Induction of Post Ruminal Acidosis Via Abomasal Infusion of Fermentable Carbohydrates

by

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

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The objectives of this experiment were 1) to directly compare the effects of two oligofructose models, a pulse dose and a staggered dose, on fecal indicators of post-ruminal acidosis and 2) to determine whether abomasal starch infusions result in similar changes to the oligofructose model. Five ruminally cannulated steers were fitted with abomasal infusion lines that were inserted through their ruminal cannulas. There were 5 treatments, a control (CON) of only water, a single pulse dose of 1g/kg of oligofructose (OL1) or starch (ST1) or 4 staggered doses of 0.25g/kg of oligofructose (OL4) or starch (ST4) administered every 6 h. Fecal samples were collected to measure fecal score, pH, dry matter (DM), VFA, endotoxin and apparent total tract nutrient digestibility. The results showed that a decrease in fecal pH from 7 to 6 occurred as a result of the OL1 and ST1 treatments and a decrease from 7 to 6.5 in the staggered doses. Fecal score also decreased from OL1 most severely but OL4 also caused a decline. An increase in total VFA and endotoxin concentrations in feces was seen across all treatments compared to CON. Both the pulse dose and staggered dose of oligofructose induced conditions similar to those seen in post-ruminal acidosis. Both starch treatments also showed similar effects to the oligofructose infusions suggesting that starch could be used as a model to induce post-ruminal acidosis.
Chapter 1

Literature Review

1.1 Bovine Digestion

Ruminant species have very complex and specialized digestive systems in order to utilize grasses and other forages as nutrient sources. The anatomy of a ruminant digestive tract is vastly different from monogastric mammals. Ruminants have four stomach compartments, each of which has a specific role in digestion. The two largest compartments, the rumen and reticulum, are the main sites of forage carbohydrate break down through the process of microbial fermentation. These compartments are often called the reticulo-rumen because some digesta is able to move back and forth during the fermentation process (Frandson et al., 2006). Rumination of feeds in the reticulo-rumen enhances microbial fermentation. During this process, the cow regurgitates feed, re-chews and re-swallows feed to increase surface area and provide salivary buffers. When particle size of feed is sufficiently small feed passes through the omasum and then into the abomasum. The abomasum is the compartment that most closely resembles the monogastric stomach. Gastric juices work to break down proteins for intestinal absorption. Water, hydrochloric acid, mucus and pepsin all make up the gastric juice. Pepsin breaks down the proteins and the acids hydrolyze ingesta. Ingesta then passes from the abomasum into the small intestines. In the small intestine, enzymes released by bot the pancreas and intestinal cells further digest carbohydrates, fats, and proteins. Intestinal epithelial cells absorb nutrients after this enzymatic breakdown. The large intestine primarily functions for water absorption, however, some carbohydrates can be further fermented and absorbed for energy (Frandson et al., 2006).
1.2 The Rumen

The reticulo-rumen is a large fermentation chamber in which initial digestion of feed occurs. Carbohydrates that are broken down in this compartment include sugars, starches, cellulose, and hemicellulose. The two carbohydrate classes that most often make up the largest proportion of bovine diets are starches and cellulose. Starches are most commonly found in cereals, and cellulose is most commonly found in forages. Both starch and cellulose consist of long chains of glucose, however the chemical bonding of these chains is very different. Starches have alpha glycosidic linkages between glucose monomers, whereas cellulose contains beta glycosidic linkages between glucose monomers (Pond et al., 2005). Those compounds that contain beta glycosidic linkages cannot be broken down by mammalian enzymes due to the orientation of the bond. When the beta bond forms, the linkage is equatorial and therefore results in a straight chain. Many of these beta-linked molecules lined up next to each other create strong fibers, like those in cotton or wood. Alpha linkages are formed so that the angle of the bond exposes the linkage, making it easier to break down (Chasin and Mowshowitz, 2001). Since alpha glycosidic linkages can be digested my mammalian enzymes they are the primary energy source for most mammals (Alberts et al., 2008). However, the rumen has specific cellulolytic bacteria which digest beta glycosidic bonds. In addition to specialized cellulolytic bacteria, the rumen is populated by a wide range of microorganisms capable of digesting most carbohydrate classes including starch, sugar, and hemicellulose (Pond et al., 2005). Microbial carbohydrate fermentation produces volatile fatty acids (VFA) which are absorbed across the rumen epithelium. The most common VFA are acetate, propionate, and butyrate and they are the main sources of energy for ruminants.
1.3 Small and Large Intestine

The digesta that enters the small intestine is comprised of rumen microbes and partially degraded feed. While in the small intestine, enzymes act on proteins, carbohydrates, and lipids and digested nutrients are absorbed across intestinal epithelium. Starch that is not digested in the rumen is hydrolyzed by amylase in the small intestine. Feed and microbial particles that are not digested in the small intestine are then passed to the large intestine. In the large intestine there are similar microbes to those in the rumen that degrade carbohydrates. Fermentation in the large intestine is referred to in this thesis as hindgut fermentation. Just like in the rumen, carbohydrate fermentation yields VFA that are absorbed across the epithelium wall. Anything not absorbed in the large intestine will be excreted in the feces. Though the majority of carbohydrates are broken down in the reticulo-rumen, some passes to the intestines. As reviewed by Gressley at al. (2012), 41% of organic matter is fermented in the rumen, 26% disappears in the small intestine, and 4% disappears in the large intestine; the remaining organic matter (29%) does not get digested and passes through to the feces. This is in direct correlation with the retention time of feed in the rumen vs the large intestine which is 30 h vs 13 h respectively (Gressley et al., 2012).

1.4 Rumen pH Control

Bacteria in the rumen increase growth rates and fermentation activity with an increase in available fermentable carbohydrates (Nagaraja and Titemeyer, 2007). Sugars are rapidly fermented, starches and hemicellulose are fermented at a medium rate, and cellulose is fermented slowly. Extents of digestion follow a similar trend, with roughly 100, 60, and 40% of dietary sugar, starch, and fiber digested in the rumen, respectively (Martin et al., 2000; Huhtanen et al., 2010; Patton et al., 2011). As a consequence, diets
containing primarily cellulose provide less energy to ruminants than diets supplemented with feedstuffs containing starch or sugar. Dairy cattle have high energy needs to produce large volumes of milk, and all-forage diets do not provide enough energy to support lactation for high producing cows. In order to meet the energy needs of lactating cows, diets containing roughly 50% forage and 50% concentrate feeds are typically fed. The difference in energy availability is reflected by differences in rumen bacteria concentrations which increase up to 10 fold in a high concentrate compared to high forage diet (Slyter, 1976).

Volatile fatty acids are weak acids, with pKa around 4.8. Ideal rumen pH for maximum microbial fermentation is between 5.8 and 6.5 (Nagaraja and Titegmeyer, 2007). Because VFA production rates can fluctuate throughout the day with consumption of meals, the ruminant has evolved physiological mechanisms to maintain rumen pH within the ideal range. First, when rumen pH decreases the rate of VFA absorption across the rumen wall increases (Dukes, 2004). In addition, there are buffers such as sodium bicarbonate present in saliva, and rumination increases saliva flow to the rumen, which buffers VFAs that are produced (Stone, 2004). Management tools are also used help maintain rumen pH. Diets should have a minimum of 19% neutral detergent fiber (NDF) to stimulate rumination and flow of saliva containing endogenous buffers (NRC, 2001). Forage processing is also important to rumination, and forages that are processed into particles that are too small will not adequately stimulate rumination (Stone, 2004). Physically effective NDF (peNDF) is a measure that combines both NDF content and particle size, and it is recommended that diets contain at least 22% peNDF (Stone, 2004). Diets that have low amounts of coarse fiber can reduce chewing activity, saliva production, and rumen buffering which can lead to rumen pH depression (Khafipour et al., 2009a). Another management tool that can be used to help maintain rumen pH is to
supplement the diet with exogenous buffers, most often sodium bicarbonate (Dohme et al., 2008).

1.5 Ruminal Acidosis

When there is an increase in fermentable carbohydrates or a decrease in buffering capacity, acidosis can develop. During acidosis, VFA absorption and buffering are not sufficient to maintain healthy pH. Acidosis can occur as acute or sub-acute depending on the magnitude of the pH depression. Sub-acute ruminal acidosis is characterized by a ruminal pH below 5.6, while acute acidosis is characterized by a pH below 5.0. Acute acidosis results in obvious clinical signs including reduced dry matter intake, loss of body weight, diarrhea, damage to gut epithelium, and lameness (Emmanuel et al., 2007; Khafipour et al., 2009b). Since feedlot steers are often exposed to high concentrate diets, these steers have a higher susceptibility to buffering problems and as a result are more likely to develop acute acidosis than dairy cows.

Acidosis results in a shift of microbial populations. A rumen pH below 5.8 can inhibit the cellulolytic activity of cellulolytic microbes and reduce fiber digestibility and feed efficiency, which decreases rumen cellulose digestion (Nagaraja and Titegmeyer, 2007; Khafipour et al., 2009b). Protozoa which normally help to slow starch fermentation are also pH sensitive and lyse as the pH drops. As pH continues to drop, gram-negative bacteria fail to thrive. However, concentrations of gram-positive starch fermenters increase and produce VFA. As pH continues to drop, acid tolerant bacteria such as lactobacilli and Megasphaera elsdenii, that are normally not competitive in the rumen begin to flourish and produce lactic acid which is a stronger acid than VFA with a pKa of 3.9 (Nagaraja and Titegmeyer, 2007; Slyter, 1976). Lactic acid further reduces the pH of
the rumen, and many bacteria that cannot live in acidic conditions are thus lysed inside the rumen.

Endotoxin is released into the rumen as a result of growth or lysis of gram-negative bacteria. Endotoxin is a broad term for toxins that are released from gram-negative bacteria; lipopolysaccharide (LPS) is a type of endotoxin that is released. Following consumption of a large meal, rapidly fermentable carbohydrates are degraded by both gram-negative and gram-positive bacteria. Up to 60% of free LPS in the rumen is produced shortly after feeding as species like *Prevotella* spp., *Ruminobacter amylophilus*, *Succinivibrio dextrinosolvens*, and *Succinimonas amylolytica* degrade the rapidly fermentable carbohydrates. During this rapid growth phase, these organisms use autolytic enzymes that release LPS as a by-product of cell division (Khafipour et al., 2009b; Gozho et al., 2007). As time increases after a rapidly fermentable meal, the rumen pH steadily declines which then causes death of these gram-negative organisms, releasing more free LPS (Khafipour et al., 2009a; Gozho et al., 2007). The combination of increased free LPS and reduced ruminal pH can damage the epithelium in the rumen and allow endotoxin to enter the blood (Emmanuel et al., 2007).

### 1.6 Sub-Acute Ruminal Acidosis

As its name implies, SARA lacks the obvious clinical symptoms found with acute acidosis. The most common symptom is bouts of mild intake depression, which can be difficult to assess in a commercial setting. Sub-acute ruminal acidosis (SARA) is defined by Li et al. (2012) as ruminal pH below 5.6 for 180 min/d or more. In a research setting, SARA can be induced by feeding a rapidly fermentable carbohydrate source such as wheat, often immediately following a mild feed restriction period (Gozho et al., 2005; Krause and Oetzel, 2005). This induces pH depression, but cows rapidly recover
following return to a normal ration. During SARA challenges, rumen pH decreases, rumen propionate concentrations increase, and intake decreases (Gozho et al., 2005; Krause and Oetzel, 2005).

Intra-ruminal and systemic effects of SARA are similar to those of acute acidosis, just milder. Measuring the rumen pH is the only definitive way to diagnose SARA. Sub-acute ruminal acidosis, like acute ruminal acidosis, is the result of increased concentrations of VFA in the rumen, which decrease the pH. Often ruminal pH will decrease after feeding and then increase after rumination causing bouts in the symptoms (Stone, 2004). The mild nature of the symptoms makes diagnosis difficult and can result in a chronic problem in dairy herds. Long-term results of SARA are decreased milk production and milk fat, unexplained diarrhea, laminitis, and increased culling rate (Nocek, 1997; Gohzo et al., 2007).

As discussed above, rapidly fermentable diets and low pH can cause lysis of gram-negative bacteria which releases endotoxins and increases free LPS. Gozho et al. (2005) fed combinations of wheat-barley pellets and chopped alfalfa to induce SARA. Results showed that feeding wheat barley pellets, in comparison to only forages, increased LPS concentrations in the rumen after SARA was induced. Increase in LPS was correlated to the time below ruminal pH 5.6, an indicator that free LPS is increased during SARA. Free LPS in the rumen can compromise the barrier function by reduced organization and thickness in the epithelial wall even without visible tissue damage (Li et al., 2012). Thus, there is a possibility that during SARA, LPS can more easily translocate through the rumen wall and into the general circulation. Detecting the presence of LPS in the blood can be an indicator of SARA, as the rumen epithelium becomes damaged and allows LPS to translocate. Laminitis is a condition characterized by the inflammation of the hoof, which results in cell death and causes the animal great discomfort and lameness.
Laminitis and other inflammatory conditions associated with SARA are believed to be a result of LPS moving from the rumen into the general circulation (Gozho et al., 2005; Li et al., 2012).

1.7 Post Ruminal Acidosis

Post-ruminal acidosis, or hindgut acidosis, is caused by the same dietary conditions as ruminal acidosis. Increased carbohydrate fermentation in the rumen often results in an increased amount of fermentable carbohydrates passing to the hindgut, thus increasing fermentation in the hindgut. Increasing the starch content of the diet (from 19% starch to 63.5% corn starch) of steers resulted in increased flow of starch out of the rumen (from 357 to 982 g/d) and increased disappearance of starch in both the small intestine (from 331 to 634 g/d) and large intestine (from 14 to 296 g/d) (Karr et al., 1966). This data shows that some starch can escape ruminal fermentation at both low and high total starch concentrations. Some of the starch that passes the rumen is not digested in the small intestine and moves into the large intestine where it is fermented. In lactating dairy cows, up to 44% of intake starch disappears post-ruminally due to digestion in the small intestines and fermentation in the large intestines (Khafipour et al., 2009a). Li et al. (2012) also found that challenging lactating cows with a SARA-inducing diet increased starch flow to the cecum compared to a higher forage diet. As in the rumen, increased fermentation in the hindgut increases production of VFA and microbes and decreases pH (Owens et al., 1986). As a result, increased microbial protein output in the feces and decreased fecal pH are also evidence of increased hindgut fermentation (Gozho et al., 2005). Just as in the rumen, growth and lysis of bacteria results in release of toxins. However free endotoxins found in the hindgut are proposed to originate exclusively from the intestinal gram-negative population. It is believed that endotoxins produced in the
rumen are neutralized by a combination of acidic conditions in the abomasum and bile salt binding in the small intestine (Khafipour et al., 2009a).

A dense layer of keratinized epithelium protects the rumen; however, the epithelium in the large intestine is only protected by a layer of mucous and mucosal cells. It has been proposed that the thinner epithelium in the large intestine may be more sensitive than rumen tissue to the pH drop and endotoxin increase that follows rapid starch fermentation (Li et al., 2012). Emmanuel et al. (2007) conducted an in vitro study to evaluate the effect of pH and LPS concentration on permeability of ruminal and intestinal mucosa. They found that LPS was capable of translocation across both ruminal and intestinal tissue and that there was a dramatic loss in the barrier function that occurred at pH 4.5 for rumen tissue and pH 5.5 for colon tissue (Emmanuel et al., 2007). These results implicate that hind gut acidosis as well as ruminal acidosis makes the epithelium much more permeable to LPS and that hindgut is more sensitive to a drop in pH than the rumen. Khafipour et al. (2009a) proposed that the hindgut would similarly be more sensitive to an increase in LPS than the rumen, but Emmanuel et al. (2007) found that both tissues were similarly affected by the presence of LPS. Entry of toxins into the blood through a breach in epithelium is proposed to cause the secondary problems associated with SARA (Li et al., 2012). Whether these breaches are more likely to occur in the rumen or the hindgut has yet to be determined.

Even small amounts of fermentation that occur in the hindgut can cause pH depression. In the small intestine the pH will be elevated from about 3.0 (abomasal pH), to a pH of 7 or 8. The increase in pH allows enzymes of the small intestine to work properly. Fermentation does not generally occur in the small intestine, as its general function is enzymatic breakdown of ingesta. However, if the small intestine were to be colonized by certain bacteria fermentation could bring the pH down to about 5.5 or 6,
stopping enzymatic activity (Frandson et al., 2006). Overgrowth of bacteria in the small intestine could cause colonization leading to deconjugation of bile salts, and alterations in structure and function of the small intestine. Without the bile salts to buffer the intestine, pH would not reach ideal level, which would decrease enzymatic activity. In a situation like this fermentative bacteria would overpower enzymes and post-ruminal conditions are more likely to form (Lifshitz et al., 1978).

1.8 Oligofructose

Starch is the most abundant rapidly fermentable carbohydrate fed to dairy cattle, and is responsible for most cases of ruminal acidosis (Nordlund, 2003). However, because starch can be fermented in the rumen, digested in the small intestine, and fermented in the large intestine, precisely measuring the amount of starch fermented in the large intestine is impossible in intact animals. Oligofructose is a fermentable carbohydrate that is not digestible by mammalian enzymes. Consequently, abomasal oligofructose delivery can be performed on animals with only ruminal cannulas and results in quantitative passage of dosed oligofructose to the large intestine.

1.9 Post Ruminal Acidosis Models

Bissell (2002) abomasally infused 5 g starch/kg BW each day over 3 days to induce post-ruminal acidosis. Each day, starch was infused over a 12 h challenge period followed by a 12 h rest period. After each starch infusion fecal pH decreased from ~7 to ≤ 5 in comparison to the control group which stayed at ~7 throughout the trial. Decrease in fecal pH was evidence of hindgut acidosis, because it reflected increased fermentation in the hindgut. Bissell (2002) reasoned that the decrease in fecal pH indicated hindgut acidosis because fecal pH is representative of large intestinal pH. In addition, Bissell
(2002) observed mucus, tissue segments, and mucin casts in feces when fecal pH was less than 6.0. These findings are consistent with epithelial layer damage, indicating that lowered pH caused tissue damage. This study demonstrated that intestinal epithelial damage following hindgut acidosis is similar to rumen epithelial damage following ruminal acidosis. Bissell (2002) also suggested that tissue damage occurs at pH below 5.5 in the rumen and 6.0 in the large intestine.

Mainardi (2009) used abomasal oligofructose infusions as a model to induce large-intestinal acidosis. Oligofructose, unlike starch, cannot be digested in the small intestine and therefore passes to the small intestine where it is fermented. Steers on this trial received a pulse dose of 1 g/kg BW oligofructose. This pulse dose was administered in 1 L water via an abomasal infusion line. Control animals received only the 1 L water. Average fecal pH for oligofructose-treated animals was 6.67 versus 7.02 in the control. Fecal pH was lowest at 6 h (6.51 vs. 6.94 in the control) and all animals infused with oligofructose developed profuse, watery diarrhea. Control animals had a higher fecal score and fecal dry matter percent compared to oligofructose treated animals. Oligofructose treated animals also had a higher total VFA concentration in feces compared to the control animals, with the greatest increase seen at 12 h. Based on these results Mainardi (2009) successfully generated a hindgut acidosis model using oligofructose. However, one criticism of the model was that the diarrhea preceded the increased VFA, potentially indicating that the single pulse dose caused an osmotic challenge that pulled body water into the large intestine and caused the diarrhea. An oligofructose challenge model that causes hindgut acidosis without a concurrent osmotic challenge needs to be developed. Additionally, the ability of post-ruminal oligofructose challenge models to successfully model hindgut acidosis caused by starch has not been evaluated.
1.10 Objectives

The objectives of this experiment were 1) to directly compare the effects of two oligofructose models, a pulse dose of 1g/kg BW and a staggered dose of 0.25 g/kg BW, on fecal indicators of post-ruminal acidosis and 2) to determine whether a pulse dose of 1g/kg BW and a staggered dose of 0.25 g/kg BW via abomasal starch infusions would result in similar changes on fecal indicators of post-ruminal acidosis to those shown by the oligofructose models.
2.1 Animals and Treatment

Five ruminally cannulated steers (947 ± 33 kg) were individually housed in 2.4 m × 4.6 m tie stalls. Each stall was equipped with an automatic waterer with a metering system, and water intake was recorded daily. Steers were fed a lactating cow ration restricted to 1.5% body weight (BW) dry matter daily. Table 1 shows the composition of the TMR fed to steers. Major dietary ingredients were corn silage (33%), alfalfa silage (16%), and ground corn grain (16%). The ration was formulated to be a relatively high energy and protein lactating cow ration and nutrient composition showed 17% CP, 33% NDF, and 19% starch. The ration was provided once daily at 0900 h, and steers always consumed all of the feed provided. All animal procedures were approved by the University of Delaware Agriculture Animal Care and Use Committee.

One week prior to the start of the experiment, steers were fitted with abomasal infusion lines that were inserted through their ruminal cannulas. Infusion lines consisted of a 1.8 m piece of tubing that was threaded through a rumen cannula plug on one end and a flexible plastic flange on the other end (Figure 1). To place the lines, the flange was folded and inserted into the rumen, through the omasal orifice, and into the abomasum. Once in the abomasum, the flange was unfolded which held it in place in the abomasum throughout the experiment.

Following a 1 week adaptation period, steers were assigned to a 5×5 Latin square experimental design with 7-d periods. Treatments consisted of different abomasal infusions and were administered on d 6 of each period. On d 6, all steers were given
abomasal infusions of 1.6 L water at 1000 h, 1600 h, 2200 h, and 0400 h on d 7. Water was mixed with different amounts of corn starch (Argo Food Companies Inc., Memphis, TN), oligofructose (Beneo P95, Orafti Active Food Ingredients, Tienen, Belgium), or no carbohydrate, depending on treatment. Treatments were 1) no carbohydrate infusion control (CON), 2) 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 3) 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 4) 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 5) 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4). For both the ST1 and OL1 treatments, water alone was infused at the 1600 h, 2200 h, and 0400 h infusion times. Infusion mixtures were placed in a plastic Nalgene bottle and pulse dosed into the abomasal infusion line using a veterinary stomach pump (Nasco, Fort Atkinson, WI). Carbohydrates were rigorously mixed with the water both prior to and during the infusions.

2.2 Feed Sampling and Analysis

Individual feed components were collected once weekly and dried for 48 h at 60°C in a forced-draft oven. Dry Matter (DM) results were used to adjust TMR mix amounts to account for DM fluctuation. Total mixed ration samples were collected on d 6 and 7 of each period. Total mixed ration samples were dried for 48 h at 60°C, ground through a 2-mm screen using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA), and analyzed for NDF, N, starch, ash and indigestible NDF. Neutral detergent fiber was determined using sodium sulfite and α-amylase (Goering and Van Soest, 1970) using the Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY). Nitrogen was determined using an Elementor Vario Max CN Analyzer (Elementor Americas Inc., Mt. Laurel, NJ). Starch was analyzed by wet chemistry (Cumberland Valley Analytical
Services, Hagerstown, MD), and ash content was measured following 5 h at 600˚C in a muffle furnace. The indigestible NDF was determined after 120 h of in vitro rumen incubation using the Goering and Van Soest (1970) method with modifications. These modifications were weighing the samples into filter bags and incubating them in buffer and rumen fluid for 120 h using a Daisy II incubator (Ankom Technology, Macedon, NY). Rumen fluid was collected from two lactating cows being fed the lactating herd ration. After 60 h of incubation, the original rumen fluid and buffer were discarded and were replaced with fresh fluid and incubation continued for an additional 60 h.

2.3 Fecal Sampling

Fecal samples were collected to measure fecal score, pH, DM, VFA and endotoxin and apparent total tract nutrient digestibility. Fecal grab samples (~300 g) were collected every 6 h during the last 2 days of each period, beginning at 1000 h on d 6 (0 h post-challenge) and ending 42 h post-challenge. Fecal score was assessed based on consistency, 1 = watery to 5 = solid (Hulsen, 2006). For measurement of fecal pH, 20 ± 0.5 g of feces were weighed into a 50 mL conical tube containing 20 mL distilled water. The mixture was shaken for 10 sec and pH measured using a portable pH meter (Extech, Waltham, MA). For analysis of fecal VFA, 25 ± 0.5 g of feces was weighed into a 50 mL conical tube and 10 mL of a 2.06% H₂SO₄ solution was added. The mixture was shaken for 10 sec and strained through cheesecloth into a 15 mL conical tube and stored at -20°C until analysis of lactate and VFA. One gram of fecal sample was placed in a 2 mL microcentrifuge tube and stored at -20°C until endotoxin analysis. The remaining feces was stored at -20°C until measurement of DM and nutrient composition.
2.4 Fecal Sample Analysis

Analysis of fecal VFA and lactate was performed using high performance liquid chromatography (HPLC) using procedures described by Muck and Dickerson (1988) as detailed by Mainardi et al. (2011).

The endotoxin concentrations of the fecal samples were evaluated by a commercial chromogenic end point Limulus amebocyte lysate assay (QCL-1000™ Endpoint Chromogenic LAL Assay, Lonza Ltd., Basel, Switzerland) based on procedures of Levin and Bang (1964). Samples were prepared for analysis by centrifugation at 10,000 $\times$ g for 30 min at room temperature, diluting the supernatant 1:100 using endotoxin-free water, and passing the diluted sample through a 0.22 µm sterile filter. For the assay, samples were further diluted 10-fold and treated with a 1:1 ratio of diluted sample to β-1, 3 glucan blocker (β-G-Blocker, Lonza Ltd., Basel, Switzerland) for a final dilution of 1:2,000. β-1, 3 glucans present in fecal samples can cause a false positive reaction in the endotoxin assay, and the blocker binds to β-1, 3 glucans to prevent this reaction. After treatment with the β-1, 3 glucan blocker, samples were analyzed for endotoxin according to manufacturer’s instructions. Briefly, a standard with known endotoxin concentration was diluted to 4 levels and placed in duplicate wells of the assay plate. Unknown samples were also placed in duplicate wells. Samples and standards were incubated with a chromogenic substrate, and results were read using a plate reader (Molecular Devices, Sunnyvale, CA) set to 405 nm wavelength. The standard curve ranged from 0 to 1 endotoxin units (EU)/mL. Endotoxin Units are arbitrary units which measure of the biological activity of endotoxin relative to a standard reference endotoxin isolated from Escherichia coli O113:H10:K0 (Rudbach et al., 1976). When unknown samples failed to fit on the standard curve, they were further diluted. Unknown samples
had final dilutions of 1:2000, 1:10,000, 1:50,000, or 1:500,000, and results were
corrected to the appropriate dilution.

Fecal samples collected for measurement of DM and nutrient composition were
thawed, and 100 g were dried at 60°C for 48 h in a forced air oven. Dried fecal samples
were composited by steer and period and analyzed for NDF, N, starch, ash, and
indigestible NDF as described for the TMR samples. Indigestible NDF in feeds and feces
was used as a marker to calculate fecal output and apparent total tract nutrient
digestibility (Oba and Allen, 1999).

2.5 Statistical Analysis

Data were analyzed using the MIXED procedure of SAS. Repeated measures
(fecal score, pH, VFA, DM, and endotoxin) were analyzed using a model that included
the fixed effects of treatment, hour, period, and the interactions of treatment × hour
period × hour and the random effects of steer and steer × period × treatment. Hour was
included as a repeated measure, steer × period × treatment was the subject, and an
autoregressive covariance structure was utilized. Endotoxin concentrations were log10
transformed prior to analysis to result in homogeneity of residual variance. Digestibility
results were analyzed using a model containing fixed effects of treatment and period and
random effect of steer. Significance was declared at $P \leq 0.05$ and trends declared at $P \leq
0.10$. 
Chapter 3
Results and Discussion

3.1 Intake and Nutrient Digestibility

To reduce over-conditioning of the steers, the ration was restricted to 1.5% of BW, roughly 14.2 kg DM/steer/day. Steers always consumed all feed provided. Rumen acidosis has been shown to reduce intake (Krause and Oetzel, 2005), and it is likely that the restricted feeding protocol masked any treatment effects on appetite. However, Mainardi et al. (2011) found that abomasal oligofructose did not affect intake when steers were fed ad libitum fed.

We recorded water intake to determine whether steers would respond to abomasal infusions by varying their water intake. Mainardi et al. (2011) proposed that abomasal oligofructose may have caused dehydration due to osmotic pressure of the infusate pulling water out of the body and into the digesta. Additionally, it has been proposed that cattle may respond to rumen acidosis challenges with increased water intake (Russell and Chow, 1993). Water intake was unaffected by treatment ($P = 0.85$) or the interaction of treatment $\times$ day ($P = 0.13$) but was affected by day ($P < 0.001$; Table 2). The day effect was due to the lowest water intake on day 7 and the highest water intakes on days 4 and 6 (Figure 2). Challenges were administered on day 6, and it is possible that the higher water intake on day 6 driven by the OL1 and OL4 treatments may have been related to the challenge, but data are too variable to draw definitive conclusions.

Total tract apparent digestibility of DM, OM, NDF and CP were not affected by treatment (Table 3). Subacute ruminal acidosis typically depresses digestibility of DM, OM and NDF (Plaizier et al., 2008). For each cow and period, we composited all fecal
samples (0 to 42 h) and determined nutrient digestibility based on those composites. The lack of treatment effects on these measures suggest that the hindgut does not substantially contribute to the digestibility depression typically seen with SARA or our compositing methods masked differences that would have been more likely to be found during the middle of the sampling period. Only starch digestibility showed differences among treatments \( (P = 0.02; \text{Table 3}) \). Starch digestibility was reduced in ST1 compared to all other treatments, indicating that some of the infused starch was neither digested in the small intestines nor fermented in the large intestines.

3.2 Fecal Samples

3.2.1 Fecal DM Content

Overall fecal DM differed by hour of sampling but there was no effect of infusion treatment on this measurement (Table 4). The hour effect was largely due to increased fecal DM at 12 and 36 hours compared to the other timepoints (data not shown). Steers were fed at roughly 0 and 24 hours, and daily patterns of intake and rumination may have driven the hour effect in fecal DM. The lack of treatment effect on fecal DM was surprising. We have previously found that abomasal oligofructose infusions decreased fecal DM (Mainardi et al., 2011), and Bissell (2002) found that abomasal starch decreased fecal DM. Additionally, one sign of SARA is increased incidence of diarrhea (Emmanuel et al., 2007).

3.2.2 Fecal pH

Hour, treatment and treatment \( \times \) hour \( (P<0.01; \text{Table 4}) \) affected fecal pH. The treatment \( \times \) hour interaction was primarily due to differences at 12 h (Figure 3). At 12 h there were treatment differences between CON and both OL1 and ST1 with CON pH at 7 and both OL1 and ST1 pH around 6. The ST4 and OL4 treatments also showed a pH
depression in comparison to CON and were intermediate with pH about 6.6. At 18 h OL1 and ST1 returned to pH near that of the CON, while OL4 and ST4 remained reduced compared to CON. Twenty-four h after the first infusion there are no marked differences between treatments at any time.

Increasing the forage to concentrate ratio of a diet can increase the amount of fermentable carbohydrate passing through the digestive system. Li et al. (2012) found a drop in cecal pH after an increased concentrate diet was fed. The decrease in fecal pH seen in all 4 treatments at 12 h compared to CON indicates that there was increased fermentation in the large intestine (Figure 3). Similarities in the depression of ST1 and OL1 suggest that the starch was in fact fermented in the large intestine to a similar extent as oligofructose. During bouts of SARA rumen pH declines from a normal pH around 6.5 to a pH between 5.2 and 5.6 (Krause and Oetzel, 2005; Nagaraja and Titgemeyer, 2007). The magnitude of the decline we observed with the ST1 and OL1 treatments is comparable to the decline seen in rumen pH with SARA. Normal intestinal pH is around 7 (Frandson et al., 2006) and the nadir in pH for the ST1 and OL1 treatments was around 6 (Figure 3). The lesser decline at 12 h found in the ST4 and OL4 treatments indicates a less dramatic increase in fermentation by splitting the infusion into 4 doses. Mainardi et al. (2011) compared the CON and OL1 treatments in an independent study and found that fecal pH declined, though the magnitude was less, 7.0 for CON vs. 6.5 for OL1 at 12 h post-infusion. Because starch but not oligofructose can be enzymatically digested in the large intestine, we expected less starch to pass to the large intestine than oligofructose and consequently expected to see a less dramatic drop in pH for the starch compared to oligofructose infusions. Contrary to this hypothesis, the magnitude of pH decline was similar for both substrates, suggesting that the majority of the infused starch bypassed small intestinal digestion and reached the large intestine. The similar change in fecal pH
suggests that both substrates, particularly when given as a single pulse dose, induced hind-gut acidosis.

3.2.3 Fecal Score

Hour, treatment and treatment × hour effected fecal score (\( P < 0.001 \); Table 4). Fecal score was depressed at 6 h with OL1 showing a fecal score of 1, which is different from CON and all other treatments which had a fecal score of about 3 (Figure 4). At 12 h there was still a low fecal score for the OL1 treatment (1.5) compared to CON and other treatments (about 3). At 30 h OL4 showed a drop in fecal score that was different from CON and all other treatments, with the OL4 score around 2 and all others at score 3.

Bissell (2002) observed dramatic changes in fecal consistency when cows were infused with 5 g/kg post-ruminal starch. Cows receiving this infusion experienced severe diarrhea (fecal score of 1), indicating a similar result as shown here by the OL1 treatment. Mainardi et al. (2011) also used the OL1 treatment to induce hindgut acidosis. As expected, the decline in fecal score in their study was nearly identical to what we observed, with a score just below 1.5 at 6 hours. The decline in fecal score observed by Mainardi et al. (2011) was mirrored by a decline in fecal DM, and the authors proposed that the osmotic pressure associated with the OL1 challenge may have induced the diarrhea. The decline in fecal score at 6 h in the current study was not accompanied by a decline in fecal DM. In the current study, we developed the OL4 treatment to provide the same total amount of fermentable substrate but split over a longer period of time to reduce the osmotic burden. This appears to have successfully avoided the rapid drop in fecal score that occurred with the OL1 treatment. However, there was likely still some osmotic burden as there was a milder decline in fecal score observed with OL4 at 30 h,
12 h after the last infusion of oligofructose. In contrast to oligofructose, neither starch infusion impacted fecal score.

3.2.4 Fecal VFA

Lactate was affected by treatment, hour and treatment × hour \( (P < 0.001; \) Table 5). The interaction was driven entirely by differences among treatments at 12 h (Figure 5). Lactate showed an increase at 12 h post infusion with the biggest peak from OL1. ST1 and ST4 also showed an increase in lactic acid relative to CON at 12 h but were substantially reduced from OL1.

Rumen lactate concentrations typically rise most dramatically in response to acute acidosis, but some have noted increased rumen lactate concentrations in response to SARA induction (Krause and Oetzel, 2005; Khafipour et al., 2009a,b). The increase in lactate levels is generally thought to be caused by a decrease in pH which allows lactobacilli to flourish in the rumen. Since large intestinal conditions are similar to ruminal conditions, it is inferred that an increase in fecal lactate levels would be a sign of post-ruminal acidosis. The starch treatments, ST1 and ST4, did show a smaller increase in lactate, at 6 h and 12 h respectively, which could also be an indicator of post-ruminal acidosis. Mainardi et al. (2011) observed a similar increase in fecal lactate at 12 h in response to the OL1 treatment. However, they observed a substantially lower increase (peak ~5 mM), which may help to explain the lesser decline in fecal pH observed in their study compared to the present study.

Acetate was unaffected by treatment or the interaction of treatment and hour but was affected by hour \( (P < 0.001; \) Table 5). Acetate concentration in the CON treatment displayed nadirs at roughly 6 and 30 h, or roughly 6 h after feeding, and the pattern over the course of the day was likely due to fluctuations associated with digesta passage
Most treatments showed patterns similar to that of CON except that at 12 h ST1, OL4, and ST4 were numerically greater than CON.

Acetate, propionate, and butyrate are the most common VFA and we expected that all 3 would increase in response to the oligofructose and starch infusions. Khafipour et al. (2009b) found that a SARA induction protocol increased rumen acetate levels from 53.9 mM in control to 74.5 mM following SARA induction. However, another SARA induction experiment found a much smaller increase in acetate level, from 61.3 mM in the control to 64.8 mM in challenged animals (Krause et al., 2009). When Mainardi et al. (2011) compared the CON and OL1 treatments, they found that acetate increased by about 50% at 12 h after abomasal oligofructose infusion.

Propionate was affected by treatment, hour and treatment × hour ($P<0.001$; Table 5). Peaks in propionate concentration can be seen at 12 h and 24 h for treatments ST1 and OL4, respectively (Figure 7). ST1 and OL4 were both greater than CON at 12 h, and OL4 remained greater than CON through 30 h. No differences among treatments were observed at any other time.

Krause and Oetzel (2005) and Khafipour et al. (2009a) found that SARA induction protocols increased ruminal propionate concentration, and Mainardi et al. (2011) found that OL1 increased fecal propionate from ~11 to ~15 mM at 12 h post-infusion. A similar difference was found in the current trial (6 vs. 10 mM for CON vs. OL1 at 12 h), but this difference was not quite significant ($P = 0.051$).

Butyrate was affected by treatment, hour and treatment × hour ($P<0.001$; Table 5). Butyrate was greater for ST1 than all other treatments at 12 h, and ST4 was greater than CON, OL1 and OL4 at 12 h (Figure 8). The ST1 and ST4 treatments were still greater than CON at 18 h, and at 24 h, ST1 was greater than ST4 but not different from any other treatment.
Others have observed an increase in butyrate in response to a SARA challenge (Krause and Oetzel, 2005; Khafipour et al., 2009a). Mainardi et al. (2011) saw an increase in butyrate at 12 h for OL1 vs. CON. In the present experiment, butyrate was increased only for the starch infusions. It could be likely that the oligofructose treatment did not get fermented into butyrate and therefore did not show the same increase.

The minor fatty acids isobutyrate, valerate, and isovalerate were all affected by treatment, hour and treatment x hour ($P<0.05$; Table 5). For isobutyrate, OL1 was lower than all other treatments at 6 h (Figure 9). At 18 h both OL1 and OL4 were lower than CON, at 24 h both OL4 and ST4 were lower than CON, and at 42 h OL4 was lower than CON. Effects on valerate were due to OL4 being greater than all other treatments at 30 and 36 h (Figure 10). Effects on isovalerate were due to OL1 being less than ST1, OL4, and ST4 at 6 h, CON being greater than all other treatments at 24 h, and OL4 being greater than all other treatments at 30 h.

Volatile fatty acid data were summarized by looking at total VFA which included all of the measured acids and total organic acids which included all VFA and lactate. Results were similar for both total VFA and total organic acids with both being unaffected by treatment but affected by hour and treatment $\times$ hour ($P < 0.01$; Table 5). The interactions were primarily due to differences among treatments at 12 h (Figures 12 and 13). When looking just at VFA, only ST1, OL4, and ST4 were greater than CON at 12 h, but when lactate was included, all treatments were greater than CON at 12 h.

The increase in total VFA seen at 12 h indicates that there was all treatment infusions increased fermentation in the large intestine. Increasing fermentation of available starch in the large intestine will increase the total VFA thus causing a decrease in intestinal pH. Bissell (2002) reported the efficient absorption of VFA at normal rumen pH, but that SARA can increase keratinization of tissue of the rumen and make it difficult
for VFA to be absorbed under chronic SARA conditions. As more concentrate is fed in the diet there is an initial increase in VFA production, then as absorption decreases this VFA build up becomes apparent. The difficulty in absorption makes total VFA a good indicator of acidosis. Though VFA are not very acidic (pKa of 4.8) when they accumulate it can lead to a decreased pH that causes acidotic conditions. As the pH drops lactate production increases, which decreases the pH even more. Krause et al. (2009) noted an elevation in lactate levels but also believes that lactate was not the primary cause of ruminal pH depression. Instead it is believed that total VFA inside the rumen would be the cause of the rumen depression. Li et al. (2012) showed that a decrease in rumen pH was associated with increased total VFA concentrations. These conditions indicate acidosis and by inference of our results we can believe the increase in total VFA found in feces indicated post-ruminal acidosis.

3.3 Fecal Endotoxin Concentration

Fecal endotoxin concentration was affected by treatment, hour and hour × treatment ($P < 0.001$; Table 6). Comparison of the reverse transformed least squares means indicated that across all sampling times, endotoxin concentration of for OL1, ST1, and ST4 treatments were 2.2 to 3.0 times that of CON and the OL4 treatment was 8.7 times that of CON. The treatment × time interaction was due to no differences among treatments at 0 or 42 h but differences among treatments at all other times (Figure 13). Both OL1 and ST1 were greater than CON at 6, 12, and 18 h. OL4 was greater than CON from 12 through 36 h, and ST4 was greater than CON only at 18 h. The maximum differences from CON for both OL1 and ST1 were observed at 12 h, with concentrations 56-times and 19-times that of CON, respectively. The greatest difference between ST4
and CON was observed at 18 h, and endotoxin concentration for ST4 was 6-times that of CON. At 24 h, endotoxin concentration for OL4 was 57-times that of CON.

It has been suggested that free rumen LPS increases as the amount of concentrate in the diet increases (Gozho et al., 2006). Gohzo et al. (2005) found LPS concentrations in the rumen were greatest when pH was below 5.6. Emmanuel et al. (2008) found that ruminal LPS concentrations were highest in those cows fed diets with greater amounts of barley grain. These results are comparable to ours as those diets or treatments with the greatest rumen fermentation showed the highest LPS concentrations. Khafipour et al. (2009a) also found an increase in free ruminal LPS with increased concentrate in the diet. The increase in free LPS combined with reduced pH could cause the gastrointestinal damage that leads to the translocation of LPS into the blood. The weaker epithelial tissue of the large intestine is likely to be more susceptible to this damage when acids and LPS accumulate in the large intestine (Khafipour et al., 2009a). Li et al. (2012) noted an increase in cecal LPS when symptoms associated with SARA occur. Our results suggest the induction of post-ruminal acidosis increased fecal LPS similarly to the increase observed by Li et al. (2012) in response to SARA. Our results demonstrate that acidotic conditions in the hind gut will increase endotoxin release.
Chapter 4

Conclusion

Sub-acute ruminal acidosis (SARA) is defined as a ruminal pH below 5.6 for 180 min/d or more and results in reduced dry matter intake, loss of body weight, diarrhea, damage to gut epithelium, and lameness. In addition the lower pH can cause lysis of bacteria releasing free LPS, which can translocate in the blood stream causing the complications associated with SARA. It has been proposed that the excessive fermentation in the large intestine that accompanies SARA coupled with the thin intestinal epithelium makes the large intestine the most likely site of LPS translocation. The objective of the experiment described in this thesis was to compare four different models to induce hindgut acidosis in ruminally cannulated Holstein steers. These models were abomasal delivery of starch or oligofructose given as one pulse dose or four small doses. The different treatments administered over the course of the experiment proved successful in inducing post-ruminal acidosis. Both oligofructose models lowered the fecal score over the course of treatment. The single pulse doses of both starch and oligofructose caused a decrease the fecal pH in steers, while the staggered dose also decreased the pH with less severity. Total fecal VFA was increased across all treatments indicating increased fermentation in the large intestine. An increase in lactic acid was also seen across all treatments. Lipopolysaccharide concentrations increased in fecal samples across all treatments indicating an increase in the production of free endotoxin in the large intestine. This experiment suggests that both the pulse dose and staggered dose of oligofructose induced conditions similar to those seen in post ruminal acidosis.
starch treatments showed similar effects to their oligofructose counterparts suggesting that starch could be used as a model to induce post ruminal acidosis.
REFERENCES


Mainardi, S. 2009. Can abomasal oligofructose infusions be used as a model to study post ruminal acidosis? Senior thesis. Univ. Delaware, Newark.


# Appendix A

## Tables

Table 1. TMR ingredients and nutrient composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% of diet DM</th>
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</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>32.9</td>
</tr>
<tr>
<td>Alfalfa silage</td>
<td>15.7</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>8.0</td>
</tr>
<tr>
<td>Ground corn grain</td>
<td>15.5</td>
</tr>
<tr>
<td>Rumen protected soybean meal</td>
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</tr>
<tr>
<td>Corn hominy</td>
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</tr>
<tr>
<td>Ground soybean hulls</td>
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</tr>
<tr>
<td>Distiller’s grains</td>
<td>3.7</td>
</tr>
<tr>
<td>Citrus pulp</td>
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</tr>
<tr>
<td>Canola meal</td>
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</tr>
<tr>
<td>Soybean meal</td>
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<tr>
<td>Molasses</td>
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<tr>
<td>Sodium bicarbonate</td>
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</tr>
<tr>
<td>Rumensin for dairy</td>
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<tr>
<td>Vitamins and minerals¹</td>
<td>1.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
<th>% of DM</th>
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</thead>
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<tr>
<td>Crude protein (CP)</td>
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<tr>
<td>NDF</td>
<td>33.2</td>
</tr>
<tr>
<td>ADF</td>
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</tr>
<tr>
<td>TDN</td>
<td>73.1</td>
</tr>
<tr>
<td>Starch</td>
<td>18.9</td>
</tr>
<tr>
<td>Ash</td>
<td>7.1</td>
</tr>
<tr>
<td>Net Energy of Lactation (mcal/kg)</td>
<td>1.7</td>
</tr>
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</table>

¹Vitamins and minerals include: Calcium carbonate, calcium sulfate, NaCl, urea, Mg, Biphos, trace minerals and vitamins
Table 2. Water intake results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water intake, L/d</th>
<th>Treatment</th>
<th>P-Value</th>
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<tr>
<td>CON</td>
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<td>OL1</td>
<td>52.3</td>
</tr>
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<td></td>
<td>54.8</td>
<td>ST1</td>
<td>55.1</td>
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<td></td>
<td>53.5</td>
<td>OL4</td>
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<tr>
<td></td>
<td>5.0</td>
<td>ST4</td>
<td>53.5</td>
</tr>
</tbody>
</table>

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)

Table 3. Nutrient digestibility results

<table>
<thead>
<tr>
<th>Digestibility, %</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-Value</th>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>DM</td>
<td>68.4</td>
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<td></td>
<td>67.3</td>
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<td></td>
<td>68.7</td>
<td>1.0</td>
<td>0.13</td>
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<td>OM</td>
<td>70.1</td>
<td>67.3</td>
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<td></td>
<td>68.9</td>
<td>70.0</td>
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<tr>
<td></td>
<td>0.18</td>
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<td></td>
</tr>
<tr>
<td>Starch</td>
<td>97.1</td>
<td>96.6</td>
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<td></td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>CP</td>
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<td>74.5</td>
<td>71.6</td>
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<td>72.3</td>
<td>73.3</td>
<td>1.6</td>
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<td>0.71</td>
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in rows with unlike superscripts are different between treatments (P < 0.05)

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)
Table 4. Fecal dry matter, pH, score, and lipopolysaccharide concentration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment</th>
<th>P-Values</th>
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<tbody>
<tr>
<td>CON</td>
<td>OL1</td>
<td>ST1</td>
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<tr>
<td>Fecal DM, %</td>
<td></td>
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<tr>
<td>Fecal pH</td>
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<td>Fecal score</td>
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superscript a-c Means in columns with unlike superscripts are different between treatments ($P < 0.05$)

1 Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)
Table 5. Fecal concentrations (mM) of volatile fatty acids and lactic acid

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<thead>
<tr>
<th></th>
<th>CON</th>
<th>OL1</th>
<th>ST1</th>
<th>OL4</th>
<th>ST4</th>
<th>SEM</th>
<th>Treatment</th>
<th>Hour</th>
<th>Treatment × Hour</th>
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<tbody>
<tr>
<td>Lactate</td>
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<td>3.48b</td>
<td>0.65a</td>
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<td>Acetate</td>
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<td>Proprionate</td>
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<td>6.57b</td>
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<td>0.02a</td>
<td>0.01a</td>
<td>0.08b</td>
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<td>0.01</td>
<td>0.001</td>
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<tr>
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<td>0.11a,b</td>
<td>0.18c</td>
<td>0.11a,b</td>
<td>0.02</td>
<td>0.04</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Total VFA²</td>
<td>54.1</td>
<td>54.0</td>
<td>58.6</td>
<td>58.4</td>
<td>54.7</td>
<td>3.4</td>
<td>0.33</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Total OA³</td>
<td>54.0</td>
<td>58.4</td>
<td>59.9</td>
<td>59.8</td>
<td>54.9</td>
<td>3.8</td>
<td>0.26</td>
<td>0.001</td>
<td>0.02</td>
</tr>
</tbody>
</table>

a-b Means in columns with unlike superscripts are different between treatments ($P < 0.05$)

1 Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)

2 Total volatile fatty acids = sum of all acids except for lactate

3 Total organic acids = sum of all acids including lactate
Table 6 Fecal LPS Concentrations

<table>
<thead>
<tr>
<th>Fecal LPS, log_{10}(EU/mL)</th>
<th>Treatment</th>
<th>P-Values</th>
<th>SEM</th>
<th>Treatment</th>
<th>Hour</th>
<th>Treatment × Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>OL1</td>
<td>ST1</td>
<td>OL4</td>
<td>ST4</td>
<td></td>
</tr>
<tr>
<td>3.39^a</td>
<td>3.87^b</td>
<td>3.81^b</td>
<td>4.33^c</td>
<td>3.73^b</td>
<td>0.22</td>
<td>0.001</td>
</tr>
<tr>
<td>Fecal LPS, EU/mL</td>
<td>2,449</td>
<td>7,341</td>
<td>6,415</td>
<td>21,345</td>
<td>5,324</td>
<td></td>
</tr>
</tbody>
</table>

^a-b Means in columns with unlike superscripts are different between treatments (P < 0.05)

1 Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)
Appendix B

Figures

Figure 1. Cannula Infusion Line Used for Treatments
Figure 2. Water Intake

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4
Figure 3. Fecal pH

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4).

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4.
Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)
Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4
Figure 5. Lactic Acid

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4
Figure 6. Acetate

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4).

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4.
Figure 7. Propionate

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4
Figure 8. Butyrate

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4
Figure 9. Isobutyrate

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4
Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)
Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4
Figure 11. Isovalerate

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4
Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4).

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4.
Figure 13. Total Organic Acids

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4).

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4
Figure 14. LPS Concentration

Treatments were no carbohydrate infusion control (CON), 1 g/kg BW oligofructose administered as a single infusion at 1000 h (OL1), 1 g/kg BW starch administered as a single infusion at 1000 h (ST1), 1 g/kg BW oligofructose administered as 4 infusions of 0.25 g/kg BW (OL4), 1 g/kg BW starch administered as 4 infusions of 0.25 g/kg BW (ST4)

Symbols denote differences from CON: § for OL1, # for ST1, ¶ for OL4, * for ST4