumen and cecum were randomly assigned to treatments in a 3x3 Latin square design and fed either a 70% forage diet (control), a 34% grain diet (high grain), or a

high forage diet (37% of DM was replaced with ground alfalfa). Samples of rumen fluid, blood, cecal digesta, and fecal samples were taken before and after feeding at the end of each period. An increase in LPS concentration in cecal digesta and an increase in LPS binding proteins in blood serum of treated cows was observed. The authors concluded that the increase in LPS binding protein in cows given the high grain diet was due to the increased growth of LPS-producing bacteria within the hindgut and not solely due to increased LPS-producing bacteria within the rumen. Bile within the small intestine leads to degradation of rumen-generated LPS, but the continued presence of LPS later on in the digestive tract, as well as in fecal matter, suggested that LPS-producing bacteria grew in the cecum (Li et al. 2012). These studies demonstrate that due to the damage of intestinal epithelial barriers caused by ruminal and hindgut acidosis, endotoxins are capable to entering the blood stream and inducing systemic inflammation.

1.7 Buffers

In an effort the decrease the effects of ruminal acidosis, many dairy farms supplement their high energy diets with buffers to mitigate the fluctuation in ruminal pH. Sodium bicarbonate is commonly used to buffer acidic conditions within the rumen in early lactation cattle when their diets are rapidly switched from a maintenance feed to a high energy feed for production (Kilmer et al., 1980). Sodium bicarbonate in high energy rations increased the pH in the rumen and nutrient

digestibility is increased which results in an increase in milk yield (Kilmer et al., 1981). Varner et al. (1972), showed that the addition of calcium carbonate and starch to the rations of steers fed a high energy diet significantly increased organic matter and cellulose digestion, as well as energy digestibility overall.

Although a significant amount of work with buffers has been done, most focused on protecting the rumen, however little has been done to see how to buffer the hindgut. Potential buffers that could be used to protect the hindgut include magnesium oxide and calcium carbonate. In studies by Christiansen et al. (1990) and Teh et al. (1985), feeding of magnesium oxide consistently increased fecal pH to levels higher than that of control groups. This outcome showed the efficacy of magnesium oxide in buffering the intestines and neutralizing acids in the intestinal environment. In a study by Wheeler and Noller (1977) on 9 Holstein steers fed high grain diets calcium carbonate and magnesium limestone led to higher fecal and intestinal pH values, as well as reduced fecal starch contents as compared to those of control cows. These studies suggest that it may be possible to effectively buffer the intestines in cows fed high starch diets using additives such as magnesium oxide and calcium carbonate. Additionally, encapsulated sodium bicarbonate products are marketed for horses to reduce the risk for hindgut acidosis. It is possible that encapsulated buffers may be of benefit for dairy cattle as well, though to date these types of buffers have not been evaluated in ruminants.

1.8 Post-Ruminal Starch Infusions

To experimentally induce post-ruminal acidosis without concurrent ruminal acidosis, carbohydrates can be infused directly into the abomasum by passing an infusion line through the rumen of cannulated cows. Mainardi et al. (2011) used abomasal infusions of 1 g of oligofructose/ 1 kg of body weight to induce hindgut acidosis in six ruminally cannulated Holstein steers. Oligofructose was used because it is indigestible by mammalian enzymes and thereby provides as a substrate specifically for gastrointestinal microbes. Animals given the oligofructose infusion had lower fecal pH than those given the control, as well as increased fecal excretion of microbial fermentation products, signs of increased hindgut fermentation. The relevance of this model to actual intestinal acidosis in cows can be questioned as oligofructose was provided as a substrate to intestinal microbes, whereas intestinal acidosis that accompanies ruminal acidosis is typically due to hindgut fermentation of starch. A follow-up study by Gressley et al. (2016) directly compared abomasal oligofructose infusion to abomasal starch infusion on fecal measures of hindgut fermentation. The authors found that abomasal starch and abomasal oligofructose caused similar decreases in fecal pH and increases in fecal LPS.

1.9 Objectives and Hypothesis

The objective of the current experiments was to compare the effectiveness of fed sodium bicarbonate (FSB), calcium carbonate (FCC), calcium carbonate with

added magnesium oxide (FCCM), and abomasally infused encapsulated sodium bicarbonate (ISB) as buffers when hindgut acidosis was induced by the infusion of 1 g of starch/1 kg of BW. It was hypothesized that the ISB, FCC, and FCCM treatments would be effective in ameliorating hindgut acidosis. The effectiveness of these treatments would be determined by rumen and fecal pH, VFA, and endotoxin concentrations.

Chapter 2

MATERIALS AND METHODS

2.1 Animals and Treatment

Ten ruminally cannulated, multiparous mid-lactation Holstein cows were individually housed in the University of Delaware's tiestall facility. At the start of the trial, average body weight was 736.8 ±74.8 kg and days in milk was 188.4 ± 50.2 days. Cows were ad libitum fed a total mixed ration typical for high producing, early lactation cows (Table 1). Cows were fed twice daily (0800h and 2000h), with 70% of daily feed in the morning and 30% fed at night. Daily offered and refused amounts were recorded and aimed for ~5% refusal. All animal procedures were approved by the University of Delaware Institutional Animal Care and Use Committee.

Prior to the experiment, all cows were fitted with abomasal infusion lines that were inserted through the ruminal cannula, as described by Gressley et al. (2006). Briefly, the infusion lines consisted of about five meters of flexible tubing with a flexible plastic disc attached to the end. The insertion device was constructed of PVC pipe, which can be placed in the rumen, passed through the omasum orifice, and into the abomasal orifice. A flexible disc was folded and placed into the PVC pipe, and was put into the proper location within the cow through the rumen cannula. Once the device entered the abomasum, the disc unfolded to hold the infusion line in place. The other end of the infusion line was threaded through a hole in the center of the cannula plug and held in place with clamps. The placement of the infusion line was checked

twice per week and remained in the abomasum for the duration of the trial. At the end of the trial, the infusion lines were removed by hand.

Cows were weighed for two consecutive days at the start of the trial to calculate the amount of starch that would be infused daily. Cows were infused with 0.5g/1kg bodyweight of corn starch twice daily at the time of feeding (1g/1kg of bodyweight per day) (Table 2). Cows were given twice daily infusions of corn starch (Ingredion, Westchester, IL) suspended in 1.5L of tap water at the time of feeding. The starch mixture was placed in plastic bottles and mixed rigorously both before and during the infusions. Infusions were administered using hand pumps at 0800h and 2000h. On average, cows were give about 736 g/d of starch, which was about 10% of their total daily starch intake.

Cows were assigned to a 5X5 Latin square design with 7-d periods for five consecutive periods. Treatments were A) control ration (CON), B) control diet plus 200g/d sodium bicarbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (FSB), C) control diet plus abomasal infusions of 336 g/d, 168g at am feeding and 168g at pm feeding, of encapsulated sodium bicarbonate (Equishure Balchem, New Hampton, NY) added to corn starch infusions (ISB), D) control diet plus 200 g/d of calcium carbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (FCC), and E) control diet plus 125 g/d calcium carbonate (87.5g at am feeding and 37.5 at pm feeding) and 75 g/d of magnesium oxide (52.5g at am feeding and 22.5g at pm feeding) hand mixed into feed (FCCM). Cows received treatments twice daily during feeding.

2.2 Feed Samplings and Analyses

Samples of the individual feeds and two independent samples of TMR were collected on day 7 of each period. A portion of each individual feed sample was dried at 60°C for 48h and the dry matter (DM) results were used to adjust TMR mix amounts to account for fluctuations in DM. The remainder of the individual feeds were sent to Cumberland Valley Analytical Services (CVAS) for nutrient analysis. The TMR was also sent to CVAS for analysis of nutrient composition and 240-h indigestible NDF (iNDF).

2.3 Sample Collection

Samples collected throughout the trial included milk, fecal, and rumen samples, all of which were collected on day 7 of each period. Milk yield was recorded at each milking throughout the study and samples were collected at both milkings on day 7 (0430h and 1530h). All samples were sent to Dairy One Cooperative Inc. (Ithaca, NY) for NIR analysis of lactose, protein, fat, somatic cell count (SCC), and milk urea nitrogen (MUN) using a Milkoscan System 4000 (Foss North American, Eden Prairie, Minnesota, USA). Fecal and rumen samples were collected every 4 hours on day 7 of each period, starting at 0630h and continuing to 0230h the following day.

Fecal samples of about 200g/cow were collected via rectal palpation at each time point. For fecal pH, 20 $\pm$ 2g of feces were added to a 50ml conical vial

containing 20ml of distilled water. The sample was vigorously shaken then passed through two layers of cheesecloth into a beaker where a pH meter was inserted into the liquid, given time to equilibrate, and the value was recorded. For VFA analysis, 25 $\pm$ 5g of feces was weighed and the exact amount recorded. The feces was added to a 50ml conical vial containing 10ml of 2% H_2SO_4 solution and vigorously mixed. The resulting solution was strained through two layers of cheesecloth and frozen at -20°C. For LPS measurement, about 1g of feces was placed in a 2ml LPS-free microcentrifuge tube and frozen at -20°C. About 100g of the remaining feces was frozen at -20°C for later DM analysis.

Rumen fluid was collected from four different locations within the ventral rumen sac and strained through two layers of cheesecloth into a beaker, where a pH meter was inserted into the liquid, given time to equilibrate, and the value was recorded. Ten ml of rumen fluid was placed into a 15ml conical vial containing 0.2ml of 50% H_2SO_4 solution and stored at -20°C until later VFA analysis. For later LPS measurements, about 1ml of rumen fluid was placed in a 2ml LPS-free microcentrifuge tube and frozen at -20°C.

2.4 Sample Analysis

The analysis for fecal and rumen VFA was performed using high performance liquid chromatography (HPLC) as described by Mainardi et al. (2011). The endotoxin concentration of both fecal and rumen samples were determined using a commercial

chromogenic end point *Limulus* amebocyte lysate (LAL) assay (QCL-1000™ Endpoint Chromogenic LAL Assay, Lonza Ltd., Basel, Switzerland). Samples were centrifuged at 10,000 x g for 30 minutes at room temperature, the supernatant was diluted 1:10 using endotoxin-free LAL reagent water, and passed through 0.22-µm microcentrifuge filter tubes (Costar Spin-X, Corning Inc. Corning, NY) via centrifugation at 13,300 x g for 2 minutes. Samples were diluted further to 1:100 using endotoxin-free LAL reagent water. The samples were treated with a 1:1 ratio of β-1, 3 glucan blocker (β-G Blocker, Lonza Ltd., Basel, Switzerland). A standard curve was created which ranged from 0 to 1 endotoxin units (EU)/mL. Samples were prepared according to kit instructions and the results were read using a plate reader (Molecular Devices, Sunnyvale, CA) set to 405nm wavelength. When samples failed to fit the standard curve they were further diluted to final dilutions of 1:5000, 1: 10,000, 1: 25,000, and 1: 50,000.

Fecal samples collected for measurement of DM and nutrient composition were thawed and dried at 55°C for 72h in a forced air oven. Samples were composited by cow and period and sent to CVAS for analysis of NDF, ADF, starch, ash, and indigestible NDF.

2.5 Statistical Analysis

Mean milk yield and dry matter intake were calculated from the last three days of each period. Milk composition for each cow within each period was calculated as

the mean of the am and pm values. Dry matter intake, milk yield and composition, and nutrient digestibility were evaluated using the Glimmix procedure of SAS. The model included the main effects of treatment, period, and square, and the random effect of cow within square. Differences among fixed effects were estimated using the pdiff option of the lsmeans statement. The overall effect of postruminal buffer was evaluated using a contrast statement with values of -1, 0, 0.34, 0.33, and 0.33 for CON, FSB, ISB, FCC, and FCCM, respectively.

Repeated measures (pH, VFA, LPS) were analyzed using the Glimmix procedure of SAS. The model included the fixed effects of treatment, period, square and hour and the interaction of treatment by hour and the random effects of cow within square and cow by period by treatment. Repeated measures were indicated using the "random _residual_" statement with a subject of period by treatment by cow within square and an autoregressive covariance structure. Differences among fixed effects were estimated using the pdiff option of the lsmeans statement. The overall effect of postruminal buffer was evaluated using a contrast statement with values of -1, 0, 0.34, 0.33, and 0.33 for CON, FSB, ISB, FCC, and FCCM, respectively. When the treatment by hour interaction was significant, the Tukey's adjustment was used to evaluate differences among treatment at each individual time

Chapter 3

RESULTS

3.1 Feed intake and Milk Component Analysis

Treatment did not affect intake, milk yield, or milk composition (Table 4). On average, cows consumed 26.0 kg DM and produced 32.4 kg/d milk with 3.54% fat and 3.08% protein.

3.2 Rumen Sample Results

3.2.1 Rumen Volatile Fatty Acids

Effects of treatment on rumen VFA and lactate are presented in Table 5. Time affected all VFA and lactate ($P < 0.05$), and the effect of time on total VFA is presented in Figure 1. Total rumen VFA were lowest at 0 h, highest at 16 h, and intermediate at the other times. There were no effects of treatment on rumen lactate, acetate, propionate, isobutyrate, valerate, isovalerate, or total VFA ($P > 0.10$). Treatment tended to affect butyrate ($P = 0.08$). As illustrated in Figure 2, this was due to lower butyrate for ISB compared to CON at 12 h (12.3 vs. 14.2 mM, $P = 0.05$) and 16 h (13.8 vs 17.1, $P < 0.001$). The contrast of CON vs. the three treatments expected to have post-ruminal effects (ISB, FCC, and FCCM) was also significant ($P = 0.02$), due to lower butyrate in those treatments compared to the CON.

3.2.2 Rumen pH

Treatment did not affect rumen pH, but an effect of time was observed ($P < 0.001$; Table 7). There were no differences in rumen pH between 4, 8, and 12 ($P >$

0.10), but all other times differed from one another ($P < 0.01$; Figure 3). Rumen pH was highest at 0 h, and lowest at 16 h.

3.3 Fecal Sample Results

3.3.1 Fecal Dry Matter

Treatment did not affect fecal dry matter ($P = 0.39$), which averaged 13.8% across treatments (Table 7). Fecal dry matter was affected by time ($P < 0.001$), due to the lowest dry matter being recorded at 0 h (13.0%), intermediate dry matter at 4, 12, and 16 h (13.4 to 13.8%), and greatest dry matter at 8 and 20 h (14.4 and 14.5%, respectively; data not shown).

3.3.2 Fecal Volatile Fatty Acids

Effects of treatment on fecal VFA and lactate are presented in Table 6. Time affected all VFAs and lactate ($P < 0.05$), and the effect of time on total VFA is presented in Figure 4. Total fecal VFAs were highest at 0 and 20 h, lowest at 12 and 16 h, and intermediate at the other times. There were no effects of treatment on fecal lactate, butyrate, isobutyrate, valerate, or isovalerate ($P > 0.10$). Treatment affected acetate ($P = 0.04$) and propionate ($P= 0.03$) and tended to affect total VFA ($P = 0.07$). Total VFAs were greater for FCC and FCCM compared to CON ($P = 0.03$ and 0.007, respectively; Figure 4). Similarly, acetate was greater for FCC and FCCM compared to CON ($P = 0.02$ and 0.003, respectively; Figure 5), and propionate was greater for FCC and FCCM compared to CON ($P = 0.01$ and 0.005, respectively; Figure 6). In addition, fecal acetate was lower in FSB compared to FCCM ($P = 0.05$). The contrast of CON vs. (ISB + FCC + FCCM) was also significant for total VFA, acetate, and

propionate, due to lower VFA for CON vs. the proposed post-ruminal buffers (Table 6).

3.3.3 Fecal pH

Fecal pH was affected by both treatment and time ($P < 0.001$; Table 7). Fecal pH was greater for FCCM compared to all other treatments ($P < 0.01$). As shown in Figure 7, the effect of time was due to lowest pH at 0, 8, and 20 h, intermediate pH at 4 and 12 h, and greatest pH at 16 h.

3.4 LPS and Digestibility

Results of LPS concentrations for both fecal and rumen samples are still pending, as well as results for starch digestibility.

Chapter 4

DISCUSSION

Starch is one of the most efficient carbohydrates that can be fermented in the rumen and promote microbial growth, however, when high starch diets are quickly digested within the rumen, pH levels can decrease, causing acidosis. There is also increased passage of fermentable carbohydrates to the intestines. Some of these carbohydrates are degraded by enzymes, but typically their levels are too high to be completely degraded, causing the excess to flow to the large intestine where microbial fermentation occurs. Therefore, acidosis typically leads to both low rumen pH and low pH in the large intestine. The epithelium of the rumen consists of stratified cell layers and is more capable of withstanding this lowered pH than the epithelium of the large intestine, which is comprised of only one layer of cells. It is possible that damage to the large intestine contributes to laminitis and other health problems resulting from acidosis. In the present study, we aimed to evaluate the impact of calcium carbonate, magnesium oxide, and encapsulated sodium bicarbonate on measures of intestinal fermentation in cows fed a typical high starch lactating cow ration and abomasally infused with 1 g/1 kg of bodyweight per day of starch to challenge the large intestines (Mainardi et al., 2011). With this challenge to the large intestines, we were expecting to see signs of hindgut acidosis. The effectiveness of the ISB, FCC, and FCCM treatments administered could then be determined. The starch in the FSB treatment was expected to be completely degraded within the rumen and this treatment was therefore used as a second control with rumen buffering activities only.

Rumen acidosis typically leads to decreased milk production and decrease feed intake, so in combating the effects of acidosis, buffers should have the ability to

mitigate these symptoms (Slyter, 1976). The effects of excessive hindgut fermentation on performance are less clear, but typically the only production measure affected is a decrease in milk fat percentage (Gressley et al., 2012). There was no effect of treatment on feed intake, milk production and milk composition, meaning that increased ruminal buffering (FSB) or increased intestinal buffering (ISB, FCC, FCCM) did not impact performance. We fed a typical high starch lactating cow diet, but this diet was balanced to contain adequate forage and buffers to prevent sub-acute ruminal acidosis. We were successful in achieving this goal, as rumen pH was never below 5.6, and was only below 5.8 at one-time point (Figure 3). The FSB treatment was expected to increase rumen pH, but this did not occur, perhaps because sodium bicarbonate inclusion in the base ration was already sufficient.

As suspected, total rumen VFA was lowest during the first sampling time (0h) when the cows had not yet been fed, and highest at 16h, 4 h after their second feeding. The only VFA that was affected by treatment was butyrate, which was lower for ISB cows as compared to the CON. In addition, the contrast of ISB, FCC and FCCM vs. CON was also significant, with lower rumen butyrate for the proposed postruminal buffer treatments compared to the CON. The impact of postruminal buffers on ruminal butyrate was unexpected, particularly for ISB, as this treatment was administered postruminally and should not have affected any VFAs within the rumen.

In order to see the effect of buffer treatments on hindgut fermentation, our study looked at fecal dry matter, fecal VFA, and fecal pH. Time affected all fecal measures. In a study done by Bissell (2002), low fecal pH was associated with increased hindgut fermentation, So, when we observed that at 0 h, fecal pH and dry matter were lowest and fecal VFA were highest, we were able to conclude that this

was the time of greatest hindgut fermentation. This time represented 12 h after postruminal starch infusion and 8 h after the greatest and lowest observed rumen VFA and pH, respectively. As seen in a study completed by Tao et al (2014b), goats fed a high concentrate diet showed a significant increase in VFA content within the colonic digesta, as well as a higher content of starch within the colonic digesta as compared to goats receiving a low concentrate diet. (Tao et al. 2014b). The results from the current study parallel the findings by these authors as increased VFA concentrations in fecal matter correlated to times of increased hindgut fermentation.

There was no change in fecal dry matter as a result of treatment, however there was an effect of treatment on both fecal VFA and pH values. Fecal pH was greater for FCCM compared to all other treatments. These findings are in agreement with those reported by Christiansen et al. (1990) and Teh et al. (1985), who found that feeding of magnesium oxide consistently increased fecal pH to levels higher than that of CON groups. To our knowledge, this was the first time that Equishure (the ISB treatment) has been administered postruminally to cows, and we expected this product to release sodium bicarbonate in the intestines and increase fecal pH. Similarly, we expected the FCC treatment to increase fecal pH as has been observed previously in cattle (Wheeler and Noller, 1977). Counter to these expectations, ISB and FCC did not increase fecal pH in the present trials. Possibly, the doses were too low to provide sufficient postruminal buffering. In the case of FCC, it is also conceivable that neutralization of acids in the rumen exhausted the carbonate supply before it could reach the intestines. DISCUSS DOSAGES? Alternatively, it is possible that some buffering action occurred in the intestinal content, but was no longer discernable in the fecal samples that were analyzed.

Total fecal VFA tended to be affected by treatment and was higher for FCC and FCCM compared to CON. In addition, fecal acetate and propionate were greater for FCC and FCCM compared to CON, and the contrast of CON vs. the proposed postruminal buffers (ISB, FCC, and FCCM) was significant for acetate, propionate, and total VFA. These results were unexpected, as it was hypothesized that increased buffering in the intestines would reduce excessive fermentation and therefore decrease fecal VFA. On the contrary, the intestinal buffer treatments actually increased fecal pH. It is possible that the buffers resulted in a more stable intestinal environment that promoted microbial fermentation. This hypothesis is supported by the observation that sodium bicarbonate can increase ruminal VFA in s concentration-dependent manner (DePeters et al. 1984). VFA levels in feces could also have increased because absorption of the acids by the intestines slows with increasing digesta pH (Myers et al., 1967).

The findings of this study suggest that when a challenge of post ruminal fermentation is imposed on the gastrointestinal tract of mid-lactation cows being fed a high grain diet, supplementing the diet with magnesium oxide and calcium carbonate treatments has the potential to alter intestinal fermentation and to possibly mitigate some of the negative effects of hindgut acidosis. The case for such positive effects will be strengthened if the data from the analyses of total tract digestibility and fecal LPS levels show increased digestibility and decreased LPS concentrations for the FCC and FCCM treatments.

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Appendix A

TABLES

Table 1. Feed Components

Components	% DM	Components	% DM
Corn Silage	39.6	Sodium Chloride	0.44
Ground Corn	14.6	Urea	0.43
Alfalfa Silage	10.8	Palm Fat	0.35
Ground Soybean Hulls	7.73	Rumensin	0.30
Canola Meal	6.13	Magnesium Oxide	0.29
Treated Soybean Meal	5.97	Monocalcium Phosphate	0.26
Citrus Pulp	5.26	Calcium Carbonate	0.23
Porcine Blood Meal	1.74	Calcium Sulfate	0.19
Sugar	1.63	Trace Minerals and Vitamins	0.12
Orchardgrass Hay	1.48	Selenium	0.06
Rumen Protected Fat	0.84	Rumen Protected Methionine	0.05
Corn Gluten Meal	0.82	Vitamin E	0.04
Sodium Bicarbonate	0.60	Biotin	0.004
Live Yeast	0.002		

2. Body Weights and Starch Dosage

Cow	Average Body Weight (BW in lbs)	Dosage (kg)
83	1752.5	795
144	1480	671
80	1565	710
148	1527.5	693
100	1490	676
108	1572.5	713
92	1785	810
138	1400	635
985	1902.5	863
106	1767.5	802

3. Chemical Composition of TMR as a percentage of DM

% DM	Alfalfa silage	Corn silage	Grain	TMR
CP	18.76	8.34	25.84	17.06
NDF	45.46	38.00	16.22	30.10
ADF	40.40	23.10	11.78	20.96
Starch	N/A[1]	32.04	18.94	21.92
NFC	25.00	47.60	47.06	44.02
Ash	10.76	3.86	10.87	7.75
Ca	1.12	0.29	1.7	0.99
P	0.35	0.35	0.54	0.43
Mg	0.33	0.26	0.86	0.52
K	2.62	1.02	1.45	1.43
Na	0.06	0.01	1.1	0.47

[1]N/A = not analyzed

Table 4. Intake and Milk Yield

	Treatment[1]						*P* values	
	Control	FSB	ISB	FCC	FCCM	SEM	Treat	Contrast[2]
DMI, kg/d	26.5	26.5	25.3	25.9	25.6	1.0	0.18	0.17
Milk, kg/d	32.0	32.4	32.8	32.3	32.5	2.6	0.96	0.50
Fat, %	3.54	3.48	3.59	3.56	3.52	0.19	0.95	0.91
Fat, kg/d	1.13	1.12	1.16	1.14	1.13	0.10	0.93	0.72
Protein, %	3.08	3.06	3.09	3.10	3.08	0.06	0.95	0.85
Protein, kg/d	0.98	0.99	1.01	1.00	0.99	0.08	0.95	0.61
Lactose, %	4.69	4.72	4.67	4.66	4.70	0.04	0.24	0.83
MUN, mg/dL	10.3	10.4	10.1	10.5	10.7	0.6	0.82	0.91
SCC, log_{10}	1.96	1.88	1.98	1.93	1.85	0.14	0.50	0.37

[1] Treatments were A) control ration (**CON**), B) control diet plus 200g/d sodium bicarbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (**FSB**), C) control diet plus abomasal infusions of 336 g/d, 168g at am feeding and 168g at pm feeding of encapsulated sodium bicarbonate (Equishure Balchem, New Hampton, NY) added to corn starch infusions (**ISB**), D) control diet plus 200 g/d of calcium carbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (**FCC**), and E) control diet plus 125 g/d calcium carbonate (87.5g at am feeding and 37.5 at pm feeding) and 75 g/d of magnesium oxide (52.5g at am feeding and 22.5g at pm feeding) hand mixed into feed (**FCCM**).

[2]Contrast compared Control (-1) to ISB (+0.34), FCC (+0.33) and FCCM (+0.33).

Table 5. Rumen Volatile Fatty Acids

	Treatment[1]						*P* values			
	Control	FSB	ISB	FCC	FCCM	SEM	Treat	Hour	Treat × Hour	Contrast[2]
Lactate, mM	0.58	0.48	0.53	0.56	0.72	0.17	0.88	0.001	0.95	0.92
Acetate, mM	78.9	76.5	75.3	77.2	79.4	1.7	0.24	0.001	0.82	0.33
Propionate, mM	27.3	26.6	25.9	28.1	26.7	1.6	0.72	0.001	0.70	0.95
Butyrate, mM	13.5_{A}	$13.0_{A,B}$	12.3^{B}	$12.9_{A,B}$	$13.0_{A,B}$	0.4	0.08	0.001	0.57	0.02
Isobutyrate, mM	1.29	1.28	1.26	1.28	1.27	0.02	0.80	0.04	0.32	0.26
Valerate, mM	1.47	1.45	1.48	1.48	1.56	0.11	0.70	0.001	0.86	0.61
Isovalerate, mM	1.84	1.76	1.84	1.91	1.72	0.19	0.73	0.001	0.82	0.90
Total VFA, mM	124.6	120.9	118.4	123.0	124.8	3.2	0.21	0.001	0.83	0.31

[1] Treatments were A) control ration (**CON**), B) control diet plus 200g/d sodium bicarbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (**FSB**), C) control diet plus abomasal infusions of 336 g/d, 168g at am feeding and 168g at pm feeding of encapsulated sodium bicarbonate (Equishure Balchem, New Hampton, NY) added to corn starch infusions (**ISB**), D) control diet plus 200 g/d of calcium carbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (**FCC**), and E) control diet plus 125 g/d calcium carbonate (87.5g at am feeding and 37.5 at pm feeding) and 75 g/d of magnesium oxide (52.5g at am feeding and 22.5g at pm feeding) hand mixed into feed (**FCCM**).

[2]Contrast compared Control (-1) to ISB (+0.34), FCC (+0.33) and FCCM (+0.33).

Table 6. Fecal Volatile Fatty Acids

	Treatment[1]						*P* values			
	Control	FSB	ISB	FCC	FCCM	SEM	Treat	Hour	Treat × Hour	Contrast[2]
Lactate, mM	1.19	1.05	0.91	1.13	1.08	0.19	0.76	0.001	0.94	0.40
Acetate, mM	58.4^{A}	$61.8^{A,B}$	$62.5^{A,B}$	64.2^{B}	66.0^{B}	2.9	0.04	0.001	0.92	0.005
Propionate, mM	12.8^{A}	$13.4^{A,B}$	$13.9^{A,B,C}$	$14.4^{B,C}$	14.7^{C}	0.7	0.03	0.001	0.88	0.005
Butyrate, mM	7.87	7.68	7.49	8.18	7.88	0.45	0.72	0.001	0.45	0.95
Isobutyrate, mM	1.34	1.31	1.27	1.34	1.36	0.04	0.20	0.001	0.70	0.67
Valerate, mM	0.50	0.51	0.52	0.49	0.54	0.03	0.67	0.001	0.73	0.52
Isovalerate, mM	0.20	0.19	0.18	0.22	0.21	0.02	0.53	0.001	0.86	0.88
Total VFA, ,mM	81.1^{A}	$84.9^{A,B}$	$85.9^{A,B}$	88.9^{B}	90.8^{B}	3.9	0.07	0.001	0.86	0.01

[1] Treatments were A) control ration (**CON**), B) control diet plus 200g/d sodium bicarbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (**FSB**), C) control diet plus abomasal infusions of 336 g/d, 168g at am feeding and 168g at pm feeding of encapsulated sodium bicarbonate (Equishure Balchem, New Hampton, NY) added to corn starch infusions (**ISB**), D) control diet plus 200 g/d of calcium carbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (**FCC**), and E) control diet plus 125 g/d calcium carbonate (87.5g at am feeding and 37.5 at pm feeding) and 75 g/d of magnesium oxide (52.5g at am feeding and 22.5g at pm feeding) hand mixed into feed (**FCCM**).

[2]Contrast compared Control (-1) to ISB (+0.34), FCC (+0.33) and FCCM (+0.33).

Table 7. Fecal and Rumen pH and Fecal Dry Matter

	Treatment[1]						*P* values			
	Control	FSB	ISB	FCC	FCCM	SEM	Treat	Hour	Treat × Hour	Contrast[2]
Rumen pH	6.05	6.12	6.10	6.09	6.03	0.06	0.57	0.001	0.78	0.65
Fecal pH	6.47^{A}	6.40^{A}	6.50^{A}	6.46^{A}	6.64^{B}	0.04	0.001	0.001	0.59	0.11
Fecal DM, %	13.7	13.7	13.9	14.0	13.7	0.3	0.39	0.001	0.91	0.24

[1] Treatments were A) control ration (**CON**), B) control diet plus 200g/d sodium bicarbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (**FSB**), C) control diet plus abomasal infusions of 336 g/d, 168g at am feeding and 168g at pm feeding of encapsulated sodium bicarbonate (Equishure Balchem, New Hampton, NY) added to corn starch infusions (**ISB**), D) control diet plus 200 g/d of calcium carbonate hand mixed into feed, 140g at am feeding and 60g at pm feeding (**FCC**), and E) control diet plus 125 g/d calcium carbonate (87.5g at am feeding and 37.5 at pm feeding) and 75 g/d of magnesium oxide (52.5g at am feeding and 22.5g at pm feeding) hand mixed into feed (**FCCM**). [2]Contrast compared Control (-1) to ISB (+0.34), FCC (+0.33) and FCCM (+0.33).

Appendix B

FIGURES

Figure 1. Rumen Volatile Fatty Acid

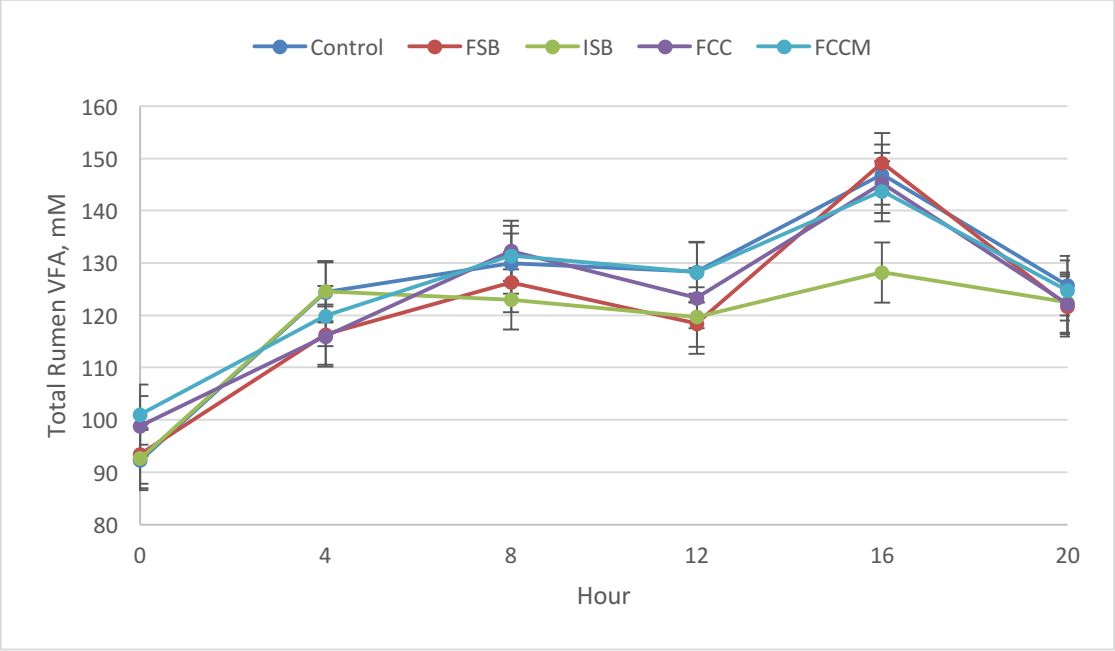

Figure 2. Rumen Butyrate

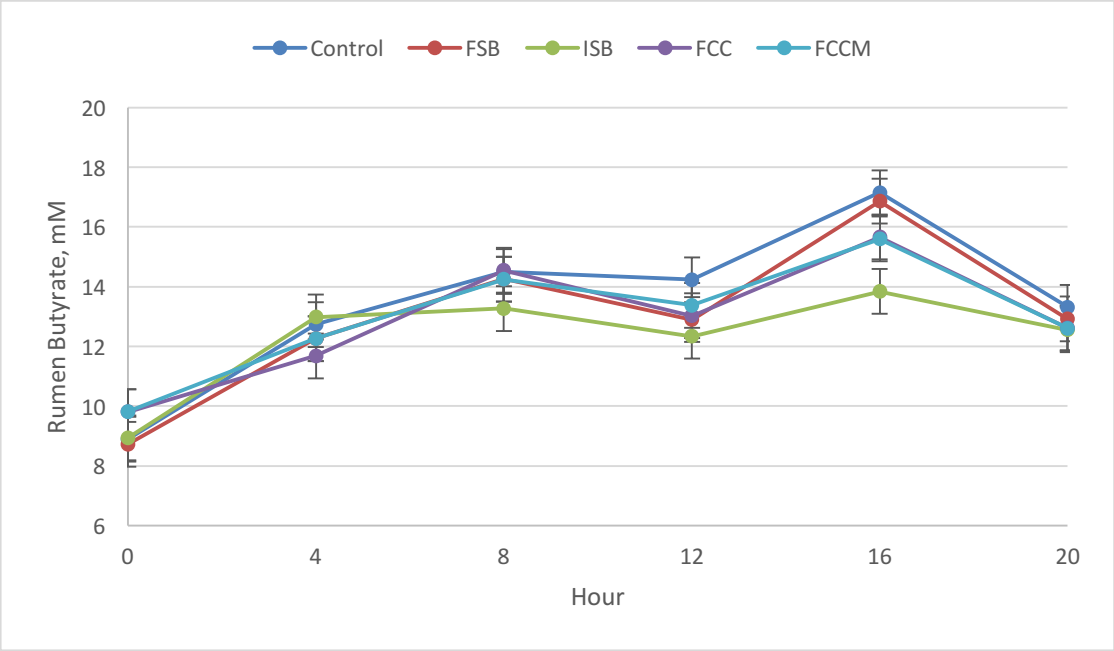

Figure 3. Rumen pH

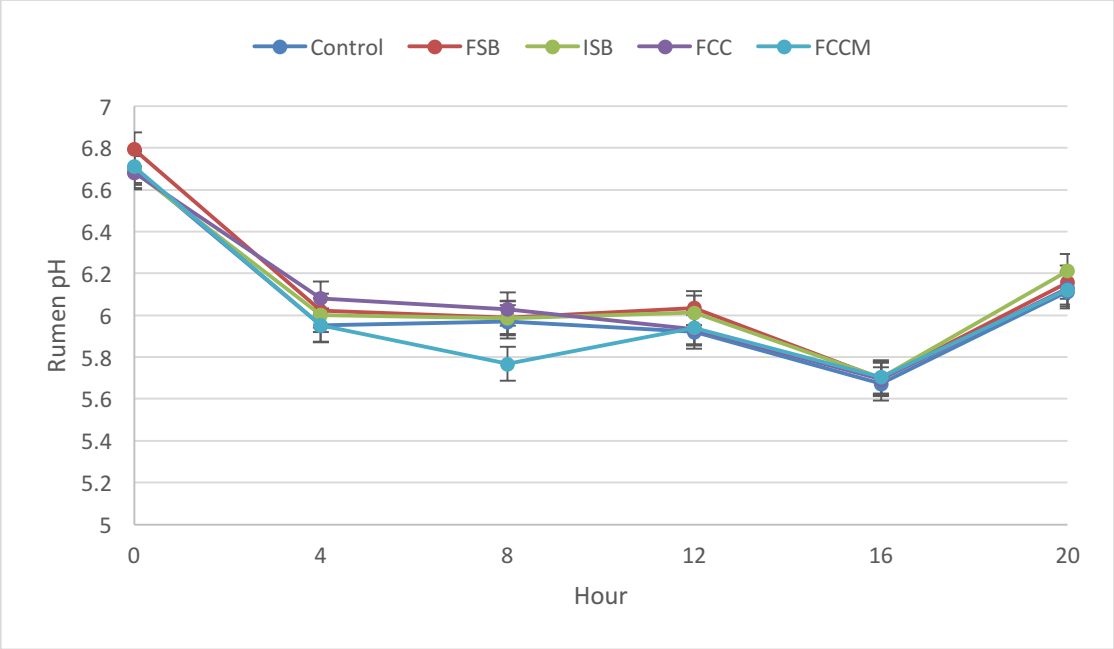

Figure 4. Fecal Volatile Fatty Acids

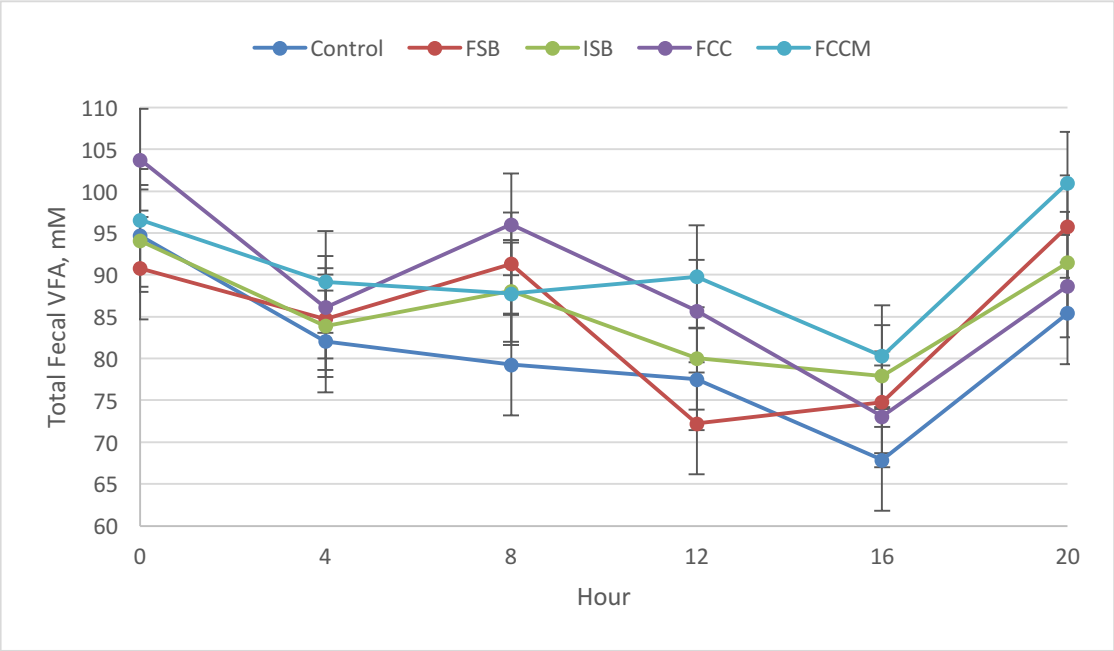

Figure 5. Fecal Acetate

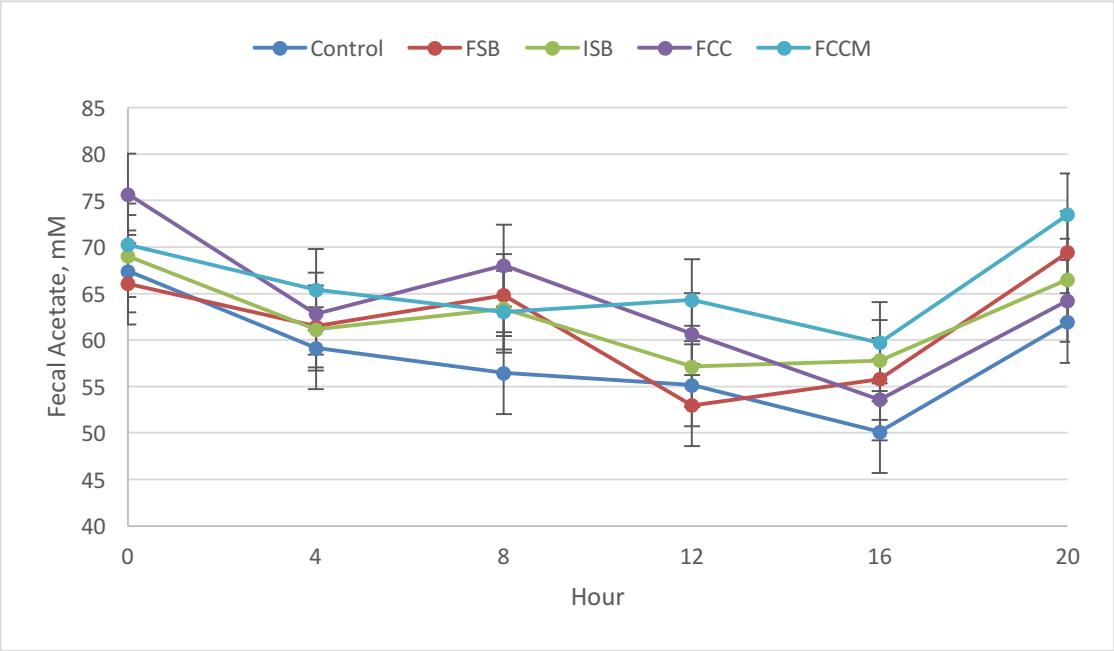

Figure 6. Fecal Propionate

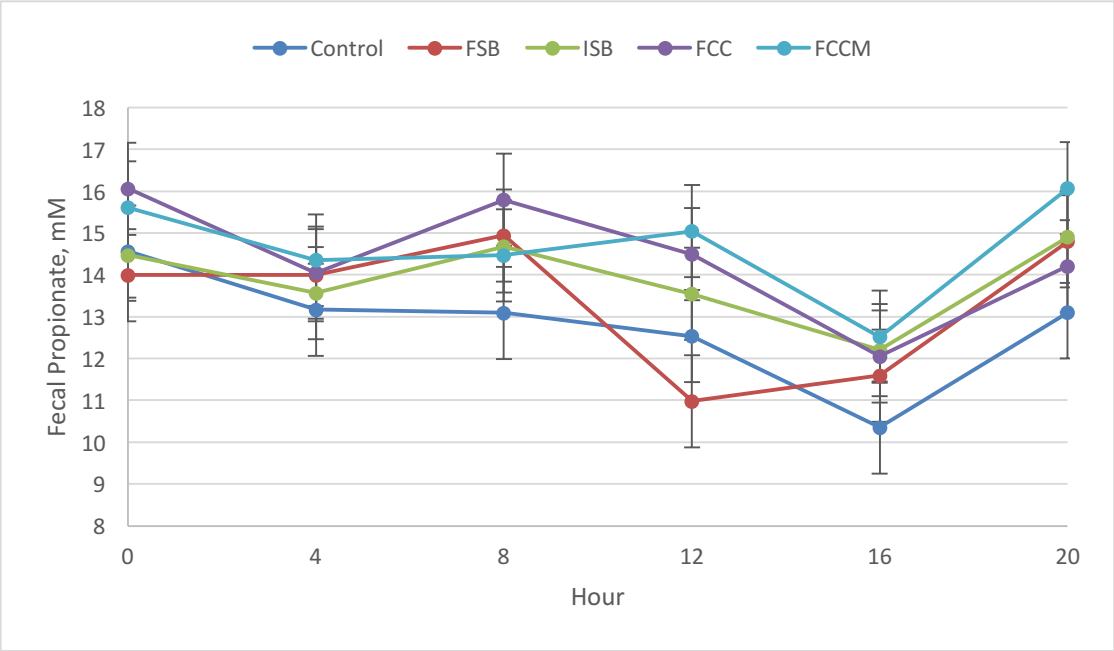

Figure 7. Fecal pH