THE POTENTIAL FOR ABOMASALLY INFUSED *S. BOULARDII* TO ALLEVIATE LARGE INTESTINAL ACIDOSIS

by

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ABSTRACT

Large intestinal acidosis occurs when excessive amounts of carbohydrates are fermented in the large intestine, an event which may contribute to laminitis and gastrointestinal damage. The objectives of this study were to: a) develop a less severe model of oligofructose-induced large intestinal acidosis than the models previously utilized and b) evaluate the ability of *Saccharomyces boulardii* (SB) ability to alleviate this oligofructose-induced large intestinal acidosis. Six ruminally cannulated steers were used in a replicated 2x2 Latin square experiment with two-18 day (d) periods. Steers were randomly assigned to one of 2 treatments: (1) Control (0 g/d SB) and (2) and SB (10 g/d SB). Control and SB treatments administered via abomasal infusion with equal amounts provided at 10 AM and 4 PM daily. On d 16 of each period, steers were abomasally infused every 6 h for 24 h with 1 L of water containing 0.25 g/kg oligofructose. Fecal samples were periodically collected for 24 h prior to and for 48 hours following the first abomasal oligofructose infusion. The oligofructose infusions resulted in large intestinal acidosis as indicated by a decrease in fecal pH and fecal score and an increase in fecal volatile fatty acid (VFA) concentrations. This large intestinal acidosis model did not change fecal dry matter content and induced a fecal pH and fecal score decline which was less severe than those seen in previous models We found that SB treatment had no effect on pre-oligofructose challenge measurements. During the oligofructose challenge however, SB tended to increase fecal pH (trend at $P=0.09$) and decrease total fecal VFA concentrations ($P=0.05$). SB treatment had no affect on fecal score or dry matter content. We conclude that our

large intestinal acidosis model was less severe than those used in previous studies as indicated by a less severe decrease in fecal score and that the oligofructose infusion had no effect on fecal DM content. Additionally we propose that SB has the potential to alleviate the severity of this large intestinal model by fasciliating higher fecal pH and lower total VFA concentrations. Higher fecal pH and lower fecal VFA concentrations suggest that SB has the potential to alleviate the severity of large intestinal acidosis.

Chapter 1

INTRODUCTION

1.1 Bovine Digestive Physiology

1.1.1 Digestion Overview

Cattle, as a ruminant species, possess a highly specialized digestive tract which allows for the use of roughages as the primary energy source. Unlike monogastric mammals, cattle have one stomach which is divided into four compartments. These compartments are the reticulum, rumen, omasum, and abomasum. The reticulum and rumen are often referred to as the reticulo-rumen as there is little anatomical difference between these two compartments. Each stomach compartment plays a different role in feed digestion and all of them are highly specialized to deal with cellulose digestion.

Feed first enters into the reticulo-rumen where it begins to be fermented by rumen microbes. It is then regurgitated and re-swallowed in a process known as rumination. Both the fermented and unfermented ingesta which bypass rumen fermentation then pass into the omasum. From the omasum, the ingesta pass into the abomasum. The abomasum contains acidic gastric juices and it is similar in function to the stomach in monogastric species. From the abomasums, the ingesta pass through to the small and large intestines and it is finally excreted as feces.

1.1.2 Rumen

The reticulo-rumen is a large fermentation chamber in which rumen microbes ferment a wide range of carbohydrates including sugars, starches, cellulose, hemicellulose, and pectins. Starches, which tend to be highest in grain and cereal crops, contain α- linked glucose whereas cellulose, which is found in roughages, contains β-linked glucose. The differences in glycosidic linkages dramatically affect digestibility. In most species, including cattle, the body produces digestive enzymes which can cleave α but not β-linkages. The rumen, however, contains cellulolytic bacteria which produce enzymes that hydrolyze the β-linkages between monosaccharide units in cellulose. The majority of rumen microbes can ferment starches, while only cellulolytic bacteria can perform cellulolysis. The normal fermentation end products are volatile fatty acids (VFA), which include acetate, propionate, and butyrate, and lactate. The body uses VFA as an energy substrate (Argenzio and Stevens, 1984). Under normal conditions lactate does not typically accumulate in the rumen as certain rumen bacterial species can ferment lactate into propionate and other VFA. These lactate fermenters and lactate-utilizing bacteria such as *Megasphaera elsdenii* prevent lactate accumulation in the rumen (Paynter and Elsden, 1970, Chiquette, 2009).

Although the ruminal pH fluctuates within a 24 hour period, optimal ruminal pH is greater than 5.5 and anytime the pH falls below this setpoin, it is considered suboptimal. The optimum pH range for rumen microbes is from 5.5 to 7.0 (Argenzio and Stevens, 1984). Rumen pH is influenced by the amount of fermentable carbohydrate present in the diet and consequently rate of VFA production, the animal's ability to buffer via saliva production, and the rate at which the acidic fermentation products are absorbed across the rumen epithelium or metabolized by other rumen

microbes. Even if one increases the amount of rapidly fermentable carbohydrate in the diet, as long as the VFA absorption rate balances VFA production, the pH will remain within the optimal range (Nagaraja and Titgemeyer, 2007). In order to counter the acidic fermentation products, the rumen is buffered by the continual influx of the salvia provided by the rumination process. While VFA have an approximate pKa of 4.8, saliva contains bicarbonates and phosphates which are alkaline in nature. In addition to the buffering capacity of saliva, the rapid rate at which VFA are absorbed across the rumen epithelium serves to keep rumen pH within the optimum range (Argenzio and Stevens, 1984).

1.1.3 Small and Large intestines

Ingesta that bypass digestion and fermentation in the stomach pass through into the intestines. A significant proportion of the remaining unabsorbed digested products are absorbed in the small intestine. The digesta is composed of unfermented and partially fermented feed as well as rumen microbes. In the small intestine, enzymatic digestion begins to break down undigested starches and the resultant glucose is absorbed. Starch digestion is achieved via intestinal carbohydrases (e.g. isomaltase), amylase activities and starch hydrolysis (Nocek and Tamminga, 1991).

The remaining undigested feed moves into the large intestine where hindgut fermentation occurs. The large intestine contains microbes which, just as those in the rumen, ferment some of the remaining carbohydrates into VFA, producing CO_2 and methane as byproducts. VFA are absorbed across the large intestinal mucosa and rumen epithelium at equivalent rates (Argenzio and Stevens, 1984). The remaining undigested carbohydrates, proteins, and microbial components pass through the intestine and they are excreted in the feces.

Although the rumen is the primary organ where carbohydrate fermentation occurs, undigested carbohydrates that bypass ruminal fermentation are subject to small intestinal digestion and fermentation by the large intestinal microbes. Nocek and Tamminga (1991) found that in lactating cows fed various rations, apparent post-ruminal starch digestion ranged from 5.6 to 44.3% of intake. Karr et al. (1966) found that increasing dietary starch resulted in an increased flow of starch to the small intestine from 16% (19% starch ration) to 38% (63.5% starch ration) in steers.

Nocek and Tamminga (1991) also observed that the percent of rumen degradable starch tended to decrease as the concentration of starch increased. However, it is clear that there is variability in this relationship as Yang et al. (2001) found that increasing the proportion of forage in the total mix ration (TMR) resulted in decreased starch digestion in the rumen, data which appear contrary to the model proposed by Nocek and Tamminga (1991) as one would expect that increased forage content would result in increased ruminal starch digestibility. Nevertheless, the general trend is that at high starch concentrations starch digestion in the rumen decreases and percentage of post-ruminal starch increases.

1.2 Oligofructose

Inulins are fructose polymers consisting of β(1-2) fructosyl-fructose linkages (Waterhouse and Chatterton, 1993). Inulins contain a mixture of fructose oligomers and polymers and inulins of different origins have different degrees of polymerization (DP). Inulins isolated from plant sources have DP <200 whereas bacterial inulins can have DP ranging from 10,000 to over 100,000 (Roberfroid and Delzenne, 1998). After starch, fructans are the most abundant natural nonstructural polyssachharides and they are found in many different plants including chicory and

jersusalem artichokes (Debruyn et al., 1992). Oligofructose is produced by a partial enzymatic hydrolysis of inulin and has a DP $\leq$ 10. The β1-2 linkages between monomers in oligofructose, as in inulins, resist hydrolysis by digestive enzymes. Oligofructose is thus designated an undigestable carbohydrate; however, oligofructose is hydrolyzed and fermented by bifidobacteria in the colon (Roberfroid 1999, Roberfroid et al., 1999).

1.3 Ruminal Acidosis

In order to support high levels of milk production, feeds contain relatively high proportions of high-energy concentrates with an adequate proportion of forage to maintain ruminal health. Starch is the predominant energy source in these high-concentrate rations. Starch is rapidly fermented in the rumen and enzymatically digested in the small intestine, thus providing the animal with a "quick energy source" to support high milk production (Karr et al., 1966). Ruminal acidosis, which can exist in either an acute or sub-acute form, occurs when cattle consume rapidly fermentable carbohydrates (RFC) in large enough amounts that VFA absorption across the ruminal epithelium and saliva production are not sufficient to maintain optimal rumen pH. This pH decrease reflects the accumulation of acidic fermentation products such as VFA and lactic acid in the rumen. In a diet low in RFC and high in forages, cellulolytic microorganisms in the rumen metabolize cellulose to the end products acetate, propionate, and butyrate. This cellulolytic activity, however, is inhibited at low pH. Cellulolytic ruminal bacteria do not have the necessary mechanisms to resist low pH and therefore, cellulolytic activity decreases when rumen pH drops below optimal levels. Thus, ruminal acidosis contributes to reduced fiber digestability by decreasing cellulolytic activity (Russell and Wilson, 1996).

Although only a few microbial rumen species are cellulolytic, many rumen species can actively degrade the starches present in RFC and the carbohydrate fermentation rate increases with RFC content. Increased fermentation increases the VFA and other acidic fermentation end-product production which decreases ruminal pH (Nagaraja and Tithemeyert, 2007). Ruminal acidosis reflects an imbalance between the rates of microbial VFA production (such as an increase in lactate producing vs. lactate utilizing bacterial populations) and microbial VFA utilization and ruminal VFA absorption. Over-feeding or sudden increase in the proportion of RFC (such as transition from dry cow diet to lactating cow diet) can cause lactic acidosis. Lactic acidosis occurs when the rate of lactate formation exceeds the rate of its fermentation, leading to lactate accumulation in the rumen (Prins et al., 1973). Long-term suboptimal ruminal pH can lead to changes in the rumen papillae and induce rumenitis, which impairs VFA absorption (McGavin and Morrill, 1976, McManus et al., 1977, Harmon et al., 1985).

1.3.1 Sub-Acute Ruminal Acidosis (SARA)

Sub-acute ruminal acidosis or SARA occurs between pH 5.0 to 5.5 (Goad et al., 1998). The major clinical manifestation of SARA is reduced or inconsistent feed intake, symptoms which are often attributed to factors other than acidosis. Additionally, in a commercial setting, it may be difficult to monitor individual feed intakes, making it difficult to diagnose SARA via inconsistent feed intake. The only reliable method to diagnose SARA is by testing ruminal pH. Sub-acute ruminal acidosis is a costly condition which can lead to decreased milk production and milk fat, unexplained diarrhea, laminitis, and even result in a high culling rate (Nocek, 1997). In an effort to study the ruminal fermentative and microbial changes associated

with SARA, Goad et al., (1998) induced acidosis in 6 ruminally cannulated beef steers. Acidosis was generated by withholding feed for 24 hours, followed by the administration of an all-grain diet for 3 days. SARA was induced in both groups, as evidenced by a decline in rumen pH to between 5.0 and 5.5. This decreased ruminal pH resulted from an increased VFA concentration rather than lactate accumulation. This indicates that the pH decrease in SARA typically results from increased fermentation and total VFA production rather than a shift towards lactate producing bacteria (Goad et al., 1998).

In order to meet the increased energy demands that lactation places on dairy cows, feeding strategies aim to maximize ruminal starch fermentability. One method to increase ruminal fermentation of starch is by increasing the degree of grain processing. As grain is more highly processed, for example by grinding, the increase in surface area allows for more of the starches to become available to fermentation by rumen microbes. Yang et al. (2001) reported that increased grain processing substantially increased the total tract digestibility due to a 33% increase in ruminal starch digestibility and a 15% improvement in post-ruminal starch digestion in lactating cows. Increasing digestibility theoretically provides more energy for the animal without increasing the feed starch content. While grain processing increased starch digestibility, neither ruminal nor post-ruminal NDF digestibility were affected. This indicates that fiber digestibility is not affected by the level of processing provided that ruminal pH is not negatively affected. Yang et al. (2001) also found that cows produced an average of 1 kg/d greater milk yield when fed more processed grain, a finding which was most likely related to the increased starch digestibility. Ruminal and post-ruminal neutral detergent fiber (NDF) digestibility was not affected by grain

processing, forage to concentrate ratio, or forage particle length. However, increasing the forage to concentrate ration resulted in a lower total tract NDF digestibility. They also found that the extent of grain processing decreased the time that pH spent above the threshold for SARA (pH 5.5) and almost doubled the time spent below. It is important to note that while processing increases starch digestibility and energy availability it also can lead to increased SARA incidence (Yang et al., 2001).

1.3.2 Acute Ruminal Acidosis

Acute acidosis is defined by a ruminal pH below 5.0 and it is considered to be the result of lactic acid accumulation (Nagaraja and Titgemeyer, 2007). Russell and Hino (1985) used *Streptococcus bovis* to study the lactate regulation and its effects on rumen acidosis. They found that *in vitro S. bovis* produced little lactate at pH 6.7, instead formate, acetate, and ethanol accounted for much of the product. At pH 4.7, simulating acute acidotic conditions, however, *S. bovis* switched to lactate production. From their data they proposed the following mechanism for the role of lactate regulation in acute ruminal acidosis. In ruminants fed forages with little starch content, *S. bovis* growth is restricted. When large amounts of starch are added to the diet, however, *S. bovis* growth is no longer restricted by energy source availability and it grows faster than other rumen bacterial species. As their growth rate increases, this results in metabolic changes which switch from acetate and formate production to lactate production (Russell and Hino, 1985). Lactic acid is 10 times more acidic than VFA and as it is less protonated, it accumulates in the rumen rather than being absorbed. Lactate accumulation decreases rumen pH more drastically than other fermentation products (Nagaragja and Titgemeyer, 2007). Low rumen pH also favors

the growth of other lactate producing bacteria, such as lactobacillus, while inhibiting the activity of lactate-fermenting bacterial species (Therion et al., 1982).

Although there are many experimental acidosis models, acute ruminal acidosis models typically involve introducing high levels of RFC to the rumen. For example, Khrebiel et al. (1995) used glucose to induce acute acidosis in lambs. Administering glucose to the rumen led to an accumulation of lactate and a decreased VFA concentration. A decrease in VFA absorption across the rumen epithelium also occurred and this decrease in absorption was still evident after 6 months.

1.3.3 Acidosis and Laminitis

In addition to disrupting normal rumen function, acute ruminal acidosis has been linked to the development of laminitis in cattle. Laminitis is a condition caused by inflammation in the hoof which results in hoof cell death. This inflammation is believed to be caused by a dysfunction of the digital vasculatory system that results in hypoxia and malnutrition within the hoof wall. These pathological alterations within the hoof cause considerable pain and lameness in afflicted animals. The term laminitis, however, is used to describe a systemic disease which affects the general condition of the animal, not just the hooves (Bergsten, 2003). While there are many risk factors for laminitis, including heat stress, walking surface, and parturition, ruminal acidosis can lead to the development of laminitis (Oetzel et al., 2004). The main theory is that endotoxin release causes capillary obstruction in the hoof which leads to cell death. Ruminal acidosis disturbs the gastro-intestinal metabolism and integrity of epithelial tissues, resulting in high levels of endotoxin production and absorption across the gastro-intestinal mucosa into the bloodstream. Once in the circulatory system, the endoxtoxins trigger a prostaglandin cascade,

leading to an increase in thromboxane and a decrease in prostaglandin cycling. Trombes are produced which obstruct the capillaries in the laminar corium of the hoof. This causes local deteroriation of the blood circulation which decreases oxygen and nutrient supply to the hoof cells, thus damaging the cells (Bergsten, 2003).

Several studies have looked at the role of acidosis and endotoxin production on laminitis development. Mullenax et al. (1966) found that generating acute acidosis (pH less than 5) resulted in a shift in the gram-positive to gram-negative bacterial ratio, where gram-positive bacteria predominated. This shift was accompanied by a fifteen-fold increase in the amount of free endotoxin in the rumen. While they did not find evidence of endotoxin in the blood, they hypothesized that the liver functions to detoxify the endotoxin. In situations where the rumen or intestinal epithelium is damaged however, this natural ability to detoxify the endotoxin would fail and result in endotoxemia.

Singh et al. (1994) found that endotoxemia can induce hoof lesions in cattle. The intravenous infusion of *Escherichia coli* endotoxin resulted in sole and hoof wall hemorrhages. Histopathological analysis showed laminae and papillae epidermal cell degeneration in the hoof, arteriosclerosis in all parts of the hoof corium, and proliferative changes in the laminae. These observations support the mechanism for endotoxin induced laminitis described previously.

Thoefner et al. (2004) examined the clinical response of cattle dosed with an alimentary oligofructose overload, particularly focusing on laminitis development. This study used 12 dairy heifers, 6 of which were control, and 6 of which received oligofructose boluses. Oligofructose treatments were divided into 3 subcategories: 13, 17, or 21 g/kg BW oligofructose; however all treatments induced similar clinical

effects. The oligofructose infusions first induced a period of gastro-intestinal disease (rumen acidosis, decreased rumen contractions, and diarrhea) which was followed by laminitis development in 4 of the 6 animals. This study demonstrated that oligofructose induced both ruminal acidosis and laminitis, indicating that the conditions that favor acidosis also favor laminitis development. Overall, these studies indicate that acidosis correlates with laminitis due to the dead of gram-negative bacteria and the consequent release of endotoxin, these endoxtoxins can lead to arteriosclerosis and hemorrhage in sole and hoof, and that the conditions that favor acidosis also favor laminitis development.

1.4 Post-Ruminal Acidosis

Although most acidosis studies focus on ruminal acidosis, the same dietary conditions that favor ruminal acidosis can also lead to post-ruminal acidosis. Both the level of starch feeding and starch availability in feed influence the amount of starch which bypasses the rumen and passes into the intestines. When early weaned lambs were fed *ad libitum versus* 70% *ad libitum* the proportion of dietary starch passing into the abomasum was significantly increased. Diet, however, had no effect on the fecal starch concentration. These data suggest that while increasing starch intake increases the amount of post-ruminal starch, small intestinal starch digestion and hindgut fermentation compensate for insufficient ruminal fermentation, resulting in no net difference in fecal starch content (Orskov *et al.,* 1968). Increased hindgut fermentation results in similar effects as those seen with increased ruminal fermentation. Karr et al. (1966) also observed an increase in post-ruminal digestion following the administration of high-concentrate rations. Increasing the starch content of feed (from 19% starch to 63.5% corn starch) resulted in an increased flow of starch

to the intestines (from 16% to 38% of daily starch consumption). These data show that starches escape ruminal fermentation at both low and high total starch concentrations and that yet undigested starches are digested in the small intestine and fermented in the large intestine.

1.4.1 Post-ruminal Acidosis Models

Reynolds et al. (2001) found that post-ruminal starch infusion significantly decreased fecal pH and apparent nitrogen digestion (from microbial protein)), indicating hind-gut fermentation. This increase in fecal nitrogen concentration results from the fact that as fermentation increases, more microbes are produced. In that study, 4 cows late in lacatation were fitted with ruminal and duodenal cannulas in order to measure starch digestion in the different portions of the digestive tract. Cows were abomasally infused with either 18 L/d of water or 1200 g/d purified wheat starch suspended in 18 L of water for the last 14 d of 5 wk periods in a balanced switchback design with 3 periods. This infusion of 1200 kg of starch into the abomasum increased total tract apparent starch digestion. Fecal starch output increased by 20 g/d, indicating that virtually all of the infused starch was digested. Increases in fecal nitrogen concentration and a decrease in fecal pH indicated that at least a portion of the starch was fermented in the hindgut. While the amount of digestion in the small intestine cannot be determined, these data indicate that when large amounts of starch are infused post-ruminally, this can overwhelm digestion in the small intestine and increase hind-gut fermentation.

While Orskov et al. (1968), Reynolds et al. (2001), and Karr et al. (1966) demonstrate post-ruminal starch fermentation, only Reynolds et al. (2001) addressed the induction of post-ruminal acidosis. Another starch induced post-ruminal acidosis

study was conducted by Bissell (2002). In this study, post-ruminal acidosis was induced on d 2 through 4 by infusing 4 kg of starch (5 g starch /kg BW) over a period of 12 h followed by a 12 h rest period. Following starch infusions, fecal pH dramatically decreased (ranging from ~7 to ≤ 5)) *versus* the control which remained constant over the course of the trial. This decrease in fecal pH was evidence of hind-gut acidosis, which was caused by increased hind-gut fermentation. Wheeler and Noller (1977) found there was no difference between small intestinal and fecal pH, concluding that fecal pH is a good indicator of intestinal pH. Therefore, in Bissell (2002)'s post-ruminal acidosis model, fecal pH is representative of intestinal pH. Bissell (2002) also observed that mucus, tissue segments, and mucin casts were often present when the fecal pH was less than 6.0. This damage was consistent with epithelial layer damage, indicating that at pH below 6.0, tissue damage occurred. This intestinal epithelial damage is analogous to the rumen epithelial damage that occurs in ruminal acidosis. In both cases, as pH decreases below 5.5 for ruminal and 6.0 for hindgut acidosis, tissue damage occurs. Although Bissell's model successfully created post-ruminal acidosis, this model is limited as the 5 g/kg starch challenge was extremely severe and the amount of starch digested in the small intestine *versus* fermented in the large intestine could not be determined.

Mainardi (2009) generated a large-intestinal acidosis model using abomasal oligofructose infusions. Oligofructose is a compound which, unlike starch, cannot be digested in the small intestine; instead it is fermented in the large intestine. Treated steers received a single pulse dose of approximately 645g (1 g/kg BW) oligofructose dissolved in 1 L water via an abomasal infusion line. Control animals received 1 L water. Oligofructose infusions decreased fecal pH and altered fecal

characteristics. Average fecal pH for oligofructose-treated animals was 6.67 *versus* 7.02 in the control, however fecal pH was lowest at 6 h (6.46 *vs*. 6.76 in the control) Control animals had a higher fecal score and fecal dry matter percent than oligofructose treated animals. Oligofructose-treated animals also had a significantly higher total VFA concentration than control animals. These data indicate that Mainardi (2009) successfully generated a hind-gut acidosis model using oligofructose.

1.5 Probiotic effects of yeast culture

1.5.1 *Saccharomyces cerevisiae*

Many acidosis studies focus on the ability of probiotic supplements to decrease the incidence of ruminal acidosis. A common dietary supplement is the probiotic yeast SC. A study conducted by Williams et al. (1991) investigated the effects of including a SC yeast culture on rumen fermentation patterns. In the first experiment 3-200 kg Holstein steers with rumen cannulas were sequentially placed on 3-35 d treatments. The first treatment was hay plus ground barley, a diet which was formulated to decrease ruminal pH and suppress cellulolysis. The second treatment consisted of hay, 7.5 g SC/d, and ground barley. Finally the third treatment was a high forage diet containing just hay. For all treatments, hay was fed *ad libitum*, barley was fed twice daily and, when applicable, SC was fed with barley once daily. This study found that barley immediately depressed the ruminal pH and the effect persisted for 4 h; SC, however, reduced this pH depression. The maximum pH depression occurred at the same time as the peak lactic acid concentration indicating that lactic acid contributed to pH depression. Two h after the barley was administered the lactic acid concentration peaked, an effect prevented by SC. Not only did SC prevent the lactic

acid peak, but the SC treatment resulted in a lower overall lactic acid concentration in the rumen fluid. In this study, SC appears to resist the pH depression caused by barley administration by eliminating the lactic acid peak and accumulation in the rumen.

While the bicarbonates found in saliva chemically buffer ruminal pH, studies have shown that SC also appears to stabilize ruminal pH. Marden et al. (2008) found that when cows were fed a diet which favored SARA, both sodium bicarbonate and SC were able to stabilize rumen pH. They also found that SC decreased the lactate content in the rumen, whereas sodium bicarbonate had no effect on lactate concentration. Additionally, SC appears to have a stabilizing effect on ruminal fermentation. Harrison et al. (1998) report that live SC cultures have a stabilizing effect on ruminal fermentation in lactating cows. Cows receiving SC had a higher concentration of cellulolytic and anaerobic bacteria and ammonia concentrations than control animals. They also observed decreased variation in ammonia, total bacteria, and cellulolytic bacteria concentrations, indicating that SC stabilized ruminal fermentation.

Although SC appears to play a beneficial role in buffering rumen pH and stabilizing ruminal fermentation, there are conflicts between studies. Williams et al. (1991) found that SC prevented a decrease in rumen pH, whereas Harrison et al. (1998) found that SC resulted in a lower pH than control. Where Marden et al. (2008) found that live SC favored fiber digestibility, Harrison et al. (1998) found no difference in fiber, starch, or hemicellulose digestibility. These discrepancies may result from the use of different SC strains, differences in diet composition, or other unknown factors.

Although the majority of yeast studies focus on ruminal acidosis, Mainardi (2009) looked at the ability of SC to alleviate large intestinal acidosis. Treated animals received 1 g SC/d sprinkled on top of feed. The addition of SC did not impact the severity of large intestinal acidosis; however the large pulse-dose oligofructose challenge may have counteracted any probiotic yeast effects. Although certain strains of SC have proved beneficial in ruminal acidosis, SC, when added to feed, may not survive *en route* to the large intestine. It is also possible that this specific strain of yeast does not exert a probiotic effect on large intestinal acidosis.

1.5.2 *Saccharomyces boulardii*

There have been extensive studies looking at the role of probiotic yeasts in the rumen; however, little research has looked at their role in hind-gut acidosis. Mainardi (2009) found that SC had no appreciable benefit in large-intestinal acidosis; however this may have resulted from a number of different factors. Another yeast, *S. boulardii* (SB), however, has been shown to have a probiotic effect in human large intestinal disorders, such as antibiotic-associated diarrhea (AAD) and acute diarrhea. Kurugol and Koturoglu (2005) administered 250 mg SB/d to children (aged 3 mo. to 7 years) who presented with acute diarrhea. By the second day, SB treatment significantly decreased both the number of stools and the duration of diarrhea compared to the control. Kurugol and Koturoglu (2005) also saw a decreased incidence of watery diarrhea. Kotowska et al. (2005) examined SB's ability to decrease the severity of AAD in children. This AAD results when the administration of a broad spectrum antibiotic disrupts the normal intestinal microflora, leading to acute inflammation of the intestinal mucosa. Kotowska et al. (2005) found that children who received SB treatment had a decreased risk of AAD.

Although SB has mainly been used as an intestinal probiotic in humans, some recent studies have looked at its probiotic ability in ruminants. Oeztuerk et al. (2005) investigated the effects of SB on *in vitro* rumen microbial metabolism. A control (0g SB) or one of two treatments (0.5 and 1.5 g/d of live or autoclaved SB) were added to a 0.75 L fermentation vessel. They found that both live and autoclaved SB did not significantly increase VFA production until d 5 of culture. At d 5, butyrate, isovalerate, and valerate increased in a dose-dependent manner for both live and autoclaved SB. Both live and autoclaved forms of SB also significantly increased NH_3 production in a dose-dependent manner at d 5. The current theory proposed by Oeztuerk et al. (2005) is that rumen microbes degrade the yeast cell and this consequent nutrient release exerts a probiotic effect (increasing short chain fatty acid production). These data indicate that SB has a beneficial function in the rumen, regardless of its status.

1.6 Objectives

The objectives of this study were to: a) develop a less severe model of oligofructose-induced large intestinal acidosis and b) evaluate SB's ability to alleviate oligofructose-induced large intestinal acidosis.

Chapter 2

MATERIALS AND METHODS

2.1 Animals and Treatment

Six ruminally cannulated Holstein steers (aged 3-4 years) were fitted with abomasal infusion lines 1 wk before the experiment began and according to the procedures established by Gressley et al. (2006). Steers were weighed at the beginning of the experiment and their weights are located in Table 1. Steers were individually housed in 2.4 m x 4.6 m pens bedded with straw and shavings. A waterer and wooden feed bin were located in the front of each pen and water was provided *ad libitum*. The ration was formulated to represent a lactating cow diet (Table 2). Steers were feed-restricted at 1.5% body weight (BW) dry matter (DM) and fed daily at 10 AM. From 10 AM to 4 PM steers were chained to the front of the pen with a 1.5 m chain allowing for access to feed, water, and laying room. During the sampling period, steers were chained from 10 AM to 4 AM. All animal procedures were approved by the University of Delaware Agricultural Animal Care and Use Committee.

The study was composed of two-18 day periods. For each period, steers were randomly assigned to 2 treatments: (1) **Control** and (2) SB yeast (**SB)** according to a 2X2 Latin square design (2 treatments, 2 periods). Treatments consisted of an abomasal infusion of water (Control) or 10 g/d SB yeast (SB; Levucell SB20, Lallemand Inc., Milwaukee, WI) dissolved in water. Infusions occurred twice daily at 10 AM and 4 PM and consisted of 50 mL of water (Control) or 5 g SB dissolved in 50

mL of water. At each infusion point, abomasal infusion lines were flushed with 50 mL of water prior to and post treatment infusion. On d 16 of each period, all animals were abomasally infused with 0.25 g/kg BW of oligofructose (Beneo P95, Orafti Active Food Ingredients, Tienen, Belgium; Table 1), every 6 h for 24 h.

2.2 Feed sampling and Analysis

Weekly samples of individual feed components were collected and dried at 60˚C for 48 h in a forced air oven to determine DM content. The total mixed ration (TMR) was adjusted weekly in response to dry matter content changes in components. Ration components and nutrient composition can be found in Table 2.

Two representative TMR samples were collected from each period for particle size analysis. Particle size was evaluated according to the procedure established by Heinrichs (1996). Briefly, each sample was placed into a Penn State Particle Separator (Nasco, Fort Atkinson, WI) containing 3 screens (0.75 in, 0.31 in., and 0.007 in. sized holes) and rotated 360º counterclockwise in 90º increments. At each 90º increment, the box was vigorously shaken 5 times.

Two representative TMR samples were collected from d 15 and d 16 of each period (total of 4 TMR samples) and frozen at -20˚C for later analysis. For analysis, TMR samples from each period were dried in a 60˚C forced-air oven for 48 h to determine DM content. The TMR samples were then ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ) with a 2 mm screen and composited for analysis of starch, nitrogen, absolute DM, organic matter, neutral detergent fiber (NDF), and indigestible neutral detergent fiber (iNDF) by period. Composite ration samples were analyzed by wet chemistry (Cumberland Valley Analytic Services, Maugansville, MD) for starch content and nutrient composition. To determine organic matter content,

TMR samples were placed in a 600ºC ashing oven for 5 h. The University of Delaware's Soil Science Laboratory (Newark, DE) analyzed ration samples for nitrogen content. TMR samples were dried in a 100ºC forced-air oven for 1 h, placed in a dessicator for 20 min, and weighed to determine absolute DM content.

Neutral detergent fiber content was analyzed according to the procedure established by Reddish and Kung (2007). To determine NDF content, triplicate samples were analyzed using sodium sulfite and α-amylase (Ankom 200 Fiber Analyzer, Macedon, NY).

Indigestible neutral detergent fiber was analyzed using the in vitro DAISY assay described by Reddish and Kung (2007). Before the addition of sample, Ankom bags (Ankom Technology, Macedon, NY) were soaked in acetone and allowed to dry for 3 h. 0.25 g of TMR was then added to each pre-soaked bag. Quadruplicate samples were run at 39ºC for 96 h in an Ankom Daisy II incubator (Ankom Technology, Macedon, NY). Rumen buffer, macromineral, and micromineral solutions and ratio of rumen fluid to buffer were prepared as described by Goering and Van Soest (1970). Rumen fluid was collected from a ruminally cannulated Holstein cow. Rumen fluid, buffer, macromineral, micromineral and cysteine HCl solutions were replenished at 48 h. After 96 h, bags were analyzed for NDF content using the NDF procedure described previously.

2.3 Fecal Sampling

On d 15 of each period baseline fecal grab samples were collected at 10 AM (0 h), 4 PM (6 h), 10 PM (12 h), and 4 AM (18 h). Fecal grab samples were analyzed for fecal score, fecal pH, DM content, VFA content, and fecal yeast concentrations. Samples were given a fecal score (based on fecal consistency) from

1(liquid) to 5(hard) (Zaaijer et al., 2005). For fecal pH, 20 ± 2 g of feces was added to a 50 mL conical tube containing 20 mL dH2O. After vigorous shaking for 20 sec, a PH110 ExStik refillable pH meter (Extech, Waltham, MA) was inserted in the fecal-water mix, allowed to equilibrate, and fecal pH was recorded. Approximately 200 g of feces was collected and frozen at -20˚C for later dry matter and nutrient analysis. Twenty-five ± 5 g of feces was weighed out for fecal VFA analysis and exact amount was recorded. Ten mL of a 2% H_2SO_4 solution was added to the feces and vigorously mixed. The resulting solution was strained through cheesecloth (Fischer Scientific) and frozen for later analysis (Gressley and Amermentano, 2005). In order to evaluate fecal yeast counts, 35 g of feces was collected and refrigerated at 4ºC for approximately 6 h before analysis.

On d 16 the animals were challenged with a 0.25 g/kg oligofructose abomasal infusion at 10 AM, 4 PM, 10 PM, and 4 AM. Fecal grab challenge samples were collected at 0, 6, 12, 18, 24, 30, 36, and 48 h relative to the first challenge infusion for analysis of fecal score, fecal pH, DM, and VFA content, as described previously.

2.4 Fecal Sample Analysis

Baseline fecal samples collected for fecal yeast count analysis were first blended, filtered through cheesecloth, and diluted in peptone solution. Dilutions 1,000 to 10,000 were plated on petrifilm (Petrifilm, 3M Microbiology Products, St. Paul, MN) for period 1 and dilutions 1,000 to 10,000 were plated for period 2. Films were then incubated at 40˚C for 72 h. After 72 h the number of yeast colonies present was counted and recorded.

Fecal VFA was determined according to the procedure established by Muck and Dickerson (1988). Fecal samples frozen for VFA analysis were thawed and centrifuged at 9400 x g for 15 min. Five hundred µL of the resulting supernatant was combined with 500 µL CaOH (3.5M) and 250µL cupric acid (0.63M $CuSO_4$ and 0.046M crotonic acid) and vortexed. The samples were then refrigerated for 30 min and centrifuged for 15 min at 9400 x g. The resulting supernatants were transferred to clean tubes and the pellets were discarded. The supernatants were then frozen for 2 h and thawed for 30 min. Once thawed, 12.5 µL concentrated H_2SO_4 was added to each supernatant and the samples were refrozen for 2 h, thawed for 30 min and centrifuged at 9400 x g for 15 min. Approximately 750 µL of each supernatant was collected and transferred to a screw cap vial (Agilent, Fischer). The supernatants were sent on dry ice to Dairyland to be analyzed for lactic, acetic, proprionic, and butryic acid content, by high performance liquid chromatography (Dairyland Laboratories Inc. Arcadia, WI).

For quantification of nutrient digestibility 150 g of thawed fecal samples were dried for 48 h in a 60˚C forced air oven. Dried samples were composited, for a total of 3 composites/steer/period, as follows: baseline samples (0, 6, 12, and 18 h during baseline measures), challenge (0, 6, 12, 18, and 24 h during challenge measurements), and post-challenge samples (30, 36, and 48 h). Composite samples were then ground using a Wiley mill with a 2 mm screen and analyzed for NDF, iNDF, starch, and nitrogen content according to the procedures described previously.

2.5 Statistical Analysis

Results were analyzed using the Mixed procedure of SAS. For fecal DM, score, and pH, baseline results (samples at -24, -18, -12, and -6 h) were analyzed

independently from challenge results (samples at 0, 6, 12, 18, 20, 24, 30, 36, and 48 h). For fecal VFA, baseline results (samples at -24, -18, -12, -6, and 0 h) were analyzed independently from challenge results (samples at 6, 12, 18, 20, 24, 30, 36, and 48 h). For those models, the fixed effects included treatment, hour, sequence, period, and the interaction of treatment x hour and the random effect was steer within sequence. Hour was included as a repeated measure, steer within treatment was the subject, and a compound symmetry covariance structure was utilized. For analysis of total tract digestibility data, the same model was utilized except that hour was be replaced by day (pre-challenge, challenge, and post-challenge). Significance was declared at $P \leq 0.05$ and a trend declared at $P \leq 0.10$.

Chapter 3

RESULTS

3.1 TMR Particle Analysis

Based on the particle separator analysis, 17.6 % of the TMR particles were greater than 0.75 in., 31.2% were between 0.31 and 0.75 in., 38.5% were between 0.07 and 0.31 in., and 12.7% were less than 0.07 in.

3.2 Fecal Samples

3.2.1 Fecal DM Content

Baseline fecal DM content (% DM) was unaffected by treatment, time, and the interaction of treatment by time (Table 3, Figure 1). Challenge fecal DM content was also unaffected by treatment, time, and the interaction of treatment by time (Table 4, Figure 1).

3.2.2 Fecal pH

Baseline fecal pH was unaffected by treatment and the interaction of treatment by time. Baseline fecal pH was influenced by time ($P < 0.0001$; Table 3). The highest pH for both treatments was at -12 h, pH was similar at -24 h and -6 h and the lowest pH was at -18 h relative to the first oligofructose infusion (Figure 2). The challenge fecal pH was unaffected by the interaction of treatment by time. Challenge fecal pH tended to be affected by treatment ($P = 0.09$). SB treated animals tended to

have a higher pH than control steers during the oligofructose challenge (6.96 vs. 6.81). Challenge fecal pH values were affected by time (*P < 0.0001*; Table 4). Fecal pH rose from 0 h to 6 h, dramatically declined from 6 h to 12 h, and then rose again from 12 to 24 h relative to the first oligofructose infusion for both control and SB treated animals. For the control animals, fecal pH rose from 12 through 48 h relative to the first oligofructose infusion. For SB treated animals, fecal pH rose from 18 to 24 h and 30 to 36 h, declined slightly from 24 to 30 h and 36 to 48 h relative to the first oligofructose infusion (Figure 2).

3.2.3 Fecal Score

Baseline fecal scores were unaffected by treatment and the interaction of treatment by time (Table 3). Baseline fecal scores tended to decrease from -24 h to 0 h relative to the first oligofructose infusion (*P = 0.08*; Figure 3). Challenge fecal scores were unaffected by treatment and the interaction of treatment by time. Challenge fecal score was influenced by time (*P < 0.0001*) Table 4). Fecal score rose from 0 to 12 h and 30 to 36 h relative to the first oligofructose infusion. Fecal score sharply declined from 12 h to 18 h, remained constant from 18 to 24 h, and declined slightly from 36 to 48 h relative to the first oligofructose infusion (Figure 3).

3.2.4 Fecal Yeast Concentrations

Only 18.8% of all fecal samples collected during baseline measurements had great enough yeast concentration to be enumerated at the dilutions utilized in this trial (1,000 to 10,000). The samples that were countable did not demonstrate any obvious pattern relative to treatment (Control vs. SB) or time (-24, -18, -12, or -6 h relative to the first oligofructose infusion).

3.4 Fecal VFA

3.4.1 Baseline VFA

During the baseline measurements, there was no detectable amount of lactate present for either Control or SB (Table 5, Figure 4). Baseline acetate, butyrate, and propionate levels were unaffected by treatment and the interaction of treatment by time. Baseline acetate and butyrate levels were affected by time (*P = 0.001* and *0.002* respectively; Table 5). Baseline acetate levels declined from -24 to -18 h and -6 to 0 h and rose from -18 to -6 h relative to the first oligofructose infusion (Figure 5). Baseline butyrate levels declined from -24 to -18 h, declined slightly from – 18 to -12 h and rose from -12 h to 0 h (Figure 6). Baseline propionate levels tended to be affected by time (*P = 0.09*; Table 5). Propionate levels declined from -24 to -6 h and rose from -6 h relative to the first oligofructose infusion (Figure 7)

Baseline total VFA concentration was unaffected by the interaction of treatment or the interaction of treatment by time. Total VFA was affected by time (*P = 0.004*; Table 7). Initial Total VFA concentration was 52 mM for Control and 55.1 mM for SB. Peak total VFA concentration occurred at -6 h relative to the first oligofructose infusion for Control (62.6 mM) and at 0 h relative to the first oligofructose infusion for SB (61.3 mM). Total VFA concentrations increased from -18 to -6 h relative to the first oligofructose infusion for Control and from -18 to 0 h relative to the first oligofructose infusion for SB (Figure 8).

3.4.2 Challenge VFA

Lactate was present during the oligofructose challenge and lactate levels were affected by time (*P = 0.004*; Table 6). Lactate was not detected until 12 h after the first oligofructose infusion and peaked at 18 h for control and between 18 and 24 h

for SB. Lactate levels declined after 30 h for both treatments (Figure 4). Lactate levels were not affected by either treatment or the interaction of treatment by time.

Challenge acetate, propionate, and butyrate levels were unaffected by treatment or the interaction of treatment by time. Acetate, propionate, and butyrate levels were affected by time ($P \leq 0.0001$; Table 6). Control acetate levels peaked at 12 h and declined from 24 h to 48 h relative to the first oligofructose infusion. SB acetate levels peaked at 12 h, declined from 12 to 18 h, rose from 18 to 24 h, and declined from 24 to 48 h relative to the first oligofructose infusion. Peak acetate levels were significantly higher for control than SB at 12 and 18 h relative to the first oligofructose infusion (Figure 5).

Butyrate levels peaked at 12 h and declined from 12 h to 48 h relative to the first oligofructose infusion for control. SB butyrate levels peaked at 12 h and 24 h, declined from 12h to 18h, 24 h to 36h and rose from 36 to 48 h relative to the first oligofructose infusion. Peak butyrate levels were significantly higher for control than SB at 12 and 18 h relative to the first oligofructose infusion (Figure 6).

Propionate levels rose from 6 to 24 h and peaked at 24 h relative to the first oligofructose infusion for both control and SB. SB propionate levels declined from 24 to 48 h, whereas control propionate levels declined from 24 to 36 h and rose slightly from 36 to 48 h relative to the first oligofructose infusion. Peak propionate levels were significantly higher for control than SB at 24 h relative to the first oligofructose infusion (Figure 7).

Challenge total VFA concentration was unaffected by the interaction of treatment by time. Challenge total VFA concentration was affected by treatment ($P = 0.05$) and by time ($P \leq 0.0001$; Table 7). Control total VFA concentration declined

from 0 to 6 hr, rose from 6 to 18h, and declined from 18 to 48 h relative to the first oligofructose infusion. SB total VFA concentration declined from 0 to 6 h and 24 to 48 h relative to first oligofructose infusion. Total VFA concentration rose from 6 to 24 h for SB. Peak total VFA concentration occurred at 18 h for control and 24 h relative to the first oligofructose infusion for SB. The 0 h VFA concentration was 52 mM for Control and 55 mM for SB. Peak total VFA concentration was significantly higher for control (18h; 95.9 mM) *versus* SB (24 h; 72.6 mM). By 48 h total VFA concentration declined to 45.1 mM for Control and 45.9 mM for SB (Figure 8).

Chapter 4

DISCUSSION

4.1 Oligofructose-Induced Large Intestinal Acidosis

In order to support high levels of milk production, dairy cattle rations contain large quantities of rapidly fermentable carbohydrates (RFC) balanced with adequate quantities of forages. While these RFC provide the animal with a quick energy source, they can lead to ruminal and hindgut acidosis. Ruminal acidosis is a condition in which ruminal pH falls below optimum (5.5) due to increased RFC fermentation and the consequent accumulation of the acidic byproducts of fermentation. In addition to altering ruminal fermentation patterns, high quantities of RFC can also affect the more distal portions of the gastro-intestinal tract. When large quantities of RFC are present in the feed, significant portions bypass the rumen and enter the intestines where they are enzymatically digested in the small intestine and fermented in the large intestine. Increased RFC fermentation in the large intestine can lead to large intestinal (or hindgut) acidosis characterized by decreased intestinal pH and damage to intestinal mucosa. Some rations and dietary treatments are believed to alleviate symptoms of hindgut acidosis. However, the efficacy of such treatments is difficult to quantify due to a lack of models to study hindgut acidosis. In this study we sought to develop a large intestinal acidosis model using abomasal oligofructose infusions. Oligofructose was used because its β (1-2) glycosidic linkages cannot be enzymatically digested in the small intestine, rather fermented in the large intestine. In

our large intestinal acidosis model, 0.25 g/kg BW of oligofructose was abomasally infused every 6 h for 24 h, an approach which was designed to induce sub-acute large intestinal acidosis. In addition to developing this large intestinal acidosis model, we also investigated whether the human intestinal probiotic yeast, *S. boulardii* (SB), has the potential to exert a probiotic effect in oligofructose-induced acidosis.

4.2 TMR particle size

According to Heinrichs (1996), ideally, 6 to 10% or greater of TMR particles should be > 0.75 in. Thirty to 50% should be between 0.31 and 0.75 in, and 40 to 60% less than 0.31 in. Our values are consistent with these values established by Heinrichs (1996).

4.3 Fecal Yeast Counts

In this experiment we evaluated the yeast concentration in the feces for both control and SB treated animals. We expected that SB treated animals would have a greater fecal yeast concentration, however, the vast majority of samples did not contain countable yeast colonies irrespective of treatment with SB. Blehaut *et al* (1989) found that less than 1% of live SB cells were recoverable in the feces of both humans and rats and that 68% and 83% of dead cells introduced to the gastrointestinal tract are destroyed within 24 h for man and rat, respectively. To account for this phenomenom, they proposed that the lack of live cells in feces may be related to the presence of bacterial hydrolyzing enzymes in the large intestine which can digest the polysaccharides present in the SB cell wall. Oeztuerk *et al.* (2005) found both live and autoclaved SB yeast exhibited the same probiotic effects on an *in vitro* rumen environment. They concluded that rumen microbes digested SB to provide an alternate

nutrient source. The lack of countable fecal yeast colonies in our experiment may be explained by intestinal microbe SB digestion. Additionally, our detection techniques could only examine the living yeast cells present in the feces and so we were unable to determine the amount of dead SB cells present in the feces. We also could not distinguish between SB colonies and other yeast colonies, which may explain the apparent discrepancies in the data.

4.4 Hindgut Acidosis Studies

4.4.1 Current Hindgut Acidosis Models

We found that our abomasally-infused oligofructose large intestinal acidosis model (0.25 g/kg BW oligofructose treatments infused every 6 h for 24 h) had the ability to induce large intestinal acidosis as indicated by a decrease in fecal pH and an increase in fecal VFA. Bissell (2002) induced severe hindgut acidosis using 5 g/kg BW of starch infused abomasally over a period of 12 h/d for 2 d. These infusions caused a dramatic drop in fecal pH (pH < 5), significantly altered the fecal characteristics, decreased dry matter content, and caused intestinal damage. While Bissell (2002) induced hindgut acidosis, this effect was much more severe than hindgut acidosis typically seen on dairy farms and so this model may not be commercially relevant. Additionally, starch can be enzymatically digested in the small intestine or fermented in the large intestine and the amount of starch fermented in the intestines cannot be determined. Another starch model created by Reynolds et al. (2001) involved continuous post-ruminal infusions of 1200 g/d wheat starch for 14 d. These starch infusions significantly decreased fecal pH, however Reynolds did not evaluate fecal DM, VFA concentration or score. Mainardi (2009) developed a post-

ruminal oligofructose model which used a single abomasal pulse-dose of 1 g/kg BW oligofructose. While this challenge did not cause symptoms of intestinal damage, this treatment significantly decreased fecal pH, decreased fecal dry matter, and altered fecal characteristics

4.2 Post-ruminal acidosis models

One method to measure the severity of post-ruminal acidosis is fecal DM content. We found that 0.25 g/kg BW abomasally infused oligofructose had no affect on fecal DM content. During the baseline measurements, average fecal DM content was approximately 14.5% (pre-oligofructose challenge) *versus* 14.2 % (DM during oligofructose challenge). While fecal DM content declined at 6 and 18 h relative to the first oligofructose infusion, fecal DM rose to pre-oligofructose challenge levels at 12 h; fecal DM content appeared to fluctuate during the oligofructose challenge. Conversely, Mainardi (2009) found that a single abomasal 1 g/kg oligofructose pulse-dose significantly reduced the percent of DM in oligofructose treated animals relative to the control. Dry matter percent decreased at 6 h post-oligofructose infusion (~12% vs. 15% in the control) but recovered at 12 h (16% vs. 15%), to a higher percent compared to the control. In another post-ruminal acidosis model, Bissell (2002) used 5 g/kg post-ruminal starch infusions to induce acidosis. While Bissell (2002) did not directly measure the fecal DM percent, they observed diarrhea and changes in fecal consistency in all starch-treated animals, observations which suggest decreased DM content. Based on the fecal photographs, control animals had dark brown feces with visible evidence of undigested material. Animals receiving starch infusions experienced either foamy or watery diarrhea. These photographs indicate that DM content was reduced in starch-treated animals. Neither Mainardi (2009) nor our

experimental models generated fecal characteristics resembling those seen by Bissell (2002), indicating that DM content was most significantly impacted by the 5 g/kg post-ruminal starch infusions. In our model, oligofructose infusions ceased at 18 h, and based on the results from Bissell (2002) and Mainardi (2009), we had expected to see a time effect during the challenge and post-challenge measurements (6 to 48 h relative to the first oligofructose infusion) on fecal DM content. The fact that we found no significant post-challenge time effects suggests that our large intestinal acidosis model was not severe enough to affect DM content.

In addition to fecal DM content, fecal score can be used to evaluate the incidence of hindgut acidosis. While our large intestinal acidosis model did not affect DM content, it did affect fecal score. Fecal score is a numerical representation of fecal consistency from 1(liquid) to 5(hard; Zaaijer *et al.*, 2005). We observed that our oligofructose model decreased fecal score from 3 at 0 h to just below 2.5 at 18 and 24 h during the oligofructose challenge period. The 1 g/kg abomasal oligofructose pulse-dose model used by Mainardi (2009) also decreased fecal score to during the challenge period (2.45 vs. 2.71). Fecal score declined from 2.8 at 0 h to 1.4 at 6 h *versus* the control (3.2 at 0 h, 2.8 at 6 h) Bissell (2002) observed dramatic changes in fecal consistency and color using a 5 g/kg post-ruminal starch infusion. Cows receiving this infusion experienced severe diarrhea and feces changed from a normal brown color to yellow. Although Bissell (2002) did not use a fecal score to quantify fecal consistency, according to our scale, fecal score would have been 1. Based on fecal score measures, our post-ruminal acidosis model was less severe than the models created by Mainardi (2009) and Bissell (2002).

In addition to fecal score, fecal pH is another method to evaluate the severity of post-ruminal acidosis and degree of large intestinal fermentation. We expected 0.25 g/kg BW abomasal oligofructose infusions would decrease fecal pH. At 0 h relative to the first oligofructose infusion, fecal pH was 6.60 and the minimum fecal pH (6.19) occurred at 12 h relative to the first oligofructose infusions. Oligofructose infusions ceased at 18 h and fecal pH returned to pre-challenge levels by 24 h (6.66) .Mainardi (2009) found that an abomasal 1 g/kg oligofructose pulse-dose caused fecal pH to drop from 6.76 at 0 h to 6.50 at 6 h in treated animals compared to 6.85 at 0 h and 6.94 at 6 h in control animals. Fecal pH in treated animals returned to pre-oligofructose infusion levels by 9 h (6.72). Bissell (2002) observed that 5 g/kg starch infusions caused fecal pH to drop from 7.0 at 0 h to < 5 at 60 h relative to first post-ruminal starch infusion. Fecal pH levels returned to pre-starch infusion levels by 120 h relative to the first post-ruminal starch infusion. Reynolds et al. (2001) found that animals receiving 1200 g/d of post-ruminally infused starch for 14 d had significantly lower fecal pH than the control (6.26 vs. 6.64). Our large intestinal acidosis model caused a decrease in fecal pH. According to fecal pHs, our model of large intestinal acidosis appears similar in intensity to those induced by Mainardi (2009) and Reynolds et al. (2001), however it was not as severe as the model created by Bissell (2002).

Finally, fecal VFA concentration is an indication of large intestinal fermentation. We found that 0.25 g/kg oligofructose administered abomasally every 6 h for 24 h resulted in a peak total VFA concentration of 95.9 mM at 18 h compared to 61.0 mM at 0 h relative to the first oligofructose infusion. Mainardi (2009) found that 1 g/kg pulse-dose oligofructose infusion caused total VFA concentration to peak at

approximately 100 mM at 12 h *versus* approximately 60 mM at 0 h relative to the oligofructose pulse-dose. In our model, lactic acid was not detectable until 6 h. Lactic acid concentrations peaked at 18 h (16.6 mM *vs*. not dectable at 0 h) relative to the first oligofructose infusion. We also observed that acetic acid concentration peaked at 12 h (60mM *vs.* 44mM at 0 h). The butyric acid concentration peaked at 12 h (7.22 *vs.* 5.67 at 0 h) relative to the first oligofructose infusion. Finally propionic acid concentration peaked at 24 h (21.9 *vs.* 11.8 at 0 h) relative to the first oligofructose infusion. Mainardi (2009) observed that the fecal lactic acid concentration peaked at 12 h (5 mM *vs.* 0 at 0 h) relative to oligofructose pulse-dose administration. The acetic acid concentration peaked at 12 h (approximately 75 mM *vs.* 50 mM at 0 h) relative to the administration of the oligofructose pulse-dose. Butyric and propionic acid concentrations peaked at 12 h (7 mM *vs.* 5 mM at 0 h for butyric and 14 mM *vs.* 12 mM at 0 h for propionic acid) relative to the administration of the oligofructose pulse-dose. While Mainardi (2002) found that a single pulse-dose of 1 g/kg abomasally infused oligofructose caused peak VFA concentrations at 12 h (lactic, acetic, butyric, and propionic acid) we saw that acetic and butyric acid peaked at 12 h whereas lactic acid did not peak until 18 h and propionate did reach peak concentration until 24 h.

Although our oligofructose induced large intestinal acidosis model did not affect fecal DM content or decrease fecal score as severely as previous models, we did see a drop in fecal pH and an increase in total fecal VFA concentrations. Minimum fecal pH in this experiment was lower than the minimum fecal pH seen by both Mainardi (2009) and Reynolds et al. (2001). Our large intestinal acidosis model also appears to induce similar peak VFA concentrations as seen by Mainardi (2009). Based

on fecal pH and VFA concentration, our oligofructose-induced large intestinal acidosis model resembled the 1 g/kg oligofructose pulse-dose developed by Mainardi (2009) and the 1200 g post-ruminal starch infusion developed by Reynolds et al. (2001). However, based on fecal DM content and fecal score during the hindgut acidosis challenge, our model was not as severe either Bissell (2002), Mainardi (2009), or Reynolds et al. (2001).

4.5 Effects of *S. boulardii* on fecal DM, pH, and score

Our large intestinal model was designed to decrease the severity of hindgut acidosis to what is more likely to occur on dairy farms. This will allow us to evaluate the efficacy of treatments to alleviate hindgut acidosis in a commercial setting. In this experiment the ability of abomasally-infused SB to reduce the severity of hindgut acidosis was also evaluated. Mainardi (2009) tested the ability of dietary *S. cerevisiae* (SC) to combat oligofructose-induced post-ruminal acidosis. However, SC did not appear to alleviate oligofructose induced post-ruminal acidosis. In fact, SC may actually have increased the severity of acidosis because the lowest fecal pH values and highest fecal lactic acid concentrations were found in the steers receiving both the acidosis challenge and dietary SC. The lack of a benefit to SC may have been due to the severity of the challenge, site of administration of SC, or a poor choice of probiotic organism.

Although SC is the most common yeast additive utilized in commercial and experimental conditions to prevent ruminal acidosis, Mainardi (2009) found that SC appeared to increase rather than alleviate the severity of large intestinal acidosis. *S. boulardii* was chosen as the yeast additive in this experiment because it, unlike SC, has been shown to exert probiotic effects in the human large intestine. While humans

can experience large intestinal acidosis, acute, chronic, and antibiotic-associated diarrhea are more common intestinal problems. *S. boulardii* appears to decrease the severity of acute, chronic, and antibiotic associated diarrhea in humans, increase resistance to *Clostridium dificile*, as well as prevent diarrhea caused by enteral feeding. SB was administered into the abomasum as previous *in vitro* rumen studies showed that SB does not survive in the rumen (unpublished data from my SES project) and SB has been extensively studied in the human intestine.

The yeast treatment in this study was utilized to determine whether SB exerts a probiotic effect in large intestinal acidosis. In this experiment we used fecal DM content and fecal score as 2 measures of severity of large intestinal acidosis. On dairy farms, watery feces and presence of excessive starch in feces is the most common way to diagnose a potential problem with hindgut acidosis. We expected that during the oligofructose challenge, SB treated animals would have a significantly higher fecal DM percent than control animals. However, DM percent was unaffected by treatment, time and the interaction of treatment by time in our oligofructose-induced large intestinal acidosis model. In addition to evaluating fecal consistency by DM basis, we used the fecal scoring system developed by Zaaijer et al., (2005) to evaluate the severity of acidosis in which normal feces had a score of 5 versus a score of 1 for diarrhea. As SB has been shown to decrease duration of diarrhea and severity, we hypothesized that there would be treatment and treatment by time interaction effects on fecal score during the post-challenge period (24 to 48 h relative to the first oligofructose infusion) where oligofructose infusions ceased at 18 h. We expected that the SB treatment would result in greater fecal scores following the oligofructose challenge and a more rapid return to baseline levels relative to the Control treatment.

Based on the challenge fecal scores, we did not observe either a treatment or a treatment by time interaction effect.

Many human SB studies have demonstrated that SB reduces the severity and incidence of diarrhea. Kurogol and Koturoglu (2005) found that SB treatment significantly decreased the duration of diarrhea and duration of hospital stay for children diagnosed with acute diarrhea. We, however, did not see a treatment effect on DM content. This may have been due to the fact that our oligofructose model was not severe enough to cause acute diarrhea. Htwe *et al.* (2008) also found that SB treatment significantly shortens the duration of diarrhea and normalizes stool consistency and frequency. The lack of a SB treatment effect in our hindgut acidosis model again may be related to the fact that we did not generate severe hindgut acidosis.

While SB has been shown to exert probiotic effects in human gastrointestinal disorders, it was not known whether SB would act as a probiotic in large intestinal acidosis in cattle. We found that SB treated animals tended to have a higher fecal pH during the oligofructose challenge period than control animals. Mainardi (2009) found that 1 g/d dietary SC did not affect the severity of oligofructose induced large intestinal acidosis. In fact SC treated animals experienced a numerical decrease in fecal pH versus animals receiving oligofructose alone. In this experiment, SB appears to decrease the severity of large intestinal acidosis as indicated by a higher fecal pH during the oligofructose challenge (6.96 vs. 6.81 in Control). Excessive large intestinal fermentation causes the fecal pH to decline sharply, however as SB treated animals did not experience as severe a fecal pH decline. The minimum pH for both Control and SB occurred at 12 h, however SB treated animals had a significantly higher fecal pH at this point (6.46 vs. 6.19 in Control) thus suggesting that SB reduces

the severity of oligofructose-induced large intestinal acidosis. Although SB's role in alleviating diarrhea in humans has been well documented, data about SB's effect on fecal pH in humans was not found.

4.6 Effects of *S. boulardii* on fecal VFA concentrations

We hypothesized that SB treatment would exert a probiotic effect on both fecal pH and fecal VFA concentrations. We expected that SB treatment would reduce excessive large intestinal fermentation as indicated by higher fecal pH and lower fecal VFA concentrations. Due to the fact that SB treatment during the oligofructose challenge resulted in increased fecal pH relative to the control, we expected that SB treatment would also have an effect on total fecal VFA concentration during the oligofructose challenge. We found that SB significantly reduced the total VFA concentration versus the Control at 12 (67.6 mM vs. 90.9 mM in Control) and 18 h (70.2 mM vs. 95.9 mM in Control). SB significantly reduced the peak VFA concentrations for lactic acid (16.60 mM vs. 8.55 mM in Control) acetic acid (48.13 vs. 60.62 in the Control), butyric acid (4.43 mM vs. 7.22 mM in the Control), and propionic acid (17.0 2 vs. 21.95 in the Control) relative to the first oligofructose infusion. SB appeared to exert a beneficial effect at 12 and 18 h for lactic acid, acetic acid, and butyric acid and at 24 h for propionic acid. In the study by Mainardi (2009) SC treatment did not affect fecal total VFA concentration following abomasal oligofructose infusion.

We expected that SB would alleviate excessive oligofructose fermentation in the large intestine, indicated by lower fecal VFA concentrations. Schneider et al. (2005) examined the effects of SB administration to human patients receiving enteral nutrition on fecal SCFA levels. They found that patients had decreased butyrate levels

before treatment and that SB treatment increased SCFA levels in patients. SB treatment did not affect SCFA levels in control patients (healthy volunteers), data which correspond with our observation that SB did not affect total VFA levels during baseline measurements (i.e. pre-oligofructose challenge) to the oligofructose challenge. The discrepancies between our VFA levels and those reported by Schneider et al. (2005) were most likely due to the intestinal disturbance model used. In our model, we induced intestinal disturbances through the introduction of large amounts of fermentable carbohydrates. However patients in the study conducted by Schneider et al. (2005) experienced diarrhea caused by a major deficiency in colonic bacteria and therefore SCFA production. SB increased SCFA concentration in these patients, presumably through its interactions with the intestinal flora. To our knowledge the mechanism by which SB is alleviating large intestinal acidosis had not yet been studied.

Chapter 5

CONCLUSION

We determined that our oligofructose-induced large intestinal acidosis model results in a less severe hindgut acidosis model than previous studies. We found that our model does not affect fecal DM content, reduces the drop in fecal consistency and pH, and increases VFA production. Based on DM content and fecal score, our oligofructose model was less severe than previous post-ruminal acidosis studies. However, based on fecal pH and fecal VFA concentrations, our oligofructose model resembled both the 1 g/kg oligofructose pulse-dose developed by Mainardi (20090 and the 1200 g/d starch model developed by Reynolds et al. (2001). However, our model was not as severe as the Bissell (2002) 5 g/kg BW post-ruminal starch model. We also found that abomasal infusions of 10 g/d SB alleviated oligofructose-induced large intestinal acidosis. We saw that fecal pH tended to be higher in SB treated animals and that SB treated animals had significantly lower fecal VFA concentrations than the Control animals. Based on this data, we conclude that SB has the ability to decrease the severity of oligofructose-induced large intestinal acidosis.

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APPENDIX: DATA

Table 1. Steer Body Weights and Oligofructose Dose

Steer	Weight (lbs)	Weight (kg)	Oligo.[1] (g)	Oligo. dose / infusion (g)
2	2292	1039	1039	260
5	2012	912	912	228
6	1976	896	896	224
9	1946	883	883	221
10	1808	820	820	205
11	1870	850	850	213

[1]Oligofructose

Table 2. Feed Components and Analysis

Item	
Ingredient	% of diet DMI
Corn Silage	32.9
Alfalfa Silage	15.7
Alfalfa Hay	8.0
Ground corn grain	15.5
Rumen protected soy bean meal	5.8
Corn hominy	5.2
Ground soybean hulls	4.9
Distiller's grains	3.7
Citrus Pulp	2.1
Canola Meal	1.6
Soybean Meal	1.6
Molasses	0.6
Sodium bicarbonate	0.6
Rumensin for dairy	0.3
Vitamins and minerals*	1.4
Nutrient Composition	% of DM
Crude protein (CP)	15.9
NDF	34.4
ADF	24.7
Starch	22.8
Particle Size Analysis, Penn State Shaker Box	% DM
>0.75 in	17.6
0.31 – 0.75 in	31.2
0.07 – 0.31 in	38.5
<0.07 in	12.7

*****Vitamins and minerals include: Calcium carbonate, calcium sulfate, NaCl, urea, Mg, Biphos, trace minerals and vitamins

Table 3. Effect of Control and *S. boulardii* treatment on fecal DM (%), pH, and score during baseline measurements. Results shown as least square means

	Treatment[1]			*P-values*		
Item	**Control**	**SB**	**SEM[2]**	**Treat.**	**Time**	**Treat. x Time[3]**
Fecal DM	14.51	14.49	0.40	0.98	0.94	0.49
Fecal pH	6.74	6.73	0.05	0.97	**<0 .01**	0.15
Fecal Score	3.59	3.80	0.11	0.15	**0.08**	0.73

[1]Treatments were abomasal infusion of water (Control) or water containing 10 g/d *S. boulardii*
[2]SEM = Standard Error of the Means
[3]Treat. x Time = interaction of treatment over time

Table 4. Effects of Control and *S. boulardii* treatment on fecal DM, pH, and score during the oligofructose challenge. Results shown as least square means.

	Treatment[1]			*P-values*		
Item	**Control**	**SB**	**SEM[2]**	**Treat.**	**Time**	**Treat. x Time[3]**
Fecal DM (%)	14.23	14.52	0.37	0.54	0.14	0.35
Fecal pH	6.81	6.96	0.06	**0.09**	**< 0.01**	0.60
Fecal Score	2.87	3.24	0.18	0.20	**< 0.01**	0.63

[1]Treatments were abomasal infusion of water (Control) or water containing 10 g/d *S. boulardii*
[2]SEM = Standard Error of the Means
[3]Treat. x Time = interaction of treatment over time

Table 5. Effects of Control and *S. boulardii* treatment on baseline VFA concentration (mM). Results shown as least square means.

	Treatment[1]			*P-values*		
	Control	**SB**	**SEM[2]**	**Treat.**	**Time**	**Treat. x Time[3]**
Lactate	ND[4]	ND	-	-	-	-
Acetate	39.87	38.52	2.77	0.74	**<0.01**	0.92
Butyrate	3.98	4.21	0.36	0.62	**<0.02**	0.82
Propionate	10.10	9.86	0.42	0.69	**0.09**	0.95

[1]Treatments were abomasal infusion of water (Control) or water containing 10 g/d *S. boulardii*
[2]SEM = Standard Error of the Means
[3]Treat. x Time = interaction of treatment over time
[4]ND = not detected

Table 6. Effects of control and S. boulardii treatment on challenge VFA concentration (mM). Results shown as least square means

	Treatment[1]			*P-values*		
	Control	**SB**	**SEM[1]**	**Treat.**	**Time**	**Treat. x Time[3]**
Lactate	4.05	2.66	1.21	0.44	**<0.01**	0.21
Acetate	42.71	38.96	1.61	0.17	**<0.01**	0.45
Butyrate	4.74	3.61	0.74	0.29	**<0.01**	0.37
Propionate	13.37	11.88	1.23	0.41	**<0.01**	0.98

Table 7. Effect of control and *S. boulardii* treatment on total VFA concentration (mM) during baseline and challenge measurements. Results shown as least square means.

	Treatment[1]			*P-values*		
	Control	**SB**	**SEM[2]**	**Treat.**	**Time**	**Treat. x Time[3]**
Baseline	53.95	52.59	3.31	0.78	**<0.01**	0.95
Challenge	65.10	57.0	2.29	**0.05**	**<0.01**	0.52

[1]Treatments were abomasal infusion of water (Control) or water containing 10 g/d *S. boulardii*
[2]SEM = Standard Error of the Means
[3]Treat. x Time = interaction of treatment over time

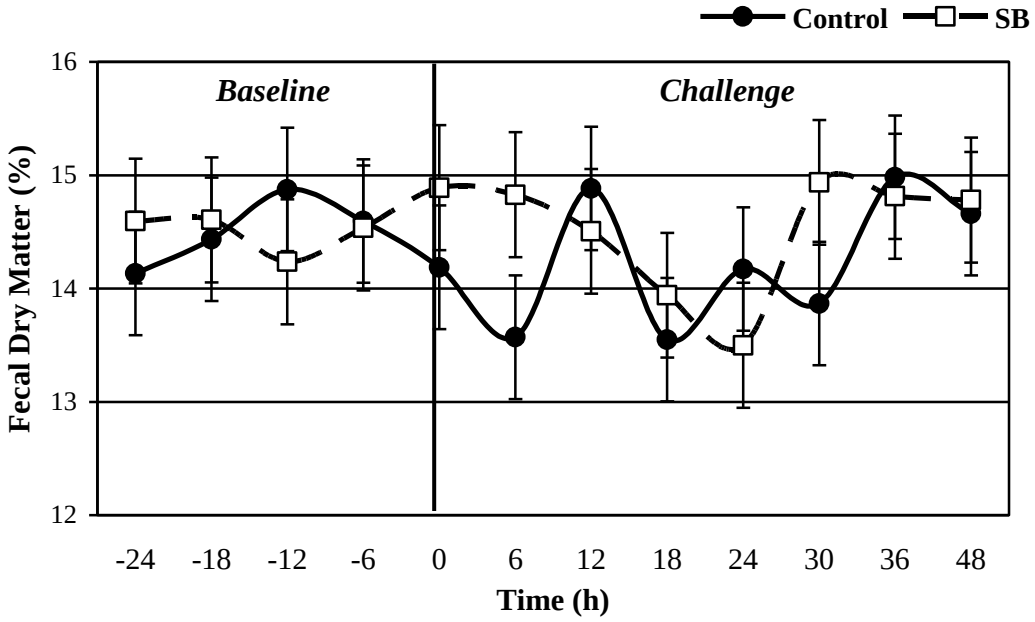

Figure 1. Fecal dry matter percent for Control (water) animals or *S. boulardii* (10 g/d) animals that were administered an abomasal oligofructose infusion (0.25 g/kg BW suspended in 1L) at hours 0, 6, 12, and 18.

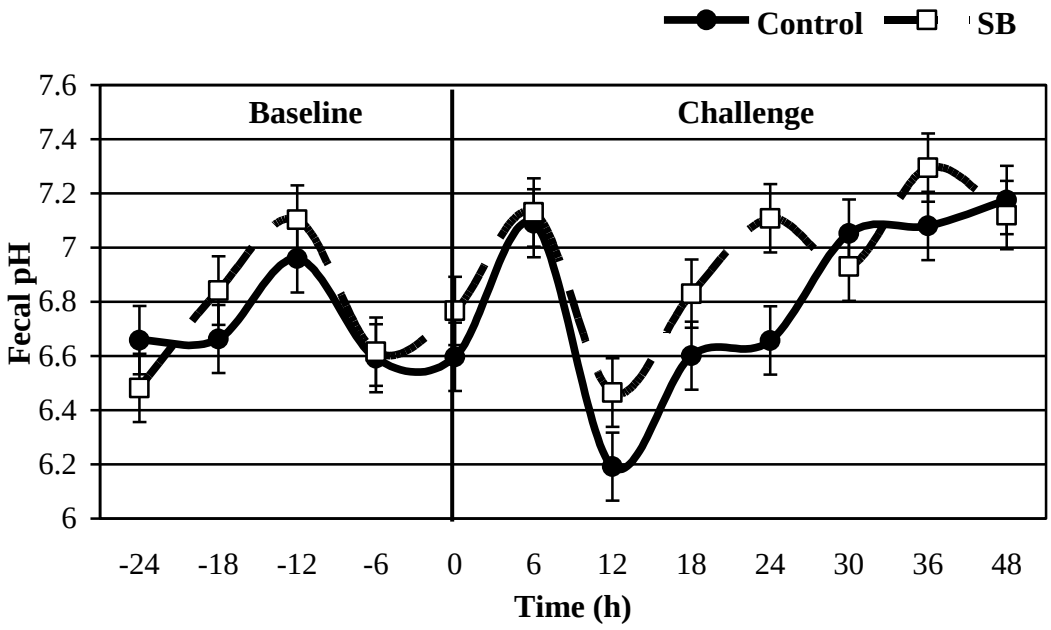

Figure 2. Fecal pH for Control (water) animals or *S. boulardii* (10 g/d) animals that were administered an abomasal oligofructose infusion (0.25 g/kg BW suspended in 1L) at hours 0, 6, 12, and 18.

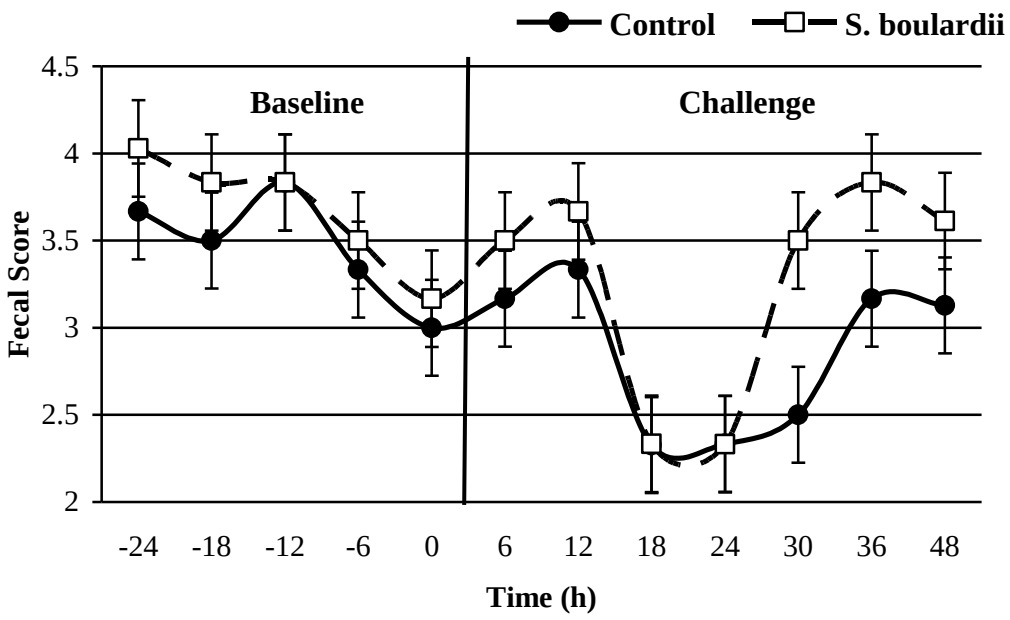

Figure 3. Fecal score for control (water) animals or *S. boulardii* (10 g/d) animals that were administered an abomasal oligofructose infusion (0.25 g /kg BW suspended in 1L) at hours 0, 6, 12, and 18.

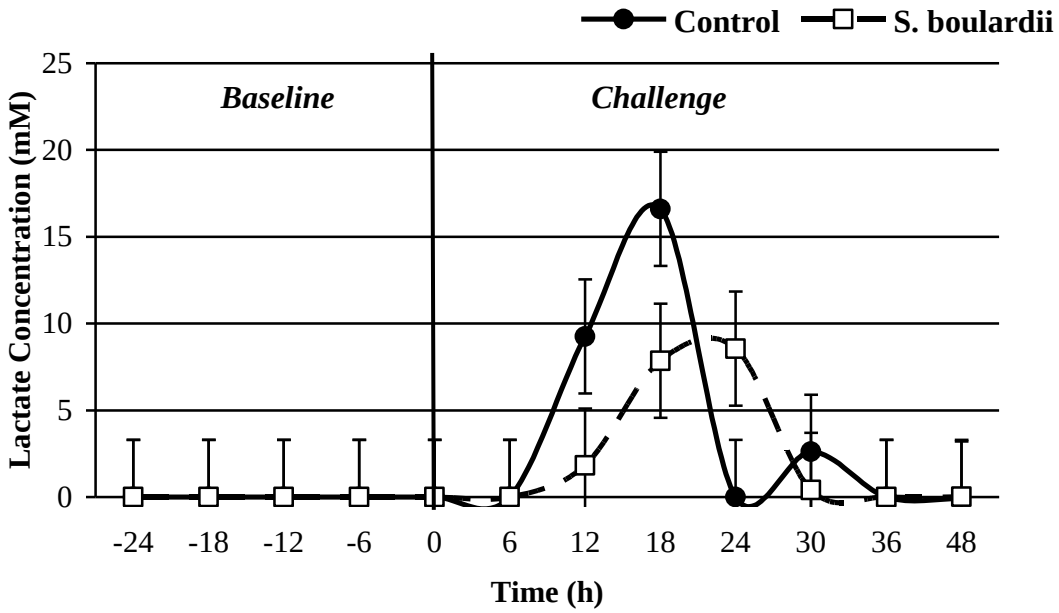

Figure 4. Fecal lactate concentration (mM) for control (water) and *S. boulardii* (10 g/d) treated animals that were administered an abomasal infusion (0.25 g/kg oligofructose) at 0, 6, 12, and 18 h.

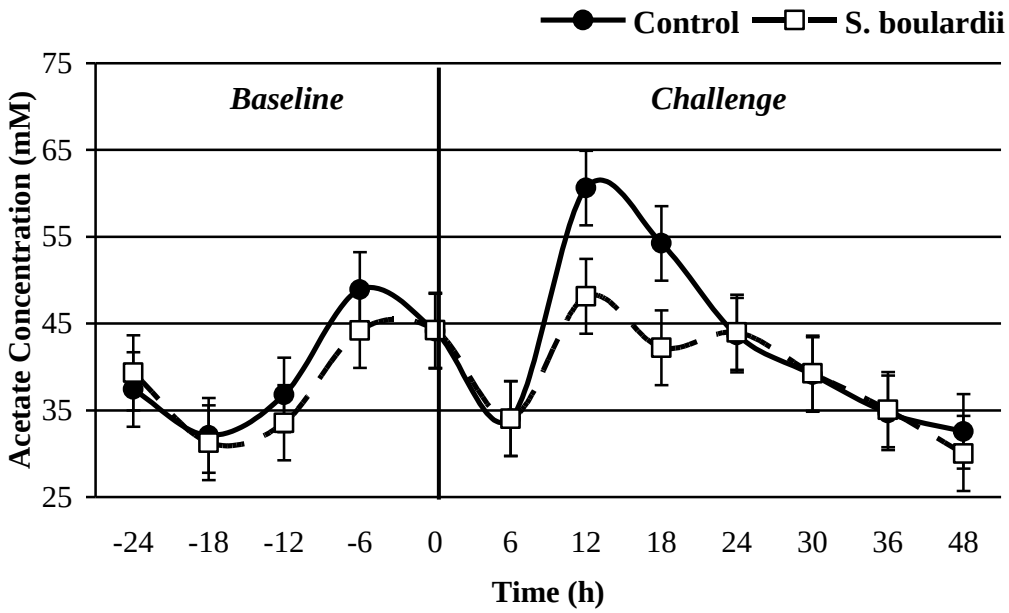

Figure 5. Fecal acetate concentration (mM) for Control (water) and *S. boulardii*(10 g/d) treated animals that were administered an abomasal infusion (0.25 g/kg oligofructose) at 0, 6, 12, and 18 h.

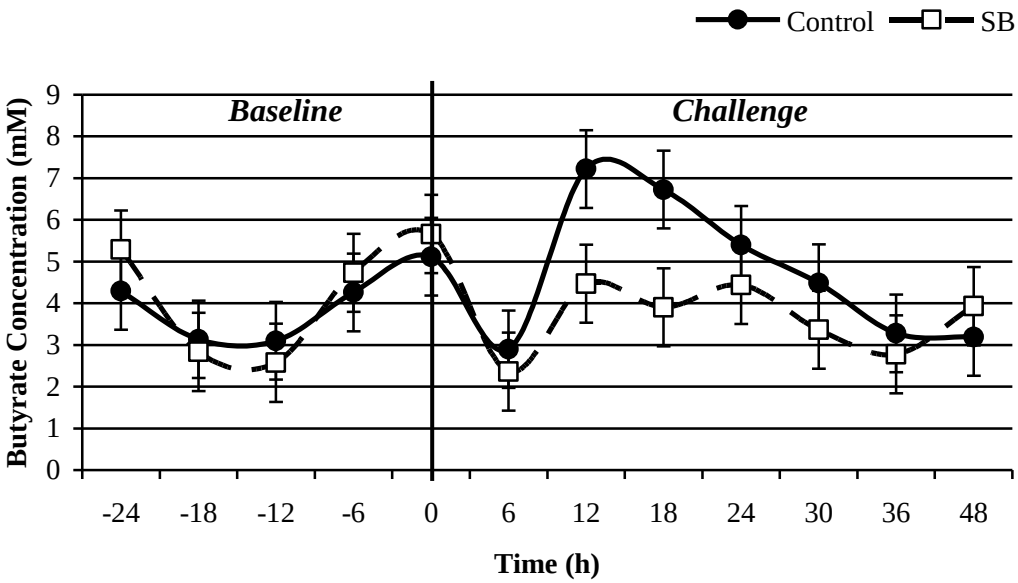

Figure 6. Fecal butyrate concentration (mM) for control (water) and *S. boulardii* (10 g/d) treated animals that were administered an abomasal infusion (0.25 g/kg oligofructose) at 0, 6, 12, and 18 h.

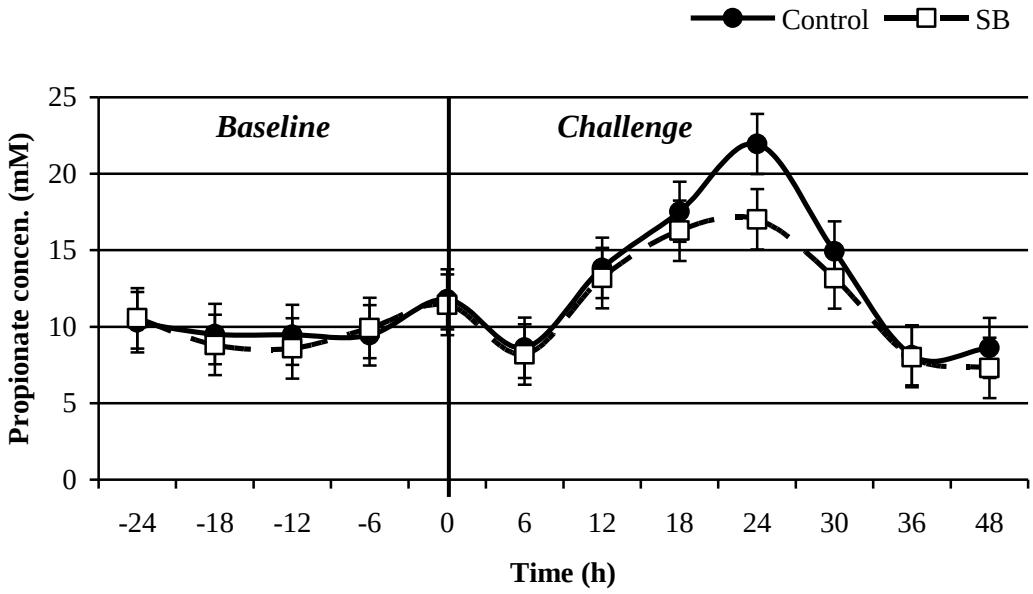

Figure 7. Fecal propionate concentration (mM) for control (water) and *S. boulardii* (10 g/d) treated animals that were administered an abomasal infusion (0.25 g/kg oligofructose) at 0, 6, 12, and 18 h.

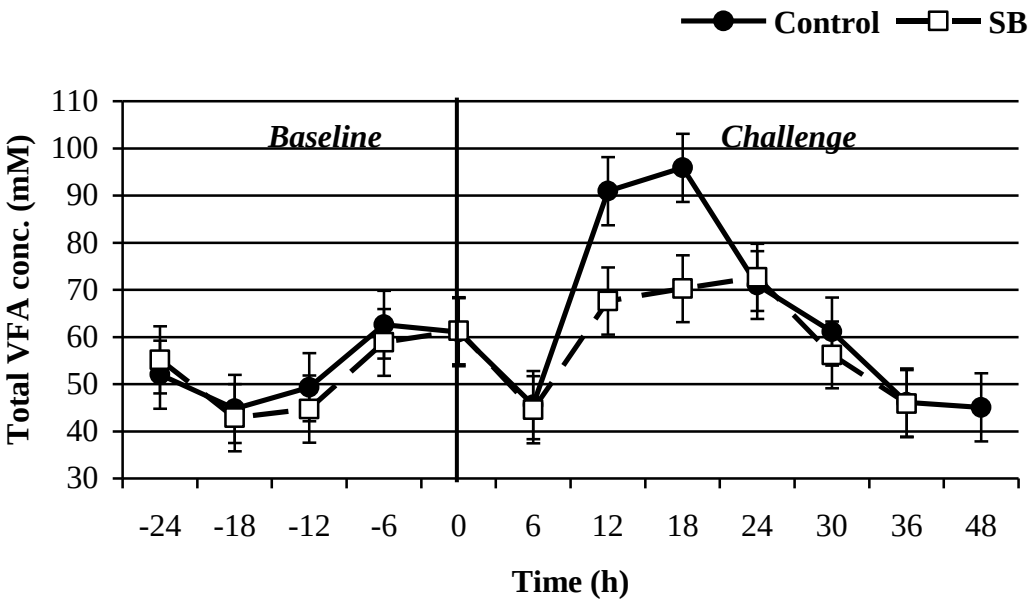

Figure 8. Total VFA concentration (mM) for control (water) and *S. boulardii* (10 g/d) treated animals that were administered an abomasal infusion (0.25 g/kg oligofructose) at 0, 6, 12, and 18 h.