

**CAN ABOMASAL OLIGOFRUCTOSE INFUSIONS
BE USED AS A MODEL TO STUDY POST-RUMINAL ACIDOSIS?**

by

Sarah Mainardi

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Sarah Mainardi

Approved: _____
Tanya F. Gressley, Ph.D.
Professor in charge of the thesis on behalf of the Advisory Committee

Approved: _____
Limin Kung, Jr., Ph.D.
Committee member from the Department of Animal and Food Science

Approved: _____
Susan J. Hall, Ph.D.
Committee member from the Board of Senior Thesis Readers

Approved: _____
Ismat Shah, Ph.D.
Chair of the University Committee on Student and Faculty Honors

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ABSTRACT

Excessive microbial fermentation of carbohydrates in the large intestine of dairy cattle can result in post-ruminal acidosis and lead to laminitis, decreased reproductive success, and damage to the lining of the gastrointestinal tract. The objectives of the experiment were 1) to develop a model to study post-ruminal acidosis, and 2) to evaluate the potential for a dietary yeast supplement to alleviate post-ruminal acidosis. Six ruminally cannulated Holstein steers were used in a duplicated 3 x 3 Latin square experiment with 14-d treatment periods. Steers were randomly assigned to one of three treatments: (1) control, (2) oligofructose, and (3) oligofructose + yeast. Steers were fed a diet containing either 0 or 1 g/d *Saccharomyces cerevisiae*. On d 13 of each period, steers were abomasally infused with 1 L of water containing either 0 or 1 g/kg body weight of oligofructose. Fecal samples, blood samples, body temperature, heart rate, and respiratory rate were periodically collected for 48 hours after the abomasal infusion. The oligofructose infusion resulted in post-ruminal acidosis as indicated by a decrease in fecal pH and fecal score ($P < 0.05$). However, blood pH, temperature, heart rate, and respiratory rate were not affected, indicating that oligofructose-induced post-ruminal acidosis was likely sub-acute. Dietary yeast did not alleviate the oligofructose-induced decrease in fecal pH and fecal score. In conclusion, abomasal oligofructose infusion appears to be an effective model to study post-ruminal acidosis in dairy cattle.

Chapter 1

INTRODUCTION

1.1 Bovine Digestion

1.1.1 Anatomical Structure

Because cattle have evolved with a specialized digestive tract, they can utilize forages and roughages as their primary sources of nutrients. The ruminant has four stomachs: the rumen, the reticulum, the abomasum, and the omasum. Each of these four compartments has its own responsibility in digestion of feed.

After being swallowed, the feed first enters into the reticulorumen and bacteria begin to ferment the ingested feed. Feed is then regurgitated, re-chewed, and re-swallowed by the process of rumination. Fermented feeds, unfermented feeds which bypassed rumen fermentation, and microbes, then enter into the omasum through an opening in the reticulum. Finally, the digesta enters into the "true stomach", the abomasum. After passing through the four compartments of the stomach, the digesta then moves through the small and large intestines before being excreted.

1.1.2 Digestive Physiology

1.1.2.1 Bovine Stomachs

The reticulo-rumen is a large fermentation vat where microbes ferment carbohydrates into volatile fatty acids (VFA). A majority of the VFA are then absorbed by the lining of the reticulo-rumen (Reece, 2004). The abomasum is similar to the stomach of a non-ruminant in that it contains HCl and enzymes necessary to continue digesting the feed and microbes. The abomasum also controls the flow of digesta into the duodenum (Reece, 2004). The omasum is responsible for the absorption of nutrients.

1.1.2.2 Small and Large Intestines

The small intestine is the site of further digestion of feed and microbes. Studies using lactating dairy cows with duodenal and ileal cannulae indicate that on average 33% of total tract apparent organic matter digestion occurs in the small intestines, with a range from 12 to 53 % (Callison *et al.*, 2001, Fernandez *et al.*, 2004, Haïmoud *et al.*, 1995, McNiven *et al.*, 1995, Palmquist *et al.*, 1993, Pantoja *et al.*, 1994, Prestløkken and Harstad, 2001, Younker *et al.*, 1998). The digesta that is not absorbed in the small intestine enters the large intestine, where fermentation of the feed continues and absorption of nutrients takes place (Hoover, 1978). In lactating dairy cattle, the large intestine is responsible for an average of 8% (range 3 to 23%) of total tract apparent organic matter digestion (Gressley, 2005). The wide range in large intestinal organic matter digestion is likely due to differences in animal production, feed intake, and ration composition. Digesta from concentrate often pass through the rumen and small intestine, resulting in high rates of digestion in the large intestine. As

is the case in the rumen, bacteria in the large intestine ferment carbohydrates into VFA. One study using Holstein steers found that roughly 9% of total metabolizable energy supplied by the diet was due to VFA absorbed from the large intestine (Siciliano-Jones and Murphy, 1989). In a study of sorghum grain, Streeter *et al.* (1991) found that between 0.35 and 7.4% of starch intake disappeared in the large intestine.

1.1.2.3 Buffering System

Chewing and rumination break down feed into smaller particles and causes an increase in saliva and bicarbonate production. The pH of the reticulo-rumen normally ranges between 5.5 and 7.0 (Oetzel, 2003). Some fluctuation of rumen pH throughout the day is normal because VFA produced after meal consumption reduces rumen pH. However, large drops in pH are not desirable because they kill rumen bacteria, decrease diet digestibility and can negatively impact animal health. The bicarbonate contained in saliva serves as a buffering system against a large change in ruminal pH. The buffering system is not controlled through negative feedback. The production of salivary bicarbonate increases as a result of increased forages present in the feed, not a decrease in rumen pH (Oetzel, 2003). Adequate concentrations and particle size of forages must be included in ruminant diets to stimulate chewing and ruminating needed for production of salivary bicarbonate to maintain rumen pH (Owens *et al.*, 1998).

1.1.3 Fermentation and VFA

In the rumen, as well as in the large intestine, carbohydrates are fermented by microorganisms. Fermentation end products include VFA, carbon dioxide, and methane (Björkman *et al.*, 1986). Carbon dioxide and methane are released through eructation, while VFA are absorbed and utilized by the body as a substrate for energy (France and Siddons, 1993). It has been determined that VFA account for 50-85% of a ruminant's energy utilization (Owens and Goetsch, 1988).

Although the majority of fermentation takes place in the rumen, fermentation also occurs in the large intestine. The large intestine contains about the same ratio of microbes, relative to size, as the rumen, although the fermentation rate is significantly lower (Hoover, 1978). The rate of fermentation depends not only on the concentration of microbes but also the quality and amount of fermentable feed that reaches the large intestine (Hoover, 1978). Dietary carbohydrates that reach the large intestine are typically less digestible than dietary carbohydrates in the rumen, leading to the lower rate of fermentation in the large intestine.

There are three main VFA that result from fermentation: acetic, propionic, and butyric acid (Reece, 2004). VFA exist primarily as dissociated acids in the blood and the rumen (Björkman *et al.*, 1986). After being produced, approximately 80-90% VFA are absorbed across the stratified squamous epithelial lining of the rumen or large intestine (France and Siddons, 1993). Rate of absorption is affected by the pH of the digesta and the chain length of the VFA (Reece, 2004).

According to France and Siddons (1993) the typical ratio of acetate : propionate : butyrate would be 70:20:10. Several studies have compared the profile of VFA collected from rumen and cecal fluid. Packett *et al.* (1966) fed 21 wethers a diet composed of either roughages (alfalfa hay) or pelleted concentrate (50% alfalfa, 40%

corn, and 10% molasses). They found that the average molar percentages of acetate, propionate, and butyrate were 64%, 23%, and 13%, respectively, in the rumen and 76%, 18%, and 7%, respectively, in the cecum (Packett *et al.* 1966). Siciliano-Jones and Murphy (1989) fed steers alfalfa and a soybean meal concentrate at a ratio of 80:20 and 20:80. They found an average of 68% acetate, 21% propionate, and 11% butyrate in the rumen and 70% acetate, 18% propionate, and 10% butyrate in the cecum (Siciliano-Jones and Murphy, 1989). In both studies, concentrations of VFA were similar in both compartments, about 62 mM (Packett *et al.*, 1966) and about 104 mM (Siciliano-Jones and Murphy, 1989) in rumen fluid and feces.

The relative proportions of VFA produced, are mainly determined by the species of microbes present in the gut which is driven by diet composition (Björkman *et al.*, 1986; Friggens *et al.*, 1998). For example, bacteria such as *Streptococcus bovis* produce lactic acid in the rumen (Plaizier *et al.*, 2009).

Polysaccharide and starch fermentation begins with hydrolysis of carbohydrates to simpler sugars (France and Siddons, 1993). Then the 6-carbon monosaccharide (glucose) is anaerobically oxidized using the Embden-Meyerhof pathway to form pyruvate (Figure 1.1). To produce acetate and butyrate, pyruvate enters the TCA cycle and are formed from acetyl CoA (Owens and Goetsch, 1988; France and Siddons, 1993). To form propionate, pyruvate is converted to either succinate or acrylate before being made into propionate (France and Siddons, 1993). Only one molecule of pyruvate is needed to form acetate and propionate, but two molecules are needed to make butyrate (France and Siddons, 1993).

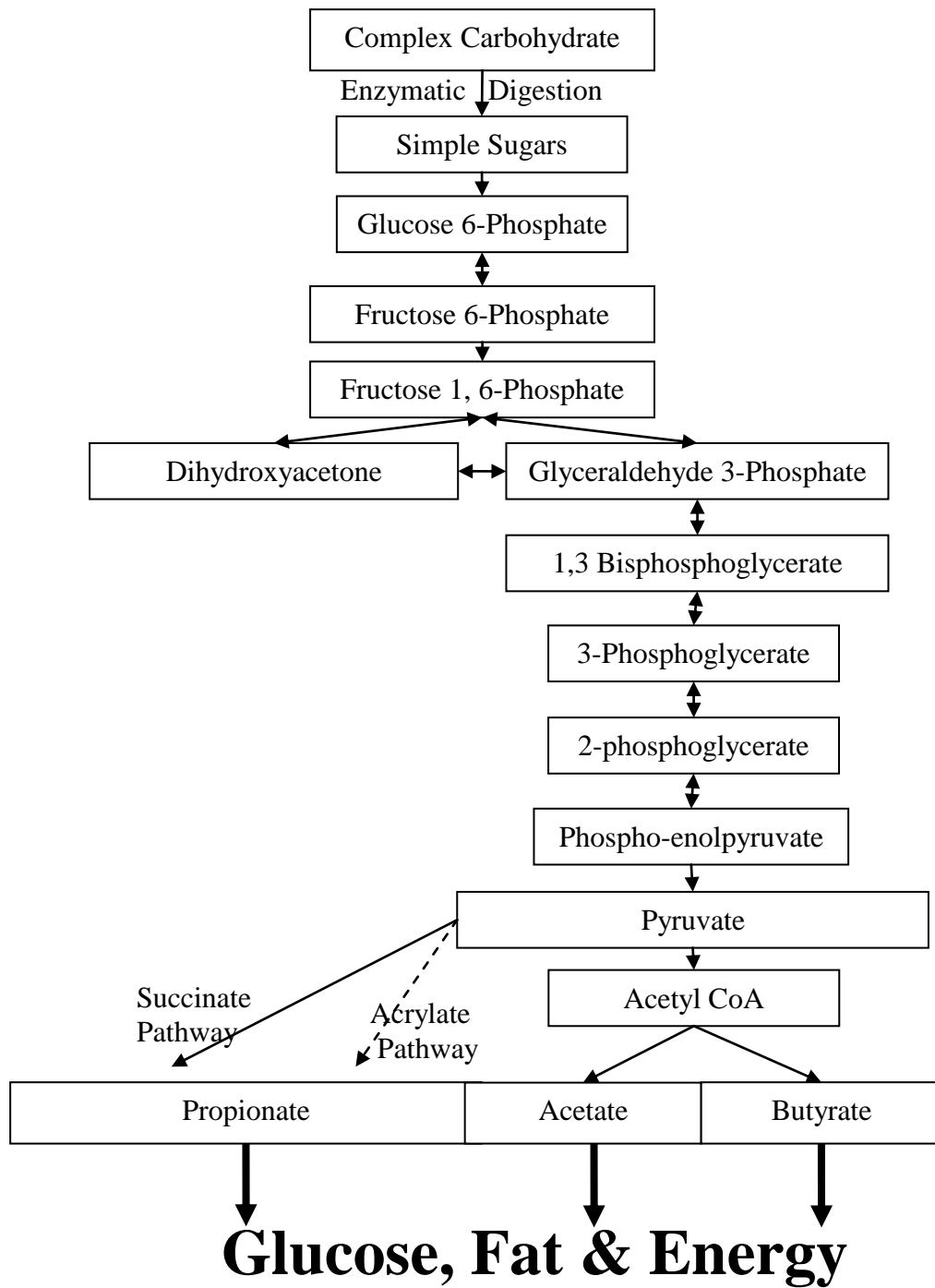


Figure 1.1 Fermentation of carbohydrates into VFA by gut biota. The common intermediate for most VFA is pyruvate. VFA are used by the body as an energy substrate.

1.2 Inulins and Oligofructose

Inulin is a fructose polymer. Inulins are found in over 36,000 species of plants, including vegetables, wheat, and chicory. Inulins are manufactured by polymerizing and altering sucrose, or by extracting it from the roots of chicory. Oligofructose (Figure 1.2) is a type of inulin that has a degree of polymerization of less than 10. It cannot be digested by the gastrointestinal enzymes because of the β 2-1 bonds that connect the sucrose monomers. Instead, it is fermented by microflora in the large intestine and, in the case of the cow, in the rumen (Niness, 1999). *Lactobacillus* and *Bifidobacteria* species of bacteria ferment inulins into acetate, propionate, and butyrate (Smiricky-Tjardes *et al.*, 2003).

There have been many studies showing that oligofructose can be used to induce lameness in horses and cattle (Danscher *et al.*, 2009, Niss *et al.*, 2009, and Thoenfer *et al.*, 2004). In cattle, oligofructose overload in the rumen results in excessive rumen fermentation and acute metabolic acidosis that induces lameness. Danscher *et al.* (2009) ruminally dosed cattle with 17 g/kg BW oligofructose and

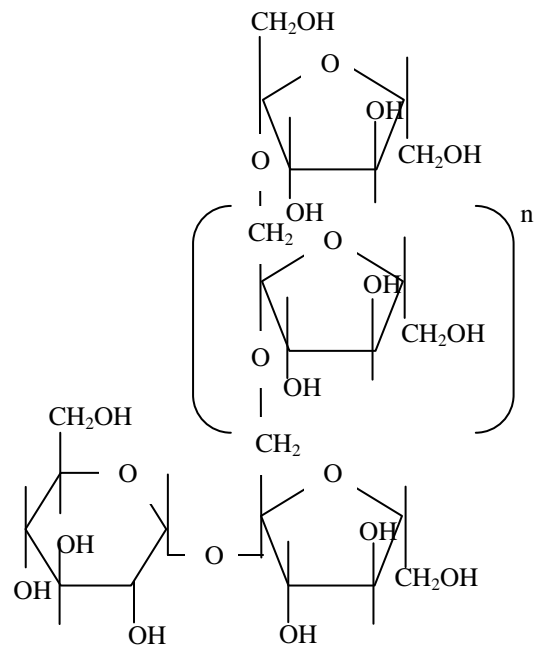


Figure 1.2. Structure of oligofructose.

Oligofructose is a polymer of up to 10 fructose molecules joined by β 2-1 linkages.

[]ⁿ = degree of polymerization.

found that locomotion scores increased from 1 (normal) to 3 (moderately lame) and weight-shifting increased. Niss *et al.* (2009) noted that when Danish Holstein-Friesian heifers were ruminally infused with 17 g/kg BW oligofructose, the time it takes to lie down was increased from 16 s to 27s. Similarly, Thoefner *et al.* (2004) found that lameness occurred after ruminal overload of oligofructose and noticed an increase in pain response in the claws of dairy cattle. Although large doses of oligofructose clearly result in lameness, the physiological effects of lower oligofructose doses, either ruminally or post-ruminally, have received little attention.

1.3 Acidosis

1.3.1 Ruminal Acidosis

Ruminal acidosis occurs when there is a significant reduction in pH in the rumen. In their review of ruminal acidosis, Owens *et al.* (1998) describe ruminal acidosis as a decrease in pH below 5.6 and 5.2 for sub-acute and acute acidosis respectively. Factors that affect the incidence of acidosis include concentration of carbohydrates in the feed, rate of glycolysis by rumen microbes, VFA utilization by the body, and osmolarity of the rumen (Owens *et al.*, 1998). Owens *et al.* (1998) suggest that a sudden increase in carbohydrates in the diets of herbivores is responsible for acute acidosis. According to Plaizier *et al.* (2009), sub-acute ruminal acidosis (**SARA**) occurs when rumen pH decreases below 5.6 for more than 3 hours a day. This decrease in ruminal pH can have various causes. In the dairy industry, SARA can occur in dairy cows around the time of parturition because of changes in dietary composition and the body's changing energy requirements (Owens *et al.*, 1998). Following parturition, a

cow will increase intake of relatively high carbohydrate rations to meet high energy demands of milk production. If the body is unable to adjust quickly to the change in diet and intake, ruminal acidosis can result (Kahn, 2005). Ruminal acidosis can also occur during other stages of the lactation cycle, particularly if high concentrations of fermentable carbohydrates are fed, dry matter intake is high, and/or there is inadequate rumen buffering (Oetzel, 2003).

Plaizier *et al.* (2009) studied the presence of acidosis on dairy farms in Wisconsin. They measured rumen fluid pH by rumenocentesis (a needle biopsy of rumen contents), removal from rumen cannula, and oral probe. When determining the presence of SARA, they used pH thresholds of 5.5, 5.8 and 5.9 for samples obtained by rumenocentesis, rumen cannula, and oral probe, respectively (Plaizier *et al.*, 2009). According to their survey, 19% of early lactation cows and 26% of mid-lactation cows had a ruminal pH below the threshold (Plaizier *et al.* 2009). They calculate that as a result of SARA, milk yield is decreased by 2.7 kg/day and can cost as much as \$400 per cow per lactation, not including veterinary bills and costs associated with culling animals (Plaizier *et al.*, 2009).

1.3.2 Acidosis Models

There have been various models used to induce either acute or sub-acute ruminal acidosis in cows. One method is to first restrict feed followed by feeding a highly fermentable diet. In the model used by Gozho *et al.*, SARA was induced by restricting access to feed for 8 hours, and then cows were fed a ration that had 25% of the total mixed ration (TMR) replaced with a ground wheat/barley grain and a total TMR concentrate level of 68% (Gozho *et al.*, 2007). Average daily rumen pH

dropped from 6.24 to 6.01 and the duration that rumen pH was below 5.6 increased from 187 min/d in control animals to 309 min/d with SARA induction. Additionally, rumen propionate concentration increased with SARA induction. However, acidosis was sub-acute because animals did not show any overt symptoms and there was no change in intake or milk production (Gozho *et al.*, 2007). Acute acidosis has also been induced through oligofructose overload; large amounts of carbohydrates are pulse dosed into the rumen, and rumen pH rapidly drops to below 5.0 (Thoefner *et al.*, 2004). In this model, animals showed acute symptoms including watery diarrhea, cessation of intake, and metabolic acidosis. For any given experiment, the choice of acute or subacute acidosis induction model will depend upon experimental objectives.

1.3.3 Health Effects and Current Findings

Over the past 50 years, milk production per cow has increased dramatically. One reason this increase has been accomplished is by feeding rations with greater proportions of grain and lower proportion of forages. Higher proportions of grain lead to greater production of VFA and increased energy supply to the cow; however, high concentrate diets tend to reduce production of saliva and bicarbonate which buffer the rumen. Reduced buffering capacity combined with increased VFA production has decreased rumen pH and increased the incidence of acidosis. Oetzel (2003) stated that SARA can cause lameness, reproductive failure, and an increase in *E. coli* shedding in feces. A decrease in digesta pH can also cause damage to the lining of the intestinal tract. Plaizier *et al.* (2009) stated that with a decrease in ruminal pH, there was an increase in osmotic pressure, which increased the potential for epithelial damage. The epithelial cells found in the rumen are susceptible to rumenitis, erosion, and ulceration when pH is very low (Oetzel, 2003). Furthermore, Clayton *et al.* (1999)

suggested that ruminal acidosis can cause abscesses to the liver and decrease milk fat percent. In severe cases, death can result.

Laminitis, is a concern in the dairy industry, as it often occurs during early lactation. It occurs when the dermal layers of the hoof become inflamed as a result of the innate immune response (Plaizier *et al.*, 2009). Although the exact connection between laminitis and increased carbohydrate intake is not clear, it has been hypothesized that the drop in rumen pH causes toxins to be released into the blood stream which ultimately damage the capillaries in the hoof tissue (Plaizier *et al.*, 2009). Productive efficiency and locomotion are severely diminished when acidosis occurs. As a result of acidosis, inflammation can also damage digestive tract tissues. Plaizier *et al.* (2009) state that when inflammation occurs, as in ruminal acidosis, there is a decrease in absorption of VFA, which increases damage to the papillae of the rumen.

Lameness affects 11.6% of dairy cattle, and laminitis is the most common cause of lameness (Cook *et al.*, 2004; Goff, 2006). The high incidence of laminitis in high producing dairy cattle has made research on laminitis imperative. In order to induce laminitis, researchers must first induce acidosis. Thoenner *et al.* (2004) found that 17 g/kg BW of oligofructose caused cattle to show signs of acute ruminal acidosis. Animals displayed low rumen pH, depression, anorexia, and watery diarrhea. This acute acidosis episode was then followed by clinical signs of laminitis (Thoenner *et al.*, 2004).

Laminitis has been well studied in horses, and some of the results can be applied to cattle, as their hoof anatomy is similar. As a result of oligofructose overload, Milinovich *et al.* (2006) found that the excess carbohydrates caused diarrhea in horses starting at hour 9 post administration. They also noted a decrease in feed

intake for horses between hour 9 and 15. Acidosis, determined by a decrease in intestinal pH, developed in all animals receiving the oligofructose treatment. Thoefner *et al.* (2004) noticed a correlation between rectal temperatures and dose of oligofructose.

Although acute acidosis clearly induces an inflammatory response, the potential for SARA to induce inflammation has also been studied. Gozho *et al.* (2007) measured the effect of SARA induction on various markers of the inflammatory response. These inflammatory markers included rectal temperature, rumen lipopolysaccharide (LPS) concentration, and serum concentrations of copper, fibrinogen, haptoglobin, and amyloid A. They found that rumen LPS and serum amyloid A were both increased by SARA while the other measures were unaffected (Gozho *et al.*, 2007).

1.3.4 Post-Ruminal Acidosis

Acidosis is not limited to the upper digestive tract, but can also occur post- ruminally. Post-ruminal acidosis occurs when large amounts of carbohydrates escape digestion in the rumen and small intestine and are fermented in the large intestine. This fermentation leads to a drop in intestinal pH and damage to intestinal tissues.

Typically, the pH of the large intestine is higher than that of the rumen, and sometimes above 7.0, but varies with the composition of feed (Hoover, 1978). In horses, Jouany *et al.* (2008) state that when the hay:grain ration was decreased, more starch entered the lower digestive tract, which in turn decreased large intestinal pH and disrupted the balance of microbes. Post-ruminal acidosis also likely increased in cattle as dietary concentrate increased. A study using Holstein steers found that cecal pH measured

over 24 h averaged 5.8 for a 20% forage, 80% concentrate diet and 6.6 for a 80% forage, 20% concentrate diet (Siciliano-Jones et al., 1989).

In cattle, dietary starch is most likely to reach the intestines if ruminal starch fermentation is incomplete due to high carbohydrate intake or inadequate feed processing with a high rate of passage. Most starch that escapes ruminal fermentation is digested in the small intestine. However, starch digestion in the small intestine is not always complete and some starch can pass into the large intestine. Reynolds *et al.* (2001) duodenally infused cows over 24 h with 700, 1400, or 2100 g of corn starch. They found that cows can digest up to 1600 g of the starch in the small intestines, with the remainder passing to the large intestine. Streeter *et al.* (1991) found that when steers with ileal cannulae were fed an 81% concentrate ration, an average of 7.2% of starch intake disappeared in the large intestine.

Unlike ruminal acidosis, there is little research that has been conducted on post-ruminal acidosis in cattle. One experiment, conducted by Bissell (2002), used abomasal infusions of starch (5 g/kg BW) to induce and study post-ruminal acidosis. In her study of post-ruminal acidosis, Bissell (2002) found that abomasal starch infusions caused a large decrease in fecal pH (as low as 4.32) due to an increase in hindgut fermentation. She also observed a change in consistency of fecal matter when cows received the starch treatment. Feces became frothy, foamy, lighter in color, pasty and sticky. Abomasal infusions also had an influence on health measures. Cows receiving starch treatments also had an increase in respiration rate and plasma haptoglobin concentration (Bissell, 2002).

Although Bissell (2002) infused cows with 5 g/kg BW starch, there is no way to determine how much starch was digested in the small intestine and how much

was fermented in the large intestine. The post-ruminal acidosis induction model described in this thesis allowed for controlled amounts of fermentable carbohydrates to reach the large intestine.

1.4 Inflammatory Mediators: Acute Phase Proteins

As indicated earlier, some of the damage to gut and hoof tissues that occurs with acidosis is due to inflammation. During inflammation, acute phase proteins are responsible for protecting the body from injury, to aid in destroying the infection, and to begin repairing damaged tissues (Plaizier *et al.*, 2009). Acute phase proteins are formed in the liver and circulate through blood. The blood concentration of acute phase proteins increases with the presence of inflammation or infection. Their release is mediated by various cytokines that are produced by macrophages, including tumor necrosis factor α (TNF- α), interleukin-6 (IL-6) and interleukin-1 (IL-1). These proteins are important because they help mediate tissue repair and infection resolution (Horadagoda *et al.*, 1999). Two acute phase proteins that are important in the cow are haptoglobin and serum amyloid A.

Haptoglobin helps with intravascular hemolysis of red blood cells. Hemolysis is an essential process that red blood cells undergo at the end of their life cycle. With time, red blood cells become less flexible and are unable to pass through small capillaries. In hemolysis, haptoglobin combines with hemoglobin, which allows the hemoglobin to be engulfed by the mononuclear phagocytic system (Reece, 2004). This process is important because it prevents the loss of iron through urine (Horadagoda *et al.*, 1999).

Normally, there are only trace amounts of haptoglobin present in the blood. An increase in concentration of haptoglobin results from an acute inflammatory response (Kaneko, 1997). In healthy cows, Eckersall *et al.* (2001) found serum haptoglobin levels to be undetectable in the majority of animals. Those animals that had a detectible level of haptoglobin ranged between 0.02 and 0.1 mg/mL (Eckersall *et al.* 2001). In an animal undergoing an inflammatory reaction, for example due to mastitis, levels of haptoglobin in serum can increase over 100 times (Eckersall *et al.* 2001). Eckersall *et al.* (2001) reported serum haptoglobin concentrations of up to 1.36 mg/mL in cows with mild mastitis and up to 1.84 mg/mL in cows with moderate mastitis. Kushibiki *et al.* (2003) treated dairy cows with recombinant tumor necrosis factor (rbTNF) to induce an inflammatory response. They found that plasma concentration of haptoglobin increased to over 1.5 mg/mL in cows as compared to trace levels in control cows.

There are two types of serum amyloid A (SAA), 'acute-phase' and 'constitutive' serum amyloid A. Acute-phase serum amyloid A (A-SAA) is the primary form of SAA present in the blood. It is a precursor for the amyloid A protein. SAA has many functions within the body. It induces many necessary enzymes to initiate repair of damaged tissue. Furthermore, it acts as an attractant for cells of the immune system. Finally, when combined with lipids, SAA has the ability to prevent atherosclerosis from accompanying an inflammatory response (Uhlir and Whitehead, 1999). In a healthy dairy cow, serum amyloid A levels range between 3.6 and 11.0 $\mu\text{g/mL}$ with a median of 5.1 $\mu\text{g/mL}$ (Eckersall *et al.* 2001). Similar to haptoglobin, serum levels of serum amyloid A can increase 100 fold with inflammation due to mastitis (Eckersall *et*

al. 2001). Levels of serum amyloid A were 13.8 µg/mL in cattle with mild mastitis and 29.9 µg/mL in cattle exhibiting moderate mastitis (Eckersall *et al.*, 2001).

1.5 Copper

Copper is a required cofactor for many enzymes in the body, including cytochrome oxidase, lysyl oxidase, ceruloplasmin, tyrosinase, and superoxide dismutase. In ruminants, only 1-5% of dietary Cu is absorbed, primarily by the mucosal cells of the small intestines (Reece, 2004). In cattle, the normal plasma concentration of copper ranges between 5.16 and 5.54 µM (Kaneko, 1999). Concentrations of copper in serum are correlated with the concentration of copper in plasma with a correlation coefficient of 0.92 (Blakley and Hamilton, 1985).

Ceruloplasmin (Figure 1.3) is a plasma protein that has many vital roles. It is responsible for binding 95% of plasma copper and is essential for both iron and copper homeostasis (Healy *et al.*, 2007). Ceruloplasmin may also be responsible for oxidation of iron. Each ceruloplasmin protein contains 6 copper molecules (Healy *et al.*, 2007). During an inflammatory response, IL-1 can cause an increase in plasma copper and ceruloplasmin concentrations (Kaneko, 1999). In a study on the effects of stress on beef cattle calves, Arthington *et al.* (2003) showed that there was an increase in the plasma concentration of ceruloplasmin in response to the stress of transportation.

Plasma copper concentrations can be used as an indicator of inflammation because they positively correlate with the activity and concentration of ceruloplasmin. Blakley and Hamilton (1985) found a correlation coefficient of 0.6 between plasma copper levels and activity of ceruloplasmin, and a correlation coefficient of 0.83 for

serum copper levels and activity of ceruloplasmin in dairy cattle. When ten Holstein cows were infused with 50 colony forming units of *Escherichia coli* to study the acute phase reaction that results from mastitis, Erskine and Bartlett (1993) found that Cu concentrations did not significantly decrease during the challenge.

1.6 Blood Metabolites

1.6.1 Non-Esterified Fatty Acids (NEFA)

Non-esterified fatty acids (NEFA) are also known as free fatty acids. They are made up of at least twelve carbon atoms in a straight chain. They are primarily synthesized by hydrolysis of triglycerides in the liver, adipose cells, and mammary cells and released into the blood. Catabolism of NEFAs can occur through desaturation or β -oxidation (Kaneko, 1999). Healthy dairy cattle typically have a serum concentration of 30-100 mg/L (Kaneko, 1999). The blood NEFA concentration is affected by various things including nutrition, time of day, and stage of lactation. Concentrations of NEFA peak immediately following feeding (Ametaj *et al.*, 2009). Moreover, the amount of free fatty acids decreases with time after feeding. During the night, blood NEFA concentrations increase when immunoreactive insulin decreases. In their research, Radloff *et al.* (1966) showed that there was a relationship blood free fatty acids increase during fasting. In a study of lactating Holstein cows, NEFA concentrations decreased as a result of duodenal starch infusion (Reynolds *et al.*, 2001). NEFA levels also tend to be higher right after parturition (Fröhli *et al.*, 1988). An excess formation of NEFA can lead to ketosis and/or fatty liver disease (Ametaj *et al.*, 2009).

Increased blood NEFA concentrations often occur at the same time as an increase in cytokines. In humans, free fatty acids cause a release of migration inhibitory factor, a cytokine that induces the inflammatory cascade (Tripathy *et al.*, 2003). After injecting 2.5µg/kg BW of recombinant tumor necrosis factor- α into 12 lactating Holstein cows, Kushibiki *et al.*, (2003) found that concentrations of NEFA increased to levels over 155 µEq/L in treated cows compared to approximately 100 µEq/L in control cows. However, it is not known whether the increase in NEFA is a result of a negative energy balance created by a decrease in dry matter intake or as a result of the cytokines produced during the inflammatory response (Kushibiki *et al.*, 2003).

1.6.2 Urea

Ammonia (NH₃) is formed when dietary proteins are deaminated during digestion and metabolism. It exists in equilibrium between ionic (NH₄⁺) and nonionic (NH₃) forms with a pKa of 9.02 (Visek, 1984). As it is the major substrate for protein synthesis for many of the bacteria in the digestive tract, ammonia in the digesta is either absorbed by the gastrointestinal tract or is incorporated into microbial protein (Reece, 2004). Absorption of ammonia, either through the wall of the rumen or other sites of the digestive tract, is influenced by many circumstances. For example, pH has a large influence on the amount of ammonia absorbed; the amount of ammonia absorbed increases with increasing digesta pH. It has been shown that the amount of ammonia absorbed increases three-fold as pH increases from 4.5 to 6.5 (Visek, 1968).

The concentration of ammonia present in a healthy cow's serum is approximately 35 ± 17 µM (Kaneko, 1999). Because NH₃ is toxic to animal tissues,

the kidneys convert ammonia to urea; however when there is excessive NH_3 absorption or production or when kidney function is impaired, blood NH_3 concentration can increase (Vissek, 1968).

In a healthy animal, plasma urea concentrations are impacted primarily by protein intake. However, post-ruminal fermentation also impacts plasma urea. Continuous abomasal infusions of 1 kg/d oligofructose were found to decrease blood urea nitrogen concentration from 13.1 to 12.2 mg/dL in lactating cows fed an adequate protein diet (Gressley and Armentano, 2007). The decrease in plasma urea was attributed to an increased conversion of NH_3 to microbial protein that was excreted in the feces.

1.6.3 Ketones and β -Hydroxybutyrate (BHBA)

Ketones are primarily formed by incomplete lipolysis of long-chain fatty acids. When butyric acid is absorbed from the rumen, it is catabolized into the corresponding ketone, β -hydroxybutyrate (3-hydroxybutyric acid), through ketogenesis (Reece, 2004). β -hydroxybutyrate is present in two forms that are stereoisomers of each other. It is easily ionized and is soluble in water (Kaneko, 1999). It is an acidic compound and is transported freely through the blood (Larsen *et al.*, 2005). In a healthy lactating cow, β -hydroxybutyrate is present in the blood in a concentration of ~1 mM while a non-lactating cow will have a concentration of ~0.3 mM (Kaneko, 1999).

Blood concentrations of BHBA can change for various reasons. Ametaj *et al.* (2009) found that plasma concentrations of β -hydroxybutyrate were lowest early in the morning and peaked four hours after feeding. At calving, there is often a spike in

blood β -hydroxybutyrate. Ketosis can result when there is a high concentration of ketones present in body fluids (Ametaj *et al.*, 2009).

1.7 Changes as a Result of Post-Ruminal Acidosis

NEFA, Cu, BHBA, urea, SAA, and haptoglobin were chosen for this study because we expect their concentrations in the blood to change as a result of oligofructose infusion and acidosis. Serum amyloid A, haptoglobin, and Cu were measured as markers of the acute phase response to inflammation. Therefore, we would expect an increase in their blood concentrations during acute acidosis, and a possible increase during sub-acute acidosis. NEFA, BHBA, and urea were chosen in order to observe metabolic disturbances as a result of post-ruminal acidosis. We would expect to see an increase in the concentration of NEFA, BHBA, and a decrease in urea concentrations.

1.8 Yeast as a Diet Additive

Traditionally, buffering agents have been added to rations to prevent the decrease in rumen pH that occurs when lactating cows are fed relatively high concentrate diets (Clayton *et al.*, 1999). Yeast has recently been added to diets to increase rumen function and energy utilization and to decrease the incidence of ruminal acidosis.

Levucell SC (Lallemand Inc, Milwaukee, WI), a commercially available feed additive, contains *Saccharomyces cerevisiae*. Although *S. cerevisiae* is not a native inhabitant of the rumen, it can survive anaerobically for a limited time in the

rumen (Durand-Chaucheyras *et al.*, 1998). In *in vitro* studies, this yeast has been shown to decrease the concentration of lactic acid in rumen fluid and reduce post consumption drop in ruminal pH in sheep (Durand-Chaucheyras *et al.*, 1998).

Desnoyers *et al.* (2009) found that cattle supplemented by *Saccharomyces cerevisiae* had a higher rumen pH, higher concentration of rumen VFA, and lower proportion of lactic acid in the rumen. In horses, Medina *et al.* (2002) found cecal and fecal VFA concentrations were not influenced by the addition of *Saccharomyces cerevisiae*. The potential for yeast to alleviate hindgut acidosis has not been extensively studied.

1.9 Objectives

The objectives of this study were: a) to create a model for studying hindgut acidosis, b) to evaluate the effect that hindgut acidosis has on fecal characteristics, c) to evaluate the effect that hindgut acidosis has on plasma and health measures and d) to determine if a yeast supplement can moderate the negative effects of hindgut acidosis.

Chapter 2

MATERIALS AND METHODS

2.1 Animals and Treatment

Six Holstein steers were ruminally cannulated prior to the experiment. Steers were approximately 2 years old and their weights are shown in Table 2.1. Steers were fed a TMR formulated for lactating cows (Table 2.2). Feed and water were provided ad libitum and amounts consumed were recorded daily. Steers were fed at 10 AM each morning and orts from the previous day were recorded. Each steer was housed in a 2.4 m x 6.4 m pen bedded with straw and wood shavings. Waterers were located at one end of the pen along with wooden feed bins. All procedures were approved by the University of Delaware Agricultural Animal Care and Use Committee.

Steers were randomly assigned to one of two blocks. The study was made up of three, two week long periods. Each period, steers received one of the three treatments according to a 3X3 Latin square design: (1) control, (2) abomasal oligofructose (Beneo P95, Orafiti Active Food Ingredients, Tienen, Belgium), or (3) dietary yeast (Levucell SC, Lallemand Inc, Milwaukee, WI) + abomasal oligofructose. Animals on treatment 3 were given 1 g of yeast daily with a small portion of the daily feed. The remainder of the diet was fed following consumption of the yeast. On d 13 at 10 AM (0 hr), all animals were given a pulse-dose abomasal infusion of 1 liter water

(treatment 1) or 1 L water containing 1 g/kg BW of oligofructose (treatments 2 and 3) using a hand pump.

Abomasal infusion lines were inserted prior to the start of the experiment using the technique described in Gressley *et al.* (2006). Lines were inserted through the ruminal cannula, through the abomasum and into the abomasum. Jugular catheters were inserted each period on the day prior to sampling (day 12 of each period). Catheters were removed at the end of each period.

2.2 Feed Sampling and Analysis

TMR was reformulated weekly based on weekly samples of roughage and concentrate dry matter analysis. Feed samples were collected daily and frozen at -20°C until analysis. At the end of each period, samples were pooled and dried at 60°C for 48 hours to determine the feed dry matter. Samples for particle size analysis were collected each week and frozen at -20°C until the end of the experiment, when they were analyzed using the Penn State Shaker box. Results for particle size are shown in Table 2.2.

Table 2.1. Diet composition and analysis.

Item	
Ingredient	(% of diet DM)
Corn silage	37.0
Alfalfa silage	16.0
Soybean meal	7.2
Corn grain	17
Citrus pulp	5.6
Canola meal	3.2
Turbo meal ¹	5.1
Distillers grains	2.3
Soybean hulls	2.2
Blood meal	1.1
Molasses	0.4
Sodium bicarbonate	0.5
Vitamins and minerals ²	2.2
Nutrient Composition	(% of diet DM)
CP	17.2
NDF	29.6
ADF	20.8
Starch	25.7
DM, %	
Particle Size Analysis, Penn	(% DM)
State Shaker Box	
Top screen	5
Second screen	26
Third screen	40
Bottom	29

¹Turbo meal = extruded and expelled soybean meal, J. L. Moyer & Sons, Inc, Turbotville, PA

²Vitamins and minerals included calcium carbonate, sodium chloride, vitamin E, selenium, and urea.

2.3 Blood Sample Collection and Analysis

Rectal temperatures, respiratory rate, and heart rate were taken at 0, 3, 6, 9, 12, 24, and 48 hours post infusion.

Blood pH was measured for all cows at 0 and 6 h post infusion during periods 2 and 3. Equipment for measuring pH was not available for period 1. For blood pH measurement, 2-3 mL of blood was collected into a 3 mL heparinized syringe (BD, Franklin Lakes, NJ). The syringe was flicked to get rid of bubbles and then capped using wax and samples were transported immediately to New London Veterinary Center (Newark, DE). A maximum of 80 minutes elapsed between sampling and pH analysis on an IDEXX Vetstat Analyzer (Idexx Laboratories, Westbrook, Maine).

At 0, 3, 6, 9, 12, and 24 h, blood was collected from the jugular catheters into two 10 ml EDTA vacutainers and one 10 ml serum vacutainer (BD, Franklin Lakes, NJ). Plasma was collected following centrifugation at 1000 xg for 20 minutes. Blood collected into the serum vacutainer was allowed to clot for 3 hours at room temperature then spun at 1000 xg for 30 minutes. Samples collected at night were refrigerated overnight, and spun the following morning. Serum and plasma were stored at -20°C until analysis.

Plasma was used to determine concentrations of Cu, haptoglobin and BHBA. Serum was used to determine SAA, NEFA and urea concentrations. Commercial colometric kits were used to analyze haptoglobin (Tridelta Development Limited, Maynooth, Ireland), SAA (Tridelta Development Limited, Maynooth, Ireland), urea (Pointe Scientific INC, Canton, Michigan), BHBA (Pointe Scientific INC, Canton, Michigan), and NEFA (Wako Diagnostics, Richmond, VA). All plates were read using a Spectra MAX 190 plate reader (Molecular Devices, Sunnyvale, CA).

Plasma samples for copper analysis were thawed prior to analysis and were spun at 800 xg for 5 minutes. 200 μ L of plasma was diluted into 9.8 mL of 0.1% HNO₃ and spun at 2200 xg for 15 minutes at 4°C. The supernatant was analyzed on a 7500 Series ICP-MS (Agilent Technologies, Santa Clara, CA).

All plasma and serum analyses are ongoing and incomplete at this time. Results reported in this thesis are preliminary.

2.4 Fecal Collection and Analysis

Approximately 100 g of feces was collected into a plastic bag at 0, 3, 6, 9, 12, 24 and 48 h and frozen at -20°C until dry matter analysis. Dry matter was measured on thawed fecal samples using 30 gram samples that were placed in a forced air oven for 48 hours at 60°C.

Samples to analyze for fecal microbes were collected in a 50 mL conical tube at 0, 3, 6, 9, 12, and 24 h and were stored at -20°C until shipped on dry ice to Lallemand, Inc (Montreal, Canada) for analysis. Fecal samples were sieved at 0, 6, and 12 h during period 2 and 3 to qualitatively assess digestibility. Samples (approximately 200 g) were wet sieved through a 2 mm screen and remaining particles were squeezed to remove excess liquids. Weight before and after sieving was recorded and percentage of wet weight remaining after sieving was calculated.

Fecal samples were collected in plastic cups at 0, 3, 6, 9, 12, 24, and 48 hours post infusion. Fecal consistency was scored from one to four according to Zaaier *et al.* (2005) with 1 meaning liquid stool and 5 meaning more solid than normal. Fecal pH was measured using a PH110 ExStik refillable pH Meter (Extech,

Waltham, MA). The meter was inserted directly into the feces and pH was recorded following equilibration.

Fecal samples were collected for VFA analysis. 25 g (\pm 0.25 g) of feces was combined with 10 mL 2.06% H₂SO₄ in a 50 mL conical tube. The tube was shaken vigorously for 10 seconds, and then contents were squeezed through cheese cloth. The liquid was collected into a 15 mL conical tube and frozen at -20°C for later analysis.

For measurement of fecal VFA, 500 μ L of each sample was combined with 500 μ L calcium hydroxide (3.5 M) and 250 μ L cupric acid (0.63 M CuSO₄ and 0.046 M crotonic acid). Samples were placed in the refrigerator for 30 minutes before being centrifuged at 9400 xg for 15 minutes. The supernatant was poured into a clean tube and 12.5 μ L of concentrated sulfuric acid was added. The sample was frozen, thawed, refrozen, and rethawed. Then the sample was centrifuged at 9400 xg for 15 minutes and the supernatant was analyzed by high performance liquid chromatography (Shimadzu Corporation, Columbia, MD). The liquid phase was a mixture of EDTA, H₂SO₄, and H₂O.

Results for fecal VFA and fecal microbial populations are pending.

2.5 Statistical Analysis

Data were analyzed using Proc Mixed in the Statistical Analysis System (SAS) program. The model included the fixed effects of treatment, period, square, hour, and the interaction of hour by treatment and the random effects of steer within square and period by treatment by steer within square. Time was included as a

repeated measure using the autoregressive covariance structure. Significance was declared at $P \leq 0.05$ and a trend at $P \leq 0.10$.

Chapter 3

RESULTS

3.1 Intake

Dry matter intake for the day prior to, the day of, and the day following abomasal infusions is shown in Figure 3.1. There were no effects of time on intake ($P = 0.103$) or the interaction of treatment by time ($P = 0.29$). Intake was impacted by treatment ($P = 0.0113$). Oligofructose had a higher average dry matter intake (15.8 kg/d) than both the control group (14.5 kg/d) and the oligofructose + yeast treatment group (14.1 kg/d).

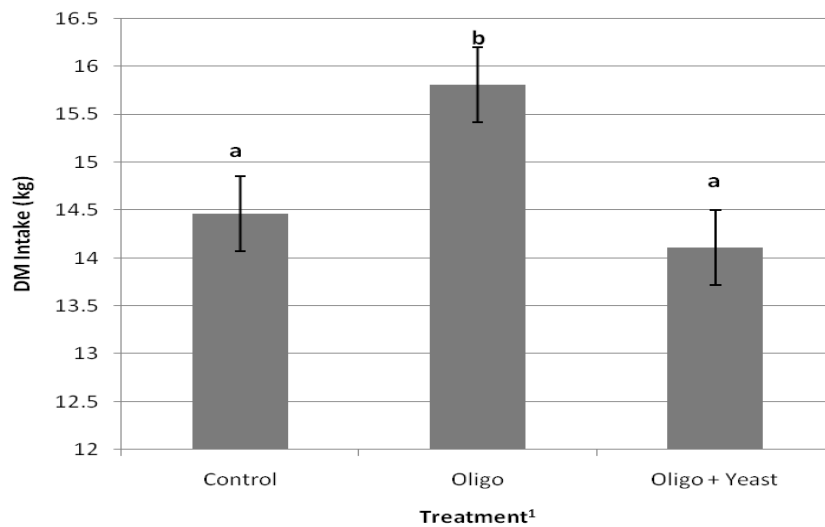


Figure 3.1. Effect of control, oligofructose, and oligofructose + yeast treatment on DM intake. SED = 0.39.

^{a, b} Treatments with different superscripts are different ($P < 0.05$).

¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast

3.2 Temperature, Heart Rate and Respiratory Rate

Body temperature was not influenced by the treatments but was impacted by time (Table 3.1). Temperature was higher than 0 hour for 6, 9 and 12 hours post infusion (Figure 3.2). Respiratory rate tended to be lower for the oligofructose + yeast than for oligofructose or control (Table 3.1). Respiratory rate was influenced by time as there was an increase at all hours compared to 0 h (Figure 3.3). Heart rate was affected by time and there was a trend for an interaction of treatment by time (Table 3.1). At 3 h post infusion, the control cattle had a higher heart rate than cattle receiving oligofructose or oligofructose + yeast (Figure 3.4). At hour 6, the oligofructose cattle had a higher heart rate than control or oligofructose + yeast cattle. Treatments were similar from 9 thru 48 h, and the 9 and 12 h heart rates were greater than the 0 h heart rate ($P < 0.05$).

Table 3.1 Effect of control, oligofructose and oligofructose + yeast treatments on vital measures. Results shown as least squares means.

Item	Treatment ¹			SED ²	Contrasts ³		
	Control	Oligo	Oligo + Yeast		Treat.	Time (<i>P</i> -values)	Treat. x Time
Body Temperature, °F	101.2	101.4	101.1	0.3	0.57	0.01	0.41
Respiration rate, breaths per minute	39 ^b	40 ^b	36 ^a	1	0.06	0.001	0.11
Heart Rate, beats per minute	89	89	86	0.3	0.32	0.001	0.06

¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast

² SED = Standard error of the differences among treatment means.

³ Treat. = effect of treatment; treat. x time = effect of treatment over time

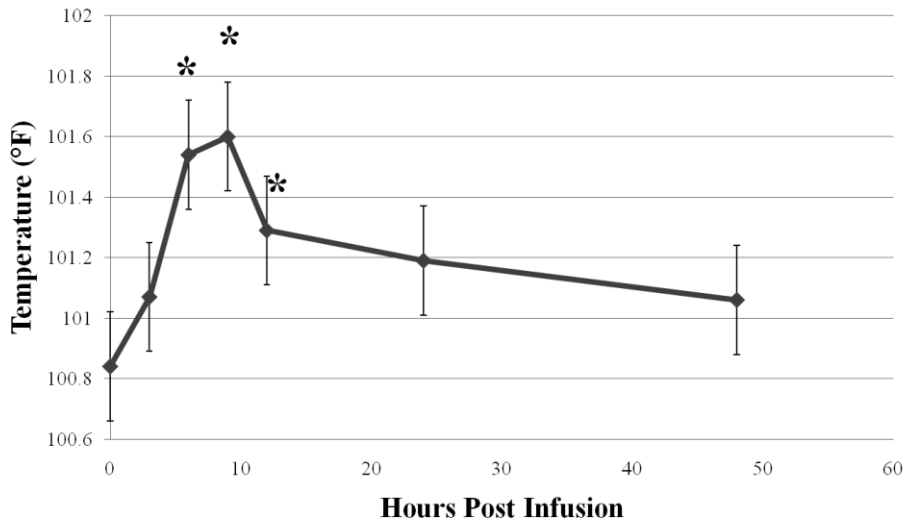


Figure 3.2. Rectal temperature vs. hour. *Indicates difference from 0 h temperature at $P < 0.05$. SEM = 0.18.

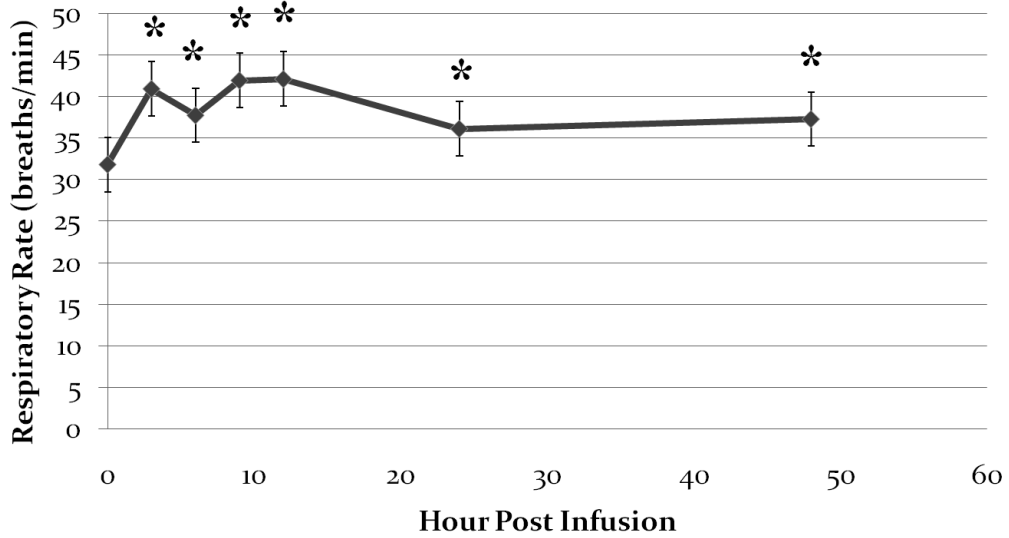


Figure 3.3. Respiratory rate vs. hour. *Indicates difference from 0 h respiratory rate at $P < 0.05$. SEM = 3.27.

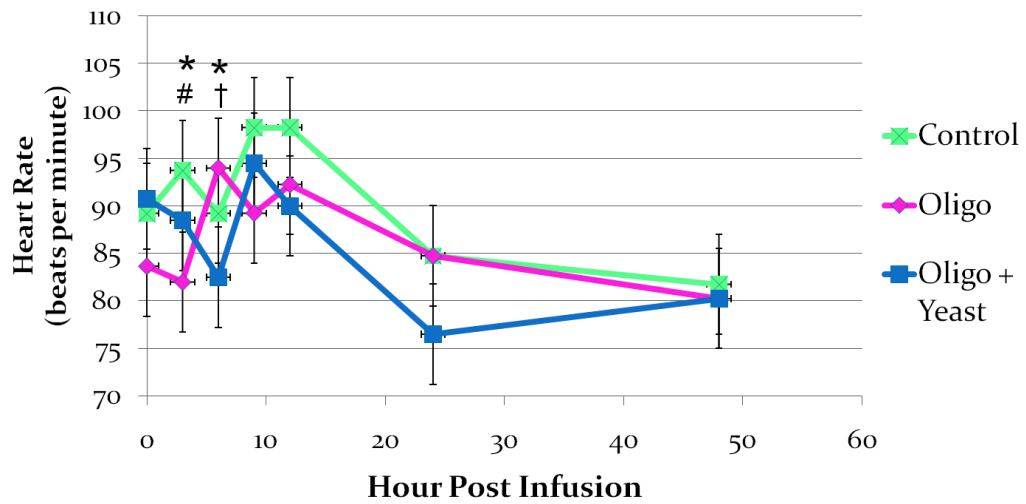


Figure 3.4. Influence of control, oligofructose, and oligofructose + yeast on heart rate over time. *Control is different ($P < 0.05$) from Oligofructose. #Control is different ($P < 0.05$) from Yeast. †Trend for difference ($P < 0.1$) between control and yeast.

¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast

3.3 Fecal Samples

The percentage of fecal wet weight after passage through a 2 mm screen was influenced by time and tended to be influenced by treatment (Table 3.2). Percent material remaining after sieving was decreased at hour 6 when compared to 0 h or 12 h ($P < 0.01$, Figure 3.5). The percent of fecal particles greater than 2 mm was higher for control cattle compared to oligofructose + yeast cattle.

Fecal dry matter was influenced by both time as well as the treatment by time interaction. There was a decrease in fecal DM relative to control for cows on the oligofructose and the oligofructose + yeast treatments at 6 h post infusion (Figure 3.6). Moreover, there was an increase in fecal dry matter relative to the control for the oligofructose + yeast treatment 12 h post infusions, as well as a trend for increase for the oligofructose treatment 12 h post infusion.

Fecal scores were affected by treatment, hour, and the interaction of treatment by hour. Fecal score for control cattle was higher at hour 3, 6 and 9 compared to cattle receiving the oligofructose + yeast treatment (Figure 3.7). At hour 6 and 9, control cattle had a higher fecal score compared to cattle receiving the oligofructose treatment.

Fecal pH was affected by treatment and by time (Table 3.2). Steers receiving the control treatment had a higher fecal pH than steers receiving the oligofructose or oligofructose + yeast treatments (Figure 3.8).

Table 3.2. Effect of control, oligofructose and oligofructose + yeast treatments on fecal pH, fecal score, fecal particles, and fecal dry matter. Results shown as least square means of treatments.

Item	Treatment ¹				Contrasts ³		
	Control	Oligo	Oligo + Yeast	SED ²	Treat.	Time	Treat. x Time
					————— (P-values) —————		
Fecal Particles > 2 mm, % wet weight	11.95 ^a	9.67 ^{a, b}	1.33 ^b	1.60	0.09	0.02	0.62
Fecal DM, %	14.72	14.90	14.70	0.46	0.87	0.0001	0.002
Fecal Score	2.71 ^a	2.45 ^a	2.11 ^b	0.15	0.01	0.001	0.001
Fecal pH	7.02 ^a	6.67 ^b	6.72 ^b	0.09	0.02	0.02	0.14

¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast

² SED = Standard error of the differences among treatment means.

³ Treat. = effect of treatment; treat. x time = effect of treatment over time

^{a, b} numbers in the same row with different letters are different ($P < 0.05$)

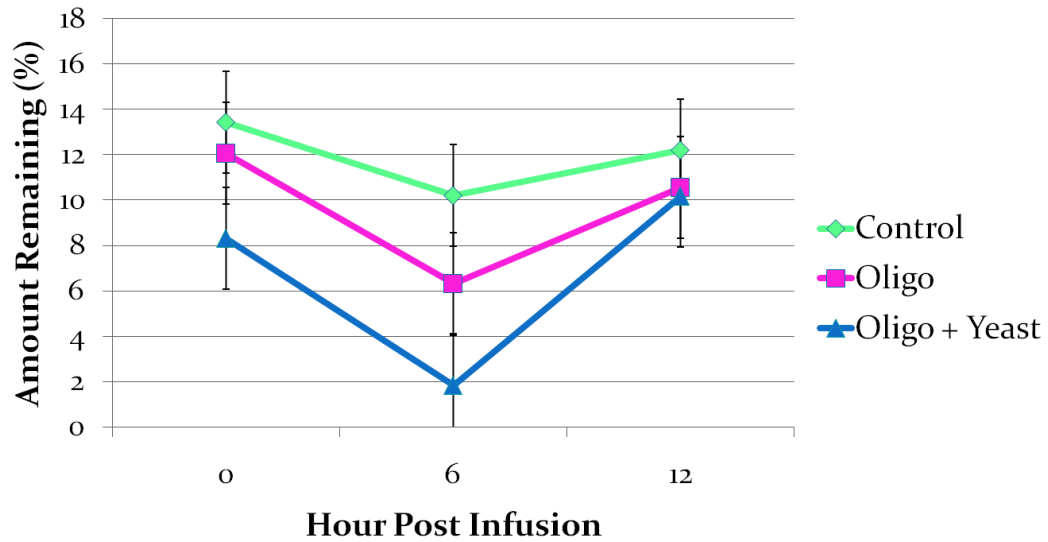


Figure 3.5. Effects of control, oligofructose, and oligofructose + yeast on fecal sieving over time. Results are shown as least squares means. SEM = 2.24.

¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast.

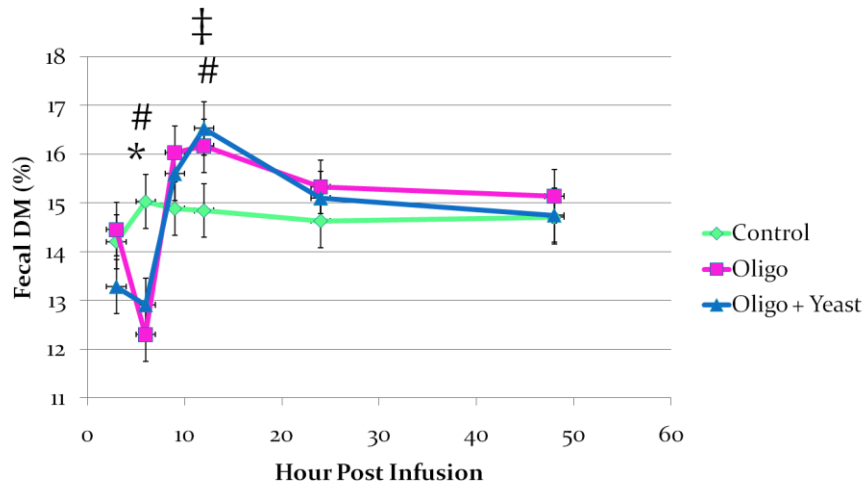


Figure 3.6. Effects of control, oligofructose and oligofructose + yeast on fecal DM over time. * Control is different from oligofructose ($P < 0.05$). # Control is different from yeast ($P < 0.05$). ‡ Control is different from yeast ($P < 0.10$). SEM = 0.55.

¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast.

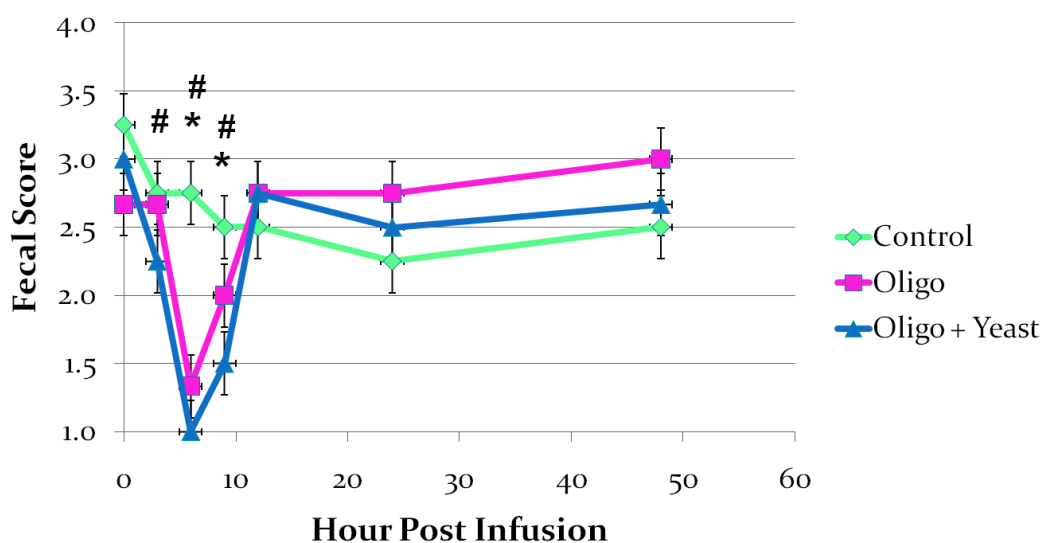


Figure 3.7. Effect of the control, oligofructose and oligofructose + yeast treatments on fecal score over time. * Control is different from Oligofructose ($P < 0.05$). # Control different from Yeast ($P < 0.05$). SEM = 0.23.
¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast.

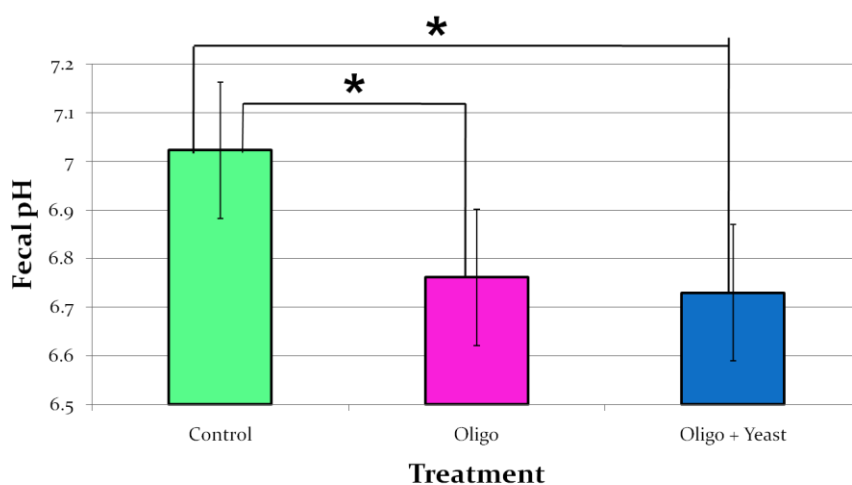


Figure 3.8. Effect of the control, oligofructose and oligofructose + yeast treatments on fecal pH over time. *Indicates difference from 0 h temperature at $P < 0.05$. SEM = 0.14.
¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast.

3.4 Blood Samples

Plasma and serum NEFA, copper and haptoglobin concentrations are still being analyzed. Preliminary results for plasma/serum urea and plasma/serum BHBA are presented in Table 3.3. Urea concentration was influenced by time (Table 3.3). At 9 and 24 h post infusion, the concentration of urea in serum was higher than at 0 h (Figure 3.9). Plasma BHBA was not impacted by treatment but tended to be impacted by time ($P = 0.07$). Concentration of BHBA in the plasma increased from 0 h to 3 h and from 0 h to 6 h (Figure 3.10). Blood pH was not influenced by treatment or time but was impacted by the interaction of treatment by time ($P = 0.047$). Blood pH decreased over time for the control and oligofructose treatments and increased over time for the oligo + yeast treatment (Figure 3.11).

Table 3.3. Effect of treatment on serum urea, plasma BHBA and blood pH. Results shown as least square means of treatment.

Item	Treatment ¹			SED ²	Contrasts ³		
	Control	Oligo	Oligo + Yeast		Treat.	Time	Treat. x Time
						(<i>P</i> -values)	
Urea, mg/dL	11.51	13.37	10.57	2.08	0.104	0.004	0.76
BHBA ⁴ , mM	1.31	1.14	1.16	0.15	0.48	0.07	0.55
Blood pH	7.44	7.44	7.44	0.01	0.94	0.86	0.05

¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast

²SED = Standard error of the differences among treatment means.

³Treat. = effect of treatment; treat. x time = effect of treatment over time.

⁴BHBA = β -hydroxybutyrate

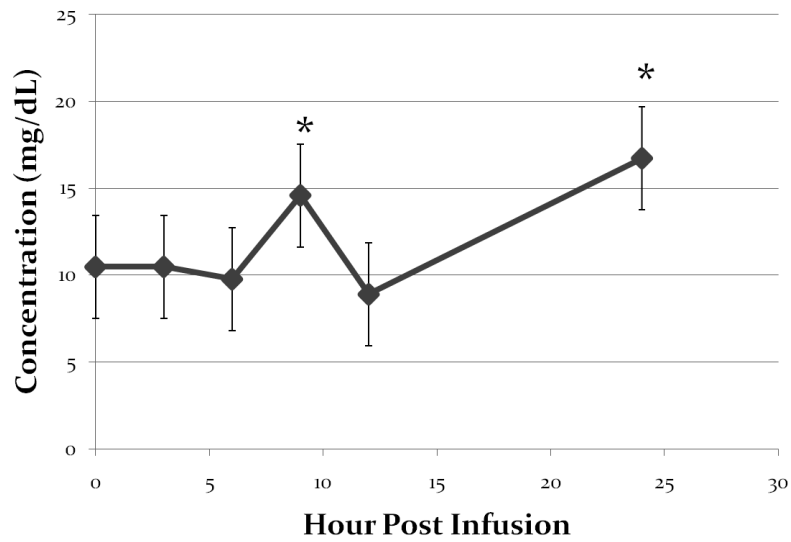


Figure 3.9. Raw means of serum urea samples analyzed to date. Results are preliminary. *Indicates difference from 0 h respiratory rate at $P < 0.05$. SEM = 2.96.

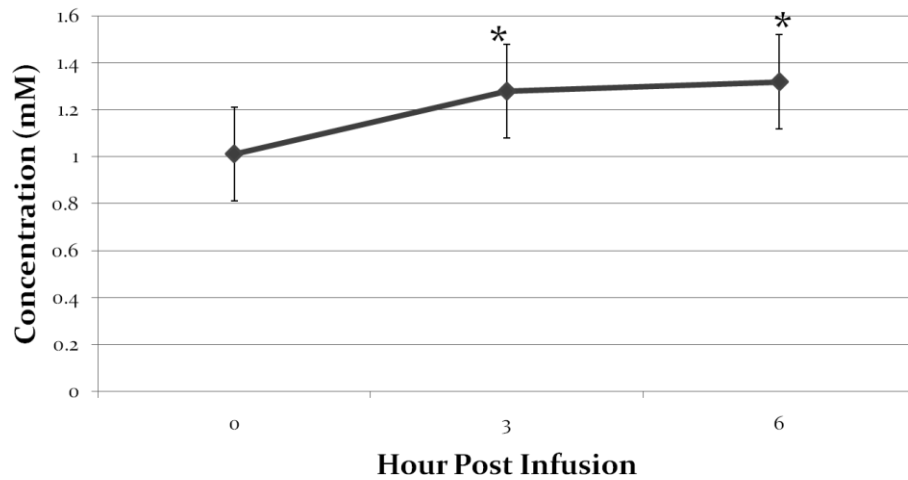


Figure 3.10. Raw means of plasma BHBA samples analyzed to date. Results are preliminary. *Indicates difference from 0 h respiratory rate at $P < 0.05$. SEM = 0.20

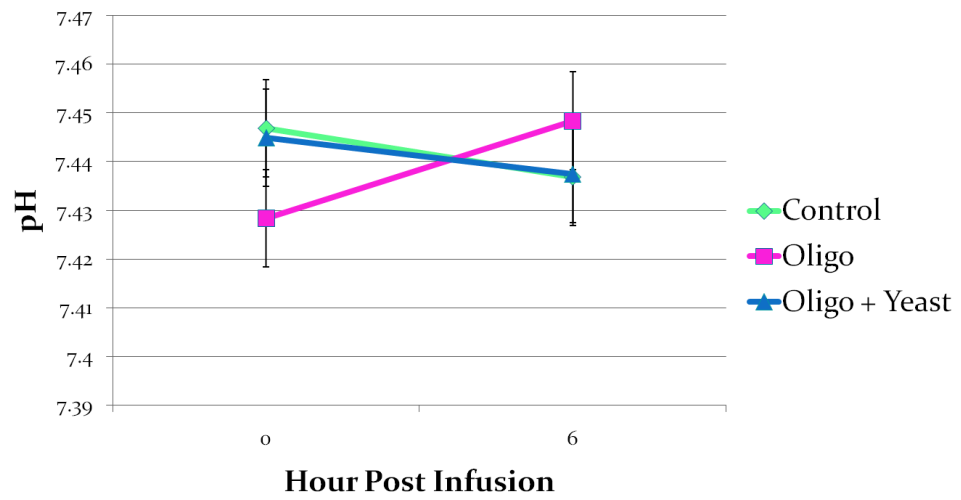


Figure 3.11. Effect of control, oligofructose and oligofructose + yeast treatment on blood pH over time. SEM = 0.01.
¹ Treatments: Oligo = oligofructose; Oligo + Yeast = Oligofructose + Yeast.

Chapter 4

DISCUSSION

4.1 Oligofructose Infusions as a Model for Post-Ruminal Acidosis

To our knowledge, this is the first time that abomasal oligofructose infusion has been used as a model to induce post-ruminal acidosis. However, one research group has utilized ruminal oligofructose to induce acute ruminal acidosis. Thoefner *et al.* (2004) infused 13, 17, or 21 g/kg BW oligofructose into the rumen to induce acute ruminal acidosis as indicated by a drop in rumen pH and development of laminitis. In this study, we used a substantially lower amount of oligofructose (1 g/kg BW) in an attempt to induce sub-acute post-ruminal acidosis. We did this because we expect that sub-acute ruminal and post-ruminal acidosis is a greater problem for dairy herds than acute ruminal and post-ruminal acidosis. Post-ruminal oligofructose infusions induced post-ruminal acidosis as was indicated by decreases in fecal pH and fecal score. However, the post-ruminal acidosis appeared to be sub-acute due to the lack of effects on respiration rate, heart rate, blood pH, and intake.

The main limitation to our model is that oligofructose was used as the substrate to induce post-ruminal acidosis whereas post-ruminal starch fermentation is the most likely substrate to induce post-ruminal acidosis on a commercial dairy. Smiricky-Tjardes *et al.* (2003) conducted a study of oligosaccharide fermentation using swine fecal inoculum and found that equal molar proportions of propionate, butyrate, and acetate resulted from bacterial fermentation. Another study directly

compared in vitro fermentation kinetics of starch and inulin using fecal inoculums from suckling pigs (Awati et al., 2006). Although organic matter disappearance over time was similar for both substrates, total VFA concentration was greater for wheat starch (7.58 mM) than for inulin (6.88 mM). Proportions of VFA were also different, with 0.0% lactate, 51.0% acetate, 35.7% propionate, and 8.0% butyrate for wheat starch and 0.4% lactate, 66.3% acetate, 19.4% propionate, and 11.2% butyrate for inulin (Awati et al., 2006).

The main benefit of oligofructose compared to starch as a model to induce post-ruminal acidosis in ruminally cannulated cattle is that we can control the amount of substrate that reaches the large intestine. Oligofructose contains β 2-1 linkages, preventing it from being digested in the small intestine. However, in the small intestine, some starches are digested (Streeter *et al.*, 1991 and Reynolds *et al.*, 1988). Because of the disappearance of starch in the small intestine, not all abomasally infused starch reaches the large intestine to cause post-ruminal acidosis. Therefore, higher infusion amounts may be necessary.

4.2 Animal Health and Stress

The effects of ruminal and post-ruminal acidosis on vital measures has been presented in several studies. Thoenfer *et al.* (2004) found that rectal temperature and heart rate were increased by acute ruminal acidosis induced by ruminal oligofructose infusions. However, it appears that SARA does not have dramatic effects like acute acidosis does. Gozho *et al.* (2007), found no difference in temperature between control cows and SARA cows.

We expect post-ruminal acidosis to cause similar animal responses as ruminal acidosis. When 5 g/kg BW starch was abomasally infused to induce post-ruminal acidosis, Bissell found no effect of infusions on respiratory rate or rectal temperature based on treatment. Bissell (2002) found respiratory rates of between 12 and 90 breathes per minute. Bissell (2002) found elevated temperatures in cattle dosed with abomasal infusions of 5 g/kg BW starch, with temperatures ranging between 37.9°C to 40.2°C, however treatment and treatment x day was not significant (Bissell, 2002). Normal rectal temperatures, heart rate, and respiration rate in dairy cattle range 38.6°F ±1, 48 and 84 beats per minute, and 26 and 50 breaths per minute, respectively (Kahn, 2005). The averages for each of our treatments fall within this range for temperature and respiratory rate. The cattle in this study had heart rates slightly outside of this range, which may have resulted from the stress of collecting samples, but there was no distinct effect of oligofructose upon heart rate. We found no effect of oligofructose on temperature, heart rate, or respiratory rate suggesting that there was no apparent stress response elicited by the abomasal infusions of oligofructose. We believe that the 1 g/kg BW oligofructose infusions caused sub-acute rather than acute post-ruminal acidosis in our dairy cattle.

Blood pH can be used as a marker for the severity of ruminal acidosis. Owens *et al.* (1998) stated that in order for a cow to be diagnosed as experiencing clinical or acute acidosis, the blood pH must fall below 7.35. In sub-clinical or sub-acute cases, a decrease in blood pH would not be expected. By looking at the blood pH of the cattle in this study, it is apparent that the 1 g/kg BW oligofructose infusions did not decrease blood pH, showing that the tissue in the gut were able to metabolize the acids before they were absorbed into the blood stream.

4.3 Yeast Additive

The oligofructose + yeast treatment was utilized in this study to determine whether dietary yeast might alleviate post-ruminal acidosis. Studies have shown that yeast supplementation increases ruminal pH (Mwenya *et al.*, 2005, Desnoyers *et al.*, 2009, Clayton *et al.*, 1999). Mwenya *et al.* (2005) used Holstein cows to determine the effects of yeast on ruminal fermentation. They found that the supplementation of 10 g/d yeast in addition to 2% galacto-oligosaccharides/kg feed increased the pH of the rumen above the pH found in cows only receiving 2% galacto-oligosaccharide/kg feed (Mwenya *et al.* 2005). Desnoyers *et al.* (2009) compiled data from 110 papers and 157 experiments and found that there was an overall increase in rumen pH for cattle supplemented with yeast compared to cattle not supplemented. Yeast may also have the similar benefits in the intestine. Clayton *et al.* (1999) found that fecal pH was increased for dairy cattle receiving 20 mg yeast/kg feed, compared to control animals receiving no yeast.

It has also been seen in studies that the addition of yeast to feed can have effects on the concentration and proportion of rumen VFA. Mwenya *et al.* (2005) found a lower concentration of VFA for the cows receiving 10g/d yeast + 2% galacto-oligosaccharide/kg feed compared to the cows only receiving 2% galacto-oligosaccharide/kg feed. Furthermore, they found a lower proportion of butyrate in cows receiving yeast compared to cows receiving galacto-oligosaccharide + yeast (Mwenya *et al.*, 2005). Finally, they found no significant change in acetate:propionate ratio of cows receiving galacto-oligosaccharids and those receiving galacto-oligosaccharides + yeast (Mwenya *et al.*, 2005). Similarly, Desnoyers *et al.* (2009)

found that supplementing rations with *Saccharomyces cerevisiae* did not result in any change in rumen acetate to propionate ratio. Unlike Mwenya *et al.* (2005), Desnoyers found that the addition of yeast caused an increase in VFA concentrations of the rumen.

The effects of yeast on blood metabolites has also been studied. Clayton *et al.* (1999) showed that there was an increase in blood concentration of BHBA increased following the addition of 30 mg/kg yeast to feed. However, various studies have found no treatment differences between calves supplemented with yeast and calves not receiving supplementation for blood concentration of BHBA (Lesmeister *et al.*, 2004 and Magalhães *et al.*, 2008). Furthermore, Wohlt *et al.* (1998) found that the addition of 10 g/d yeast caused an increase in blood concentration of urea during the first four weeks post-partum. We found no effect of yeast supplementation on plasma metabolites, but this may change after we complete sample analysis.

Chapter 5

CONCLUSION

From the data analyzed to this point, a pulse dose of 1 g/kg BW abomasal oligofructose appears to have been successful in inducing sub-acute post-ruminal acidosis. In the steers, acidosis was indicated by a decrease in fecal DM, decrease in fecal pH, and a decrease in fecal score. Acidosis was not likely acute because temperature, heart rate, and respiratory rate were not affected by treatment. The extent to which the steers' health and metabolism were affected cannot be assessed until analysis of the remaining samples. Furthermore, the 1 g/d yeast treatment appeared to have no affect on the incidence of post-ruminal acidosis in the steers.

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APPENDIX

YEAST DATA

Yeast Sampling and Analysis

Disappearance of yeast from feces and rumen fluid was measured only for the two steers on treatment 3 each period. Fecal and rumen samples for yeast analysis were collected at -24, 0, 8, 24, 48, 72, and 96 hours relative to the end of each period when the dietary treatment changed. Rumen fluid samples were collected by inserting a 50 mL conical tube through the ruminal cannula and through the rumen mat. Fecal samples were collected from the rectum. 1 mL of rumen fluid or 1 g feces was added to 9 mL of autoclaved diluent (0.1% peptone) and vortexed. The solution was squeezed through cheese cloth and a serial dilution was performed. One mL of 10^{-2} , 10^{-3} , and 10^{-4} dilutions were plated onto petrifilm (Yeast and Mold Count Plates, 3M, St. Paul, Minnesota). Petrifilm was then incubated for 48 hours at 30°C. At the end of incubation, colonies were counted using the dilution that contained between 30 and 300 colonies.

Yeast Retention Results and Discussion

The removal of yeast from the feed caused a decrease in the number of colony forming units (CFU) found in rumen fluid samples (Figure 3.11). There was a 1 log reduction in the amount of yeast present in the rumen fluid at 0 h (the last time the

yeast was fed) compared from the average of to 24 to 96 h. Fecal yeast concentrations were not impacted by yeast removal. The average for all hours was 3.41cfu/mL.

Yeast did not lessen the severity of the oligofructose induced decline in fecal pH or dry matter. The lack of effect on fecal pH may have been due to an inability of the yeast cells to survive in the gastrointestinal tract. At 0 h there was a log difference between colony forming units in the rumen compared to colony forming units in the feces. The reduction in yeast cfu found in the feces compared to yeast cfu found in the rumen is not consistent with the data found by Durand-Chaucheyras *et al.* in sheep (1998). They found that there was approximately 1×10^5 CFU mL⁻¹ in the rumen and 2.2×10^5 CFU/mL (Durand-Chaucheyras *et al.*, 1998). As expected, ruminal yeast concentration decreased following removal from the diet. However, there was no change in feces yeast counts over time after removing yeast from the diet. The relatively low fecal yeast concentrations combined with no change in fecal yeast following removal of yeast from the diet suggest that passage of live yeast to the large intestine was low. We plated only one sample at each dilution, and increased replicates may have reduced experimental error. Moreover, we did not measure rumen and fecal counts in the control animals.

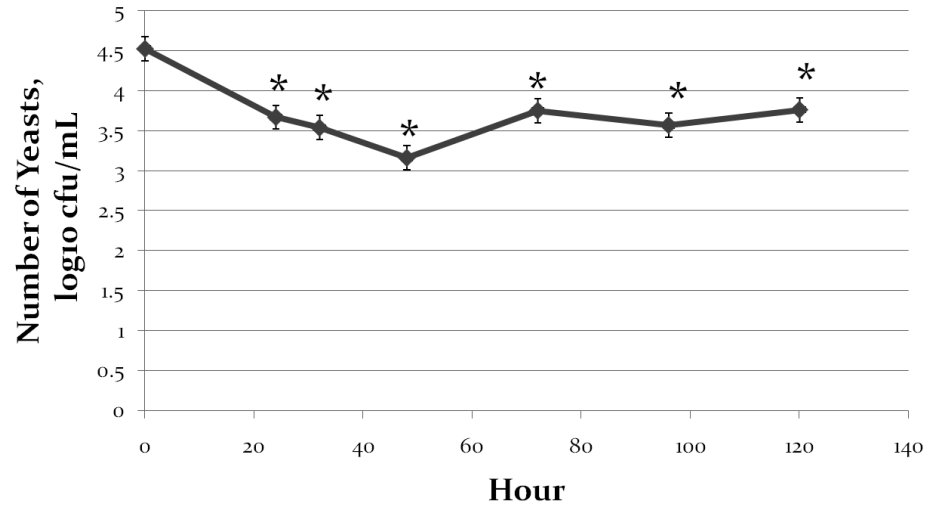


Figure 3.12. Yeast plating results for samples taken from the rumen. 0 h represented day 14 of the period when the steer received yeast, while 24-96 h represented day 1 through 5 of the following period. *Significance was determined at $P < 0.05$ compared to 0 h. SEM = 0.15