THE EFFECT OF ABRUPT DIETARY ALTERATIONS WITH AND WITHOUT A PROPRIETARY SUPPLEMENT ON BIOCHEMICAL PARAMETERS IN THE CECUM OF THE EQUINE

by

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Abstract

Abruptly increasing concentrate in the ration of horses results in altered cecal dynamics which can culminate in digestive distress. Nine Quarter horses previously fitted with cecal cannulae were utilized for 3 consecutive 22-d experiments, each separated by 2 d of rest. During Exp. 1 and 2 horses were acclimated to the same ration for the initial 21 d of each period, followed by a concentrate challenge on d 22. The acclimation ration consisted of a morning meal of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed with 1.5% BW prairie grass hay divided evenly between a morning and evening meal. On d 22 of Exp. 1, horses were fed a morning meal consisting solely of 1.0% BW concentrate while 1.25% BW concentrate was fed on d 22 of Exp. 2. Cecal samples were obtained through cecal cannulae from d 19 to 22 of each experiment every 4 h for h 24 following the morning meal each day. Cecal pH during Exp. 1 was recorded and decreased at h 12 following the concentrate meal on d 22 in comparison to cecal pH at h 12 on d 19 to 21 ($P = 0.009$). During Exp. 2 cecal pH increased at h 4 ($P = 0.02$) and decreased at h 12 and 20 ($P < 0.0001$) following this concentrate challenge compared to cecal pH recorded at the same time points during the acclimation period. Experiment 3 differed from that of Exp. 2 only in the respect that during the acclimation period horses were fed, in addition to the acclimation ration, either a proprietary supplement (n = 5) or a placebo (n = 5). Cecal samples from d 19 to 22 were analyzed for pH, concentration of lactate, and concentration of VFA. Horses consuming the supplement had increased cecal pH at h 4 ($P = 0.009$), concurrently decreased cecal lactate ($P = 0.02$), increased ratio of (acetate+butyrate)/propionate at h 8 and 16 ($P \leq 0.006$), and decreased VFA concentration at h 24 ($P \leq 0.05$) compared to horses in the control group following the concentrate challenge.

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Chapter 1 - Review of the Literature

1.1 Introduction

As a non-ruminant herbivore, the horse's digestive system is designed to utilize the nutrients ingested from its natural diet, which consists primarily of forage in post-weaned horses (Dyer et al., 2009; NRC, 2007). Forage consists of pasture grasses, legumes, and forbs that can be grazed directly or provided in a conserved form (NRC, 2007). Structural carbohydrates (SC), primarily the polysaccharide cellulose, are found in significant quantities in forage and are fermented in the gastrointestinal tract to provide energy to the horse (NRC, 2007; Ralston, 2007; Varloud et al., 2004). However, due to constraints in pasture space, increases in work demand, and increased confinement, less forage is being fed to domesticated horses in favor of more energy dense grains that are primarily comprised of nonstructural carbohydrates (NSC; Clarke et al., 1990; Daly et al., 2012; de Fombelle et al., 2001).

Starch is the primary polysaccharide component of the NSC fraction of grains (NRC, 2007). Fructans are NSC that accumulate in cool-season grasses (Lopes et al., 2004; NRC, 2007; Secombe and Lester, 2012). Researchers implicate excessive ingestion or rapid increases in dietary starch and fructans as causative factors in the onset of the digestive disorders colic and laminitis (Lopes et al., 2004; NRC, 2007; Secombe and Lester, 2012). Dietary starch levels exceeding 3.4 g·kg BW^{-1}·$meal^{-1}$ have been observed to saturate the digestive capacity of the foregut and can culminate in digestive upset (Medina et al., 2002; Potter et al., 1992). Digestive disturbances resulting from fructan overload has been observed following nasogastric administration of 10 g oligofructose/kg BW (Milinovich et al., 2006). To avoid digestive disturbances caused by overconsumption of starch and fructans, the National Research Council (NRC, 2007) recommends making gradual dietary changes if the quantity of feedstuff given, type of feed, or the physical form will increase starch or fructan availability or digestibility. Dietary changes should take place incrementally over several days or weeks to allow small intestinal morphology, as well as the microbial population of the digestive tract, adequate time to acclimate to the novel diet.

This review analyzes the effects of carbohydrate overload, most often in conjunction with laminitis-induction, in the equine. Specifically, it addresses the physiological responses,

primarily in the hindgut, that precede or are associated with major disorders, such as laminitis or colic, and supplemental feeding strategies that may help prevent these disorders. However, it also addresses prececal digestion of NSC because the percentage of NSC reaching the hindgut is directly related to the percentage of NSC utilized prececally (Varloud et al., 2004).

1.2 The Foregut

1.2.1 Carbohydrate Digestion

Monosaccharides, disaccharides, starch, oligosaccharides (including fructooligosaccharides), and fructan polysaccharides comprise the nonstructural fraction of carbohydrates (NRC, 2007). While the more common hexose monosaccharides of glucose, fructose, and galactose are found in relatively lesser concentrations in nature than other NSC, they are important in the formation of the more commonly found oligo- and polysaccharides (NRC, 2007). The type of linkages between monosaccharides largely determines the site of digestion and absorption, as well as subsequent energy yielded (NRC, 2007). Despite the fact that both starch and cellulose are glucose homopolymers, starch yields more energy than cellulose. This is because both linkages in starch, α1-4 and α1-6, can be enzymatically digested in the foregut, which yields greater energy than the β linkages in SC, such as the β1-4 linkages in cellulose, that must be broken down by microbes in the hindgut (NRC, 2007). However, while total tract disappearance of starch is greater than 90 percent, the amount of starch digested pre-cecally is determined by, among other factors, the structure and origin of the starch, level of intake, intake in proportion to forage, and method of processing used (Kienzle et al., 1997; NRC, 2007; Potter et al., 1992).

Starch is composed of a crystalline and amorphous layered structure containing amylose and amylopectin. Although glucose units comprise both amylose and amylopectin, they are structurally different. Amylose is a straight chain formed by α-1,4 linked glucose residues, while amylopectin is branched, containing α-1,4 straight chains with α-1,6 branches linked approximately every 20 glucose residues. Salivary and pancreatic α-amylase cleave only α-1,4 linkages, leaving intestinal brush border enzymes to cleave α-1,6 linkages found in amylopectin (Gray, 1992).

Fructans are a complex group of carbohydrates composed of one α-glucopyranosyl linked to varying numbers of β-fructofuranosyl units in a branched or unbranched chain (Nilsson et al., 1988; Pollock, 1986). The simplest fructan, monofructosyl sucrose, has 3 isomers: isokestose, kestose, and neokestose, which comprise the basis of the fructan families (Pollock, 1986). The simplest fructan family, inulin, contains β-1,2 linkages between terminal glucose and fructose residues (Pollock, 1986). The fructan family commonly referred to as phlein or levan contains β-2,6 linkages between terminal glucose and fructose residues, while a third family utilizes β-1,6 linkages between internal glucose and fructose residues located on both the 1 and 6 positions (Pollock, 1986). Mixed-type fructans have also been observed (Pollock, 1986). Because β-fructofuranosidase, required to remove terminal fructose units from fructans, has not been identified in the equine small intestine (Nilsson et al., 1988; Pollock, 1986), fructans must be fermented by microbes in the hindgut, along with resistant starches and oligosaccharides that escape small intestinal digestion (NRC, 2007).

1.2.2 The Stomach

Following prehension, mastication, and deglutition digesta moves from the oral cavity, down the esophagus, and into the stomach. As much as 40% of the digestion of pelleted starches and sugars has been observed to occur in the stomach of ponies due to a combination of acid hydrolysis and microbial fermentation, while negligible digestion of β-linked fructans occurs (Nilsson et al., 1988; NRC, 2007; Varloud et al., 2004). The squamous, non-glandular fundic region surrounds the esophageal sphincter and encases the upper third of the equine stomach. This area does not produce enzymes or acids, while the glandular pyloric region in the lower two-thirds of the stomach secretes acids and enzymes from glandular cells, resulting in a pH gradient (fundic pH = 5.4, pyloric pH = 2.6; Kern et al., 1974). Regardless of pre-prandial pH of gastric fluid collected by gastric cannulae in ponies, within 30 min following a concentrate meal pH was observed to be at least 5.0 (Healy et al., 1995). Additionally, microbial populations have been identified in the stomach, with concentration varying by region. Kern et al. (1974) observed ponies to have 20 times more viable bacteria in the body or fundic region of the stomach than the glandular, pyloric region. Presumably, this difference is due to the pH gradient between these regions (Kern et al., 1974). Most bacteria prefer a pH near neutral, while the equine stomach,

particularly the glandular region, maintains acidity beyond what most bacteria can tolerate (Asanuma and Hino, 1997).

Increases in glucose and L-lactate, the presence of volatile fatty acids (VFA), and a population of Gram-positive cocci have been observed in gastric fluid (Healy et al., 1995; Kern et al., 1974; Varloud et al., 2004). Strains of bacteria from the lactate-producing genera *Streptococci* and *Lactobacilli*, specifically, *L. bifidus*, *S. bovis*, *S. equinus*, and strains resembling *S. lactis*, which utilize starch as a substrate in the hindgut, have been isolated from equine gastric contents (Al Jassim et al., 2005; Alexander and Davies, 1963). The lactate-fermenting strains, *Veillonella gazogenes* and *Escherichia coli*, also have been isolated from the stomach of horses (Alexander and Davies, 1963). These bacterial populations, coupled with the presence of biochemical parameters noted earlier, are indicative of microbial fermentation of starch and sugar in the stomach of horses and ponies (Kern et al., 1974).

Horses acclimated to 74 g starch/100 kg BW in their morning meal had a greater concentration of D- and L-lactate in their stomach than those acclimated to 286 g starch/100 kg BW in their morning meal. Although counter intuitive, the lesser concentration of lactate in the gastric contents of horses receiving a greater amount of starch is likely due to an increased number of lactate-utilizing bacteria that was observed in the stomach (de Fombelle et al., 2003). A concurrent decreased ratio of (acetate+butyrate)/propionate in the stomach of horses consuming a greater amount of starch compared to the stomach of horses consuming a lesser amount of starch provided evidence that lactate-utilizing bacteria fermented lactate to propionate in the stomach, as has been evidenced in the cecum of horses following a diet containing a large amount of concentrate (de Fombelle et al., 2003).

1.2.3 The Small Intestine

Pre-cecal apparent digestibility coefficients for starch and sugar are near 0.90, but vary depending on botanical origin, extent of processing, and level fed (Kienzle et al., 1997; Varloud et al., 2004). Following microbial fermentation in the stomach, starch enters the small intestine where the majority of starch and sugar digestion occurs (Varloud et al., 2004). Anaerobic bacterial populations reside in the small intestine at levels similar to that of the cecum, but the majority consist of lactic acid bacteria and the presence of cellulolytic bacteria appears to be limited (de Fombelle et al., 2003). Limited quantities of VFAs have been detected in the small

intestine; thus microbial fermentation likely does not play a major role in small intestinal digestion. Instead, enzymes are the primary catalysts of digestion in the small intestine (Roberts, 1974).

The equine pancreas secretes a large volume of pancreatic juice containing a limited amount of enzymes into the lumen of the duodenum (Roberts, 1975). Pancreatic α-amylase is critical in the digestion of starch. While salivary α-amylase is capable of starch digestion as well, it is to a much more limited extent. In comparison to other species, activity of salivary and pancreatic derived amylase is limited in the equine. Both salivary and pancreatic α-amylase degrade starch into maltose, maltotriose, and α-dextrins in the small intestine (NRC, 2007; Richards et al., 2004; Roberts, 1975). The brush-border enzymes maltase, sucrase, and lactase degrade the disaccharides maltose, sucrose, and lactose respectively into single D-glucose units and the counterpart monosaccharides fructose and galactose. However, the abundance of these brush-border enzymes varies by small intestinal region, as well as with age (Dyer et al., 2009; Roberts, 1974; Roberts et al., 1974). Activity of maltase is similar in all 3 regions of the small intestine while sucrase is more active in the duodenum and jejunum than the ileum (Dyer et al., 2009). Activity of both maltase and sucrase increase in the proximal jejunum from birth until approximately 3 yr of age (Roberts, 1974). Conversely, lactase activity declines in the proximal jejunum following birth, and similarly to sucrase, is greater in the duodenum than the ileum of adult horses (Dyer, 2009; Roberts, 1974).

D-glucose, liberated from starch and sugar, is transported across the brush-border membrane of the small intestine via the high-affinity low-capacity sodium-dependent glucose transporter-1 (SGLT-1) into intestinal enterocytes (Dyer et al., 2009). Greater absorption of glucose occurs in duodenal enterocytes than in either jejunal or ileal enterocytes of forage-fed horses (Dyer et al., 2002). The affinity of SGLT-1 for glucose (K_m) remains constant throughout the small intestine, while SGLT-1 protein abundance declines from jejunum to ileum. It is likely that the decrease in SGLT-1 protein limits glucose transport in the mid and distal small intestine.

Following absorption by SGLT-1, glucose subsequently exits the enterocyte via the facilitative glucose transporter GLUT-2 on the basolateral membrane, independent of sodium, and enters the portal vein (Dyer et al., 2009; Shirazi-Beechey, 1995). Glucose is transported to the liver where it can enter general circulation for utilization. Alternatively, glucose may be stored in the form of muscle or liver glycogen, or it may be stored in adipose tissue (NRC, 2007).

Utilization of glucose under aerobic conditions begins with glycolysis, conversion of resulting pyruvate into acetyl-coA, entrance of acetyl-coA into the citric acid cycle, and finally, electrons undergo oxidative phosphorylation. The final products of the metabolism of 1 molecule of glucose are 4 H_2O, a theoretical yield of 38 ATP, and 4 CO_2. Under anaerobic conditions, such as in heavily contracting muscles, glucose undergoes homolactic fermentation. The first step of homolactic fermentation is glycolysis, which yields pyruvate. Pyruvate is rapidly converted to lactate and 2 ATP are generated (Nelson and Cox, 2008).

1.2.3.1 Small Intestinal Response to Increased Starch Load

Increasing carbohydrates ingested by herbivores and omnivores leads to increased expression of SGLT-1 on the brush-border membrane of the small intestine and, therefore, an increased ability to absorb monosaccharides (Ferraris and Diamond, 1989; Margolskee et al., 2007; Shirazi-Beechey, 1996). The increase in SGLT-1 expression is initiated by type 1 sweet receptor subunit-3 (T1R3) and gustducin (Ferraris and Diamond, 1989; Margolskee et al., 2007; Solberg and Diamond, 1987). Gustducin and T1R3 are located in the enteroendocrine cells. Both taste receptors sense monosaccharides, but not di-, oligo-, or polysaccharides, in the lumen of the intestine. Margolskee et al. (2007) proposed that when T1R3 and gustducin detect monosaccharides in the small intestine of mice, enteroendocrine cells release the hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic peptide (GIP). Glucagon-like peptide-1 and GIP then act on enterocytes where SGLT-1 is located to increase its abundance.

The hyperplastic effect of carbohydrates on SGLT-1 has also been observed in the equine (Dyer et al., 2009). In horses fed a concentrate (oats:corn in 2:1 ratio) and hay diet (6.0 g starch·kg BW-1·day-1) uptake of glucose by enterocytes increased 1.9- and 3.7-fold in the jejunum and ileum, respectively, compared to glucose uptake in horses fed an all-forage diet (< 1.0 g starch·kg BW-1·day-1). The affinity (K_m) of SGLT-1 for glucose was the same for both dietary treatment groups, which thus does not account for the increased glucose uptake in the jejunum and ileum of horses fed concentrate. However, there were 2- and 3.5-fold increases in the saturation capacity of the transporter (V_{max}) in the jejunum and ileum of concentrate-fed horses, respectively, which indicates increased expression of SGLT-1 along the jejunum and ileum. Western blot analysis confirmed that SGLT-1 protein increased in abundance in the mid-

and distal intestine when horses were fed concentrate rather than the forage-only diet. Thus there appears to be intestinal modification of transporter abundance in response to diet (Dyer et al., 2002; Dyer et al., 2009).

A step-wise increase in the concentrate to forage ratio in the diet also results in increased mRNA levels of SGLT-1 in the equine intestine (Dyer et al., 2009). Horses previously acclimated to a forage-only diet were introduced to a ration with 40% concentrate (2:1 ratio oats: corn; 60% hay; 3.3 g starch·kg BW-1·day-1). After 1 wk there was a 2-fold increase in SGLT-1 mRNA abundance in the ileum, while it required a month of acclimation to this ration for the quantity of SGLT-1 mRNA in the duodenum to increase similarly (Dyer et al., 2009). Increasing the concentrate to 60% of the ration (6.0 g·kg BW-1·day-1) for an additional month caused no further increases in the expression of SGLT-1 mRNA in the duodenum, but led to 3.3-fold greater expression of SGLT-1 mRNA in the ileum compared to horses on an all forage ration (Dyer et al., 2009). Dyer et al. (2009) concluded that SGLT-1 expression was increased in response to increasing dietary carbohydrates, with the effect becoming more significant over time. Because the equine brush border membrane, specifically SGLT-1 protein, requires more time to adapt to a dietary change than in species such as mice, man, and other omnivores which frequently have varying levels of carbohydrates in their diet (Buddington and Rashmir-Raven, 2002; Ferraris and Diamond, 1989), it is important to avoid scenarios that induce a dramatic increase in starch without allowing time for small intestinal adaptation in the horse (Dyer et al., 2009).

In addition to small intestinal morphological limitations in the equine, enzymatic limitations may contribute to the escape of starch from the small intestine to the hindgut. Less α-amylase activity is observed when starch is added to the diet of the equine in comparison to the response recorded in omnivores (Kienzle et al., 1994). Exogenous administration of α-amylase was associated with an increase in the mean plasma concentration of glucose in horses, which is indicative of increased starch digestion (Richards et al., 2004). Conversely, administration of exogenous amyloglucosidase (AMG), a brush-border enzyme that reduces polysaccharides to monosaccharides, did not lead to an increase in plasma glucose (Richards et al., 2004). Roberts (1974) observed brush-border enzyme activity in the equine to be similar to that in other species, and the lack of response to exogenous AMG provides evidence that this brush-border enzyme is not limiting to starch digestion in the equine. Combined administration of α-amylase with AMG

resulted in an increase in plasma glucose concentration similar to the response observed after administration of α-amylase alone, but co-administration of AMG prevents a decline in efficacy over time that has been noted when amylase is administered alone.

1.3 The Hindgut

1.3.1 Overview of Hindgut Fermentation

The microbial population in the equine hindgut is critical to the normal functioning of the horse's gastrointestinal tract (Costa and Weese, 2012; Daly et al., 2001). Structual carbohydrates and fructans containing chains of β-linked monosaccharides are neither digested enzymatically nor fermented microbially in large quantities in the foregut, which allows them to pass intact to the hindgut (NRC, 2007). Bacteria, protozoa, and fungi are the 3 microbial classifications that degrade feedstuffs reaching the hindgut. Although studied less thoroughly than the microbial population of the rumen, microbial organisms inhabiting the equine hindgut function similarly (Costa and Weese, 2012; Daly et al., 2001; Mackie and Wilkins, 1988).

Protozoa in the equine hindgut play a smaller role in the degradation of SC than either fungi or bacteria, and instead appear to be more involved in starch degradation (Moore and Dehority, 1993). Fungi in the hindgut ferment fibrous feedstuffs (Bauchop and Mountfort, 1981; Moore and Dehority, 1993), while anaerobic bacteria secrete extracellular enzymes that degrade β-linkages and any α-linkages that escaped pre-cecal digestion (NRC, 2007; Russell and Hespell, 1981). The Embden-Meyerhoff pathway is then utilized to catabolize the liberated monosaccharides into 2 molecules of pyruvate (Shirazi-Beechey, 2008). Microbes ferment pyruvate into the VFAs acetate, propionate, and butyrate; the gases CO_2, CH_4, and H_2; as well as the intermediate products lactate and succinate (Argenzio et al., 1974; Bergman, 1990; Wolin, 1981). Ultimately, VFAs are the major end products of microbial fermentation and are used to meet greater than half of the horse's energy requirements (Argenzio et al., 1974; Bergman, 1990; Daly et al., 2001).

1.3.2 Total Anaerobic Bacterial Populations

Anaerobic bacteria constitute the bulk of the microbial population in the hindgut, the population size varying with the type of meal fed, season, and from horse to horse (Costa and Weese, 2012; de Fombelle et al., 2003). The bacterial population reached 3.98×10^7 CFU/ml in

the cecum of horses sacrificed 2 h following a meal containing 0.74 g starch/kg BW during each of 3 meals and 5.01 x 10^7 CFU/ml in the cecum of horses sacrificed 2 h after a meal containing 2.86 g starch/kg BW in a morning meal and 1.65 g starch/kg BW in an evening meal (de Fombelle et al., 2003), which is within the reported limit of the small intestine's capacity for digestion of starch (3.4 g starch/kg BW; Potter et al., 1992). Others have reported total anaerobic bacteria in the cecum of cannulated horses to increase from 7.9 x 10^7 CFU/ml on a diet containing 1.26 g starch·kg BW^{-1}·$meal^{-1}$ to 4 x 10^8 CFU/ml when horses were acclimated to a diet containing 3.35 g starch·kg BW^{-1}·$meal^{-1}$ in each of 2 meals/d (Medina et al., 2002). Medina et al. (2002) designed the diet containing 3.35 g starch·kg BW^{-1}·$meal^{-1}$ to approach the upper limit of small intestinal digestive capacity for starch, potentially explaining the increase in the total anaerobic bacterial population in the cecum when horses were fed the diet containing a greater amount of starch. Accordingly, abruptly switching a cannulated pony from a diet of chopped alfalfa (2400 g alfalfa/day as-fed) to ground corn and soybean meal (1350 g corn and soybean meal/day as-fed), although isocaloric, also led to an increase in cecal counts of total viable bacteria. The number of viable bacteria in the cecum increased each day during the 6 wk that the pony was consuming corn and soybean meal compared to values obtained while the pony consumed only alfalfa. The most notable increase occurred 48 h post-dietary change (30.07 x 10^9 bacteria/g of cecal contents compared to 5.28 x 10^9 bacteria/g of cecal contents 24 h prior; Goodson et al., 1988). Goodson et al. (1988) observed a greater population of bacteria the in cecum of the pony than either de Fombelle et al. (2003) or Medina et al. (2002), which could be due to diet, species, number of animals, or culturing techniques utilized. Despite these differences, it is clear that exceeding small intestinal capacity for starch digestion can lead to increased anaerobic bacterial populations in the equine cecum.

Similarly, total concentrations of colonic anaerobic bacteria increased following an abrupt dietary change from a 70% pelleted feed with 30% wheat straw (0.08% BW starch/meal, 3 meals/d) to a barley meal containing 0.28% BW starch (6.3 x 10^6 CFU/ml at -19 h, 2 x 10^7 at +5 h, 2.5 x 10^7 at +29 h; Respondek et al., 2008). There are also regional differences throughout the colon, as the left dorsal colon contained the greatest population of anaerobic bacteria (1.3 x 10^9 CFU/ml) in the hindgut of horses fed minimal starch 3 times per d (0.74 g/kg BW per meal), while the right ventral colon contained the greatest population of anaerobic bacteria (7.9 x 10^8 CFU/ml) in the hindgut of horses fed a diet with a greater starch content in 2 meals (2.86 g/kg

BW in the morning meal; 1.65 g/kg BW in the evening meal; de Fombelle et al., 2003). Presumably, horses fed 2.86 and 1.65 g starch/kg developed a greater microbial population in the anterior portion of the hindgut than in the posterior portion due to the readily fermentable nature of the diet.

1.3.3 Cellulolytic Bacterial Population

The size of the cellulolytic bacterial population in the hindgut is greater than that of the foregut, regardless of diet. However, horses consuming a diet with elevated starch content reportedly have smaller populations of cellulolytic bacteria in the cecum (1×10^5 CFU/ml), with the right ventral colon containing the greatest population of cellulolytic bacteria (3.2×10^5 CFU/ml) and the left dorsal colon containing the least cellulolytic bacteria (5×10^4 CFU/ml). De Fombelle et al. (2003) concluded that there was a greater proportion of cellulolytic bacteria relative to total bacteria in the cecum, indicating the cecum is the major site of cellulolysis. In contrast, Goodson et al. (1988) suggested that the large colon is largely where cellulolysis occurs due to the lack of cellulolytic bacteria observed in the cecum during their experiment (< 1% of the total bacterial population). However, Goodson et al. (1988) used a 2% agar medium to culture the resident bacterial population, which the authors indicated may have inhibited several cellulolytic bacteria from exhibiting zones of clearing, subsequently resulting in an underestimate of total cellulolytic bacteria in the cecum. Of the $4.2 \times 10^8 \pm 1.7 \times 10^8$ CFU of bacteria/ml of cecal fluid in the cecum of cannulated ponies fed a 70% hay and 30% concentrate diet, $1.6 \times 10^7 \pm 0.4 \times 10^7$ bacteria/ml of cecal fluid has been reported to be cellulolytic. Similar bacterial counts were reported in the cecum of cannulated donkeys on the same diet: $5.7 \times 10^8 \pm 2.4 \times 10^8$ CFU of bacteria/ml of cecal fluid while $1.3 \times 10^7 \pm 0.6 \times 10^7$ bacteria/ml of cecal fluid were cellulolytic (Julliand et al., 1999). Cellulolytic bacteria appear to make up a small percentage of total anaerobes in the cecum for both species, comprising 2.3% in donkeys and 3.8% to 9% in ponies (Julliand et al., 1999; Kern et al., 1974).

Increasing dietary starch has a depressive effect on the numbers of acid-sensitive cellulolytic bacteria, subsequent microbial activity, and thus fermentation of fiber in the horse (de Fombelle et al., 2003; Hill, 2007; Julliand et al., 1999). A rapid change from an all-forage diet to an all-concentrate diet elicited similar depressive effects on cellulolytic bacteria in the cecum of a cannulated pony (Goodson et al., 1988). Drogoul et al. (2001) found that as the

dietary ratio of barley relative to hay increased, retention time within the equine digestive tract increased, but this was not associated with more efficient fiber utilization. This may be due to a decrease in cellulolytic bacteria, as is typical when dietary concentrates increase, such as barley and oats (de Fombelle et al., 2003; Drogoul et al., 2001; Kern et al., 1973). Altering the dietary ratio of barley to hay from 0:100 to 30:70 and, finally, to 50:50 caused numerical decreases in cellulolytic bacteria 29 h post-dietary change in cannulated ponies. Although these decreases were not significant, researchers concluded that fibrolytic activity was most likely affected as a change in the VFA profile was reported with a decrease in the molar percentage of acetate and an increase in the molar percentage of propionate (de Fombelle et al., 2001). In the equine hindgut, a decrease in the (acetate + butyrate)/propionate ratio indicates lesser fibrolytic activity, which coincided with the decrease in the number and concentration of cellulolytic bacteria observed (de Fombelle et al., 2001; de Fombelle et al., 2001; Garner, 1978). However, individual VFA concentrations were not reported, which would be more telling than using percentages alone as to whether there was a decrease in the concentration of acetate or rather a much greater increase in the concentration of propionate, causing the molar percentage of acetate to decrease in comparison.

Three cellulolytic bacterial species dominate the rumen: *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* (Julliand et al., 1999). Due to similarities between the rumen and equine hindgut, these species of fibrolytic bacteria are hypothesized to play an important role in the fermentation of structural carbohydrates into VFA, particularly acetate, in the hindgut of healthy horses (Julliand et al., 1999). However, because both *Ruminococcus* and *Fibrobacter* are "obligate fibrolytic, predominantly acid-intolerant bacteria whose growth is greatly suppressed at acidic pH," the role they play during the fermentation of carbohydrates in the equine is altered if the hindgut becomes too acidic following a carbohydrate overload (Daly et al., 2012). Utilization of oligonucleotide 16S rRNA probes confirmed that *R. flavefaciens*, *R. albus*, and *F. succinogenes* are present in the cecum, although in different proportions than in the rumen as *R. albus* was at the minimum detection limit (Julliand et al., 1999). In addition to significant quantities of *Ruminococcacaea*, the cellulolytic bacterial genera *Clostridium*, *Trepenoma*, and *Eubacteria* have been detected in the hindgut of both healthy and chronically laminitic ponies (Steelman et al., 2012).

Four strains of cellulolytic bacteria morphologically resembling ruminal *R. flavefaciens* but differing in fermentation products, as they produce primarily acetate, formate, and ethanol, have also been isolated from the cecum of cannulated donkeys and ponies (Julliand et al., 1999). The rRNA of these strains hybridized to a probe specific to *R. flavefaciens*, indicating genetic similarity to *R. flavefaciens* strains FD1 and 007, which have been identified in the rumen (Julliand et al., 1999). Strains of *R. flavefaciens* constituted the bulk of cellulolytic bacteria in the hindgut of healthy donkeys and ponies, comprising up to 9% of total rRNA, and contributed to the large proportion of acetate generated when forage is consumed (Julliand et al., 1999; Mackie and Wilkins, 1988). Hastie et al. (2008) utilized real-time PCR and agreed that *R. flavefaciens* is the predominate cellulolytic bacteria in the cecum. However, they reported *R. flavefaciens* comprises only 5.8% of total cecal bacteria.

Despite variations in the reported numbers of total *R. flavefaciens*, it appears to be more abundant than *F. succinogenes* in the equine cecum. Julliand et al. (1999) reported *F. succinogenes* to range from 0.21 to 4.3% of total bacterial RNA in ponies and donkeys, while Hastie et al. (2008) reported *F. succinogenes* to vary from 3.38 to 6.43% of total bacterial DNA in horses, depending on whether the cecal samples were frozen or lyophilized. Lin and Stahl (1995) used 16S rRNA targeted oligonucleotide probes and reported 12% of total rRNA extracted from the cecum of a pony was *F. succinogenes*. In contrast, Daly et al. (2001) analyzed PCR-amplified 16S rRNA gene sequences from the hindgut of grass fed horses and detected no traces of *F. succinogenes*. However, the authors reported that PCR amplification of *F. succinogenes* DNA may be less efficient than amplification of other bacterial DNA and potentially accounts for their lacking of findings. Differences in levels of cellulolytic bacteria reported could be due to species differences, method of analyses, and dietary variation, as *F. succinogenes*, unlike other cellulolytics, thrives on low-quality roughage (Daly et al., 2001; Hastie et al., 2008).

Meanwhile, the colon appears to contain a greater population of *Fibrobacter* (0.4 to 2.7%) than *Ruminococcaceae* (0.3 to 1.4%; Daly et al., 2012; Lin and Stahl, 1995). Daly et al. (2012) utilized oligonucleotide-RNA hybridization technology and noted that *Ruminococcaceae* comprised 1.4% of the total microbial population in the colon of horses maintained on grass compared to 0.3% in horses fed an all-concentrate diet. The *Fibrobacter* group constituted 0.4% of the total microbial population in concentrate-fed horses when compared to 2.7% in horses fed

perennial rye grass (Daly et al., 2012). Lin and Stahl (1995) reported *Fibrobacter*, primarily *F. succinogenes*, represented 4% of colonic rRNA in a pony maintained on alfalfa hay. Lin and Stahl (1995) analyzed contents from the colon of 1 female pony while Daly et al. (2012) collected colonic contents from 6 horses. Variability from horse to horse, differing species, as well as dietary differences, may explain differences noted in the microbial populations. Concurrent with the decrease in *Fibrobacter* and *Ruminococcaceae* noted when dietary concentrate is introduced to horses is an increase in Lachnospiraceae (cluster XIVa of the Clostridiceae), *Bacteroidetes*, and lactic-acid producing bacteria (Daly et al., 2012).

1.3.4 Lactate-Producing Bacteria

It is generally accepted that when horses are fed novel sources of NSC, or levels that exceed the digestive capacity of the foregut (3.4 g starch/kg BW; Potter et al., 1992), those carbohydrates significantly impact the cecum and colon (Julliand, 2005; Milinovich et al., 2007). When NSC escape digestion in the small intestine and reach the hindgut, they are rapidly fermented, causing a shift in the bacterial population of the cecum and colon from that of predominantly Gram-negative bacteria, the classification to which at least 2 important lactate-utilizing bacteria belong, to primarily Gram-positive bacteria, many of which produce lactate (Julliand, 2005; Milinovich et al., 2007). While the sheer numbers of lactate-utilizers and lactate-producers both increase in response to a meal containing an increased quantity of NSC, the increase in lactate-utilizers is not sufficient to counteract the effects of increased lactate-producers (Daly et al., 2012; Mackie and Gilchrist, 1979; Medina et al., 2002).

Following an abrupt dietary change in a cannulated pony from chopped alfalfa to a ground corn and soybean meal, starch-utilizers comprised 85.2% of total cecal bacteria compared to 73.1% during hay feeding (Goodson et al., 1988). Similarly, rapidly increasing barley in the diet from 0% to 30% and 50% with the balance of the diet consisting of timothy hay, resulted in increases in concentrations of *Streptococci* in the cecum of cannulated ponies 5 h following feeding. While there were no changes observed in the concentration of *Lactobacilli* in the cecum when barley comprised 30% of the diet, 50% barley inclusion led to rapid increases of both *Lactobacilli* and *Streptococci* in the cecum 5 h following the dietary change. When the diet was maintained, a slight decrease was observed in cecal *Lactobacilli* from 5 to 29 h following the dietary change (de Fombelle et al., 2001). Alterations in the cecal microbial profile result not

only from abrupt dietary changes, but differences are also apparent between horses acclimated to a diet containing a large quantity of starch and horses fed only forage. Medina et al. (2002) acclimated horses to a morning meal for 21 d that contained 3.35 g starch·kg BW^{-1}·$meal^{-1}$ and observed increased concentrations of *Lactobacilli* (5 x 10^7 CFU/ml) and *Streptococci* (3.2 x 10^7 CFU/ml) in the cecum 4 h following the meal as compared to concentrations of *Lactobacilli* (2.5 x 10^6 CFU/ml) and *Streptococci* (4 x 10^6 CFU/ml) observed in the group fed a diet containing a large quantity of forage. Both abrupt alterations in dietary starch content, as well as acclimation to a diet containing a large amount of starch, result in an increase of lactate-producing bacteria in the cecum compared to those in the cecum of horses fed forage.

While cecal concentrations of *Lactobacilli* and *Streptococci* increased 5 h following introduction to a 50% barley meal, colonic concentrations of both *Lactobacilli* and *Streptococci* decreased 5 h following inclusion of 50% barley (de Fombelle et al., 2001). Concentrations of *Lactobacilli* and *Streptococci* rapidly increased in the colon 29 h following the dietary change, compared to cecal, pre-prandial, and 5 h values (de Fombelle et al., 2001). De Fombelle et al. (2001) proposed that the effects of abruptly increasing dietary barley are most apparent in the colon due to the rapid passage of barley through the cecum. However, Respondek et al. (2008) analyzed the effects of an abrupt dietary change from a pelleted feed (0.08% BW starch in each of 3 pelleted meals/d) with wheat straw fed at a ratio of 70:30, before switching to a barley meal containing starch at 0.28% BW on the bacterial population of the equine colon. An increase was observed in *Lactobacilli* (3.2 x 10^5 CFU/ml at -19 h, 1 x 10^6 at +5 h, 5 x 10^6 at +29 h) and *Streptococci* (5 x 10^5 CFU/ml at -19 h, 1 x 10^6 at +5 h, 2.5 x 10^6 at +29 h) as time following the meal was compared to pre-prandial values (Respondek et al., 2008). In a study conducted by Medina et al. (2002), *Lactobacilli* was the only genera observed to increase (7.9 x 10^6 CFU/ml to 3.98 x 10^7) in the colon of horses acclimated to a meal containing 3.35 g starch·kg BW^{-1}·$meal^{-1}$ in each of 2 meals/d in comparison to horses receiving a diet containing primarily fibrous feedstuffs. Discrepancies between the studies may be explained by the differences in acclimation diets. Medina et al. (2002) had acclimated horses to starch and balanced it with an equal amount of NDF, Respondek et al. (2008) had acclimated horses to a pelleted feed containing 0.08% starch, while de Fombelle et al. (2001) fed only hay 2 wks prior to abruptly changing the diet.

1.3.5 Lactate-Utilizing Bacteria

Lactate-utilizing bacteria convert lactate to VFA under normal conditions in the equine cecum (Ushida et al., 2002), typically leaving little trace of lactate and large concentrations of VFAs (Kern et al., 1974). Differences among lactate-utilizers are apparent, as the pathways utilized, substrates, and pH influence the conversion of lactate to end products of fermentation (Counotte et al., 1981; Ushida et al., 2002). *Megasphera elsdenii* is the only known lactate-utilizing bacteria in the gastrointestinal tract to selectively ferment lactate to propionate via the acrylate pathway. Other lactate-utilizing bacteria utilize the succinate pathway. It is unique among lactate-utilizers in the sense that it is uninhibited by monosaccharides, which suppress activity of many lactate-utilizing bacteria, and it ferments 60 to 80% of lactate in the rumen into VFA (Counette et al., 1981; Ushida et al., 2002). In addition to converting lactate to propionate, *M. elsdenii* converts lactate to butyrate. Butyrate may improve hindgut health and function, and it is produced more readily at a depressed pH (Counotte et al., 1981; Ushida et al., 2002). However, the formation of butyrate from lactate requires the presence of acetate. Conversion of lactate to acetate is less efficient than the conversion of lactate to propionate due to the large energy input required and the requirement for hydrogen acceptors (Ushida et al., 2002). Hence, propionate is generally produced in greater quantities by *M. elsdenii* than acetate.

Researchers have observed increased concentrations of cecal lactate-utilizing bacteria 4 h following a large concentrate meal in comparison to horses receiving a primarily forage diet (1 x 10^7 CFU/ml to 5 x 10^7 CFU/ml; Medina et al., 2002). Goodson et al. (1988) supported this observation, noting that an average of 26.1% of the total bacterial population consisted of lactate-utilizers when horses were maintained on a forage diet before increasing to 69.2% lactate-utilizers 7 d after concentrate was first introduced. Conversely, De Fombelle et al. (2001) observed lactate-utilizing bacteria to remain relatively stable in horses following an abrupt incorporation of 30% and 50% rolled barley into a diet previously composed solely of meadow hay. There were large standards errors for the microbial analyses performed by de Fombelle et al. (2001), leading to few statistically significant findings.

1.3.6 Lactic Acid Bacteria in Various Species

Lactate-utilizing bacterial concentrations vary with diet, but there are similarities in bacteria found across species of livestock (Ghali et al., 2011; Goad et al., 1998; Medina et al.,

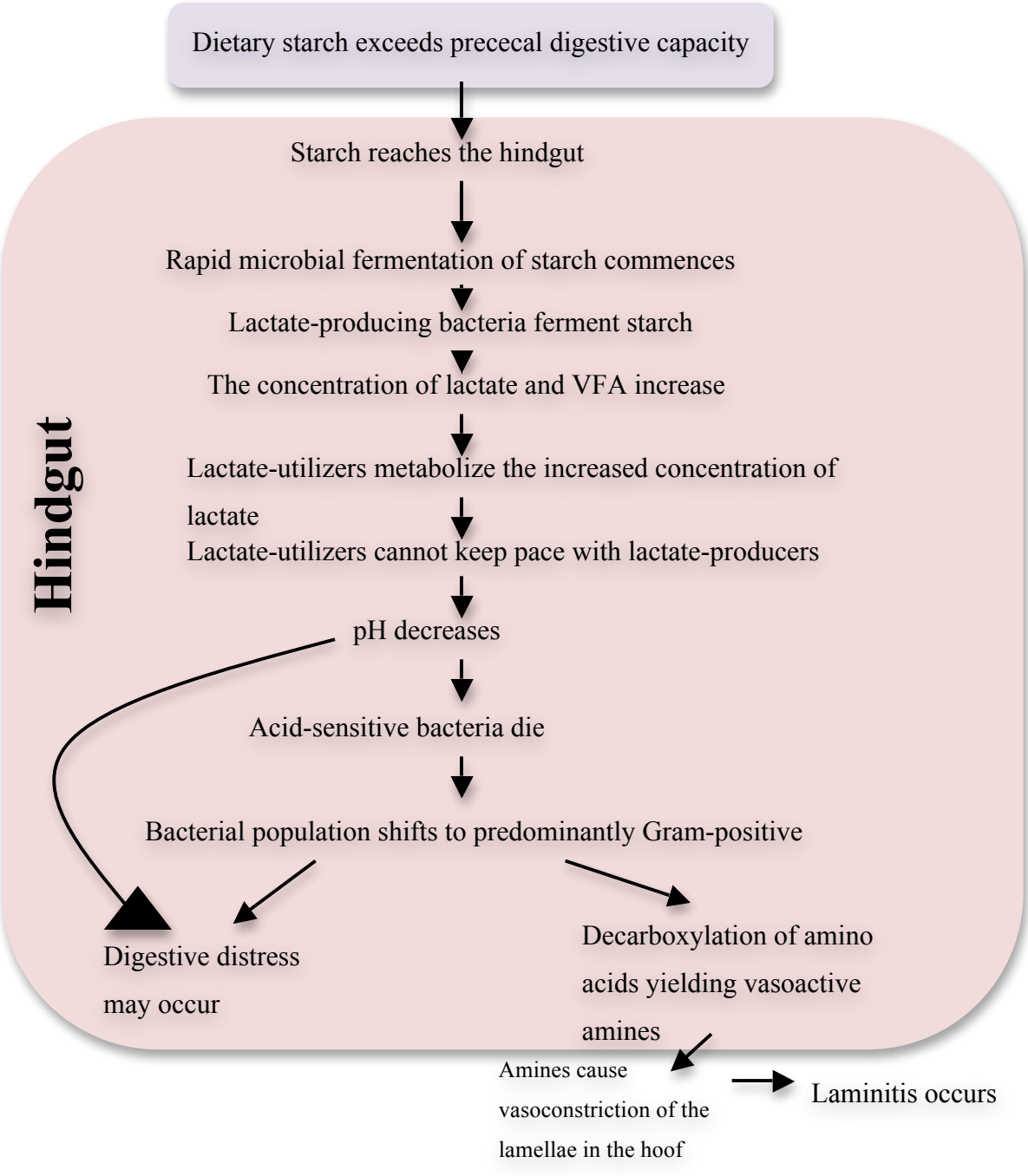
2002). A trial involving subacute acidosis in cattle revealed that grain-adapted steers housed greater concentrations of lactate-utilizing bacteria than did the rumen of hay-adapted steers (Goad et al., 1998). Ghali et al. (2011) examined the foregut of camels, which is similar to that of ruminants, for lactic acid bacteria. Researchers observed similar L-lactate-producing bacteria in the camel, such as those related to *S. bovis*, *Selenomonas ruminantium*, and *L. garvieae*, as have been reported in the horse and ruminant (Daly et al., 2001; Ghali et al., 2011). D-lactate producing bacteria in the camel were closely related to *L. pectinoschiza* (M60) and *S. ruminantium* (Ghali et al., 2011). Two relatives of *S. ruminantium* both produced (one L-lactate, the other both D- and L-lactate) and utilized lactate before converting it to propionate (Ghali et al., 2011). Mackie and Gilchrist (1979) also observed strains of lactate-utilizing bacteria in sheep that underwent step-wise adaptation to a 71% grain diet. Lactate-utilizing bacteria observed included *Selenomonas*, *Veillonella*, *Anaerovibrio*, *Megasphaera*, and *Propionibacterium*. Concentrations of these bacteria varied and shifts in the populations were observed when increasing concentrate was fed and, probably as a result, ruminal pH was reduced. When the diet contained greater than 24% grain and molasses, *Veillonella* was no longer present in the samples. Grain and molasses concentrations above 48% resulted in disappearance of *Selenomonas*. The loss of these 2 acid-sensitive bacteria was followed by increases in the more acid-tolerant bacteria: *Megasphera* (d 40), *Anaerovibrio* (d 54), and *Propionibacterium* (d 54). It took 21 d for lactate-utilizers to be roughly equivalent in concentration to lactate-producing bacteria (Mackie and Gilchrist, 1979), which supports the conventional wisdom of providing an acclimation period for any dietary alterations that involve an increase in dietary concentrates.

1.3.7 Etiology of Laminitis

Laminitis is a debilitating disease affecting the hoof of equids that may be due to, among other reasons, overconsumption of starch or oligofructose (Fig. 1.1; Katz and Bailey, 2012). The disease is characterized by lameness, typically in the front hooves although the back hooves may be affected, due to inflammation and, in some cases, separation of the laminae in the hoof (Katz and Bailey, 2012). A classification system is in place to categorize laminitis as developmental, acute, subacute, refractory, and chronic (Baxter, 1986; Hood et al., 1993; Katz and Bailey, 2012). Severity of lameness is classified using the Obel grading system, which consists of a scale ranging from 1 to 4 (least to most severe, respectively; Obel, 1948). Depending on the type of

laminitis and severity of the case, euthanasia may be performed to alleviate the suffering of the animal (Baxter, 1986). Reviewers of the epidemiology of laminitis have suggested between 1.5 and 34% of the equine population is affected by laminitis (Wylie et al., 2011). A better understanding the underlying mechanisms behind the etiology of laminitis may hold the key to decreasing the morbidity of this disease.

Figure 1.1. Flow Chart Illustrating Current Theory Regarding How a Carbohydrate Overload in the Horse Leads to Laminitis



1.3.7.1 Microbial shifts associated with laminitis

Both oligofructose- and cornstarch-induced laminitis models result in the same clinical signs of laminitis, as well as a shift from a predominantly Gram-negative bacterial population to a predominantly Gram-positive population of bacteria in the hindgut (Onishi et al., 2012). *Streptococcus* and *Lactobacillus* are Gram-positive bacterial genera associated with production of lactate in both models of laminitis (Garner et al., 1977; Onishi et al., 2012). *Streptococcus bovis* and *S. equinus* produce only the L-lactate isomer and appear to be the most abundant lactate-producing bacteria in the equine hindgut (Al Jassim and Rowe, 1999; Al Jassim et al., 2005). *Lactobacillus salivarius* and *L. mucosae* are the predominant members of the *Lactobacillus* genus native to the equine and were detected in the hindgut following oligofructose-induced laminitis (Al Jassim et al., 2005). Of 25 bacterial species isolated along the entire gastrointestinal tract, 3 isolates related to *L. salivarius* produced the greatest levels of L-lactate, averaging 21.67 ± 1.86 mM (Al Jassim et al., 2005). Both *L. salivarius* and *L. mucosae* have been implicated in lactic acid accumulation, as well as decarboxylation of amino acids, both of which have been associated with laminitis (Al Jassim et al., 2005; Bailey et al., 2003).

Relatives of *Mitsuokella jalaludinii* are also lactate-producers, and they produced the greatest levels of D-lactate following oligofructose administration to horses (23.13 ± 2.0 mM; Al Jassim et al., 2005). D-lactate levels in the blood of oligofructose-induced laminitic horses peaked between 20 and 24 h post-oligofructose administration (POA) at a concentration of 2.815 mM, presumably as a result of rapid proliferation of D-lactate producing bacteria in the hindgut (Al Jassim et al., 2005). Identification of *Mitsuokella* as a major producer of D-lactate is important in the control of acidosis because it cannot be treated like either *Streptococci* or *Lactobacilli* due to its Gram-negative nature (Al Jassim et al., 2005).

Onishi et al. (2012) utilized cornstarch-wood flour gruel and oligofructose laminitis induction models to examine the resulting alterations in both Gram-negative and Gram-positive bacterial populations in the cecum of horses. Following establishment of Obel grade 2 lameness, researchers cultured cecal fluid obtained from said horses and observed an increase of 2 to 3 orders of magnitude in Gram-positive bacteria on media selective for *Lactobacilli*, *Streptococci*, and other Gram-positive cocci (Onishi et al., 2012). The mean time for horses to reach Obel

grade 2 lameness was 29 h following administration of the cornstarch-wood flour gruel and 26 h POA (Onishi et al., 2012). Garner et al. (1978) recorded decreased numbers of cecal *Streptococci* from 0 to 8 h post-carbohydrate overload using cornstarch-wood flour gruel, with populations returning to control levels by 24 h. Concurrently, cecal *Lactobacilli* counts increased from 0 to 8 h post-carbohydrate overload, tended to continue to increase until 24 h, and Obel grade 3 lameness was observed by 32 h (Garner et al. 1978). These results indicate that *Lactobacillus* outcompetes *Streptococcus* as the primary starch-utilizing organism under such conditions and the population has increased by the time Obel grade 2 lameness is observed.

Streptococcus is the predominant oligofructose-utilizing organism (OUO) POA in the cecum of cannulated horses, which establishes itself as early as 2 h POA and increases from 8 to 24 h compared to values prior to oligofructose administration (Milinovich et al., 2007). While particulates would not typically reach the hindgut 2 h following administration (Argenzio et al., 1974), it seems likely that liquid oligofructose flows with the liquid phase digesta to cause the increased proportions of *Streptococci* 2 h POA noted by Milinovich et al. (2007; Argenzio et al., 1974; Argenzio, 1975; Drougol et al., 2001). However, the other predominate lactate-producing bacteria in the equine hindgut, *Lactobacilli*, did not establish a significant population until 32 h POA in the same study (Milinovich et al., 2007). When all of these results are considered together, it seems that *Streptococci* is out-competed by *Lactobacilli* following a starch overload, and the opposite is true following an oligofructose overload (Garner et al., 1978; Milinovich et al., 2007).

When fecal samples were utilized, numbers of *Streptococci* followed a similar pattern as had been observed in cecal specimens of cannulated horses, increasing from 8 to 16 h POA, while cultivatable OUO counts declined, likely due to a decrease in fecal pH (Milinovich et al., 2006; Milinovich et al., 2007). *Streptococci luteinsis*, a member of the equine hindgut streptococcal species which had previously been classified within the *S. bovis/S. equinus* complex, proliferated rapidly before declining sharply immediately prior to diagnosis of oligofructose-induced laminitis. Thus, *S. luteinsis* may play an integral role in the laminitic process (Milinovich et al., 2006; Schlegel et al., 2003).

Gram-negative bacteria contain lipopolysaccharide in their cell walls, which, when released following cell death, acts as a toxin and causes widespread inflammation in the equine (Hood et al., 1993). Researchers previously postulated that the gram-negative genus,

Enterobacteriaceae, is acid-sensitive and dies when subjected to an acidic environment (Garner et al., 1978). Lipopolysaccharide residue (endotoxin) is subsequently released from the cell wall of gram-negative bacteria and has been proposed to cause laminitis (Garner et al., 1978). However, *Enterobacteriaceae* has since been shown to increase numerically in cecal fluid cultured following starch- and fructan-induced laminitis (Onishi et al., 2012). Researchers using fluorescence in-situ hybridization (FISH) confirmed an increase in cecal *Enterobacteriaceae* following oligofructose-induced laminitis (Milinovich et al., 2007). Conversely, Garner et al. (1978) observed a continued decrease in *Enterobacteriaceae* ($P < 0.10$) from 8 to 24 h following a carbohydrate overload. While Garner et al. (1978) used cecally cannulated horses, Onishi et al. (2012) did not, potentially contributing to the 7-log difference in their enumerations of this bacterial species. Both potentially pathogenic bacteria within the *Enterobacteriaceae* family (*Escherichia coli* and *Salmonella*) possess lactate dehydrogenase (Garvie, 1980; Onishi et al., 2012), which may play a part in resistance to, and subsequent survivability in an acidic environment. Therefore, *Enterobacteriaceae* likely survives the harsh hindgut conditions following carbohydrate overload and thus its cellular death is not the causative factor in the onset of laminitis (Asanuma and Hino, 1997; Garvie, 1980; Onishi et al., 2012; Rowbury, 2001).

Bailey et al. (2002; 2003) proposed an alternative pathogenesis to laminitis. They noted that in vitro fermentation of carbohydrates in cecal contents yields amines. Amines have the potential to be vasoactive, leading these researchers to speculate that amines may be absorbed into the bloodstream following a carbohydrate overload, triggering the release of compounds that cause separation of the hoof from the lamellae. Both *S. bovis* and members of *Lactobacilli* have been demonstrated to produce 1 or more vasoactive amines (Bailey et al., 2003). The proliferation of *Streptococci* and *Lactobacilli* following carbohydrate overload (Garner et al., 1975; Onishi et al., 2012) lends credence to Bailey et al. (2003) theory.

1.3.8 Biochemical Parameters in the Hindgut in Response to Starch

Lactate-producing bacteria rapidly ferment NSC, leading to production of propionate and lactic acid, an intermediate product of fermentation (Mackie and Gilchrist, 1979). An imbalance between lactate-producers and lactate-utilizers often follows a carbohydrate overload and results in the accumulation of VFA and intermediate fermentation products in the hindgut. The pK_a of lactic acid is 3.86 whereas the pK_a of acetic acid, butyric acid, and propionic acid is 4.76, 4.82,

and 4.87, respectively. Thus, the accumulation of lactate creates an acidic environment which favors further proliferation of lactate-producing bacteria (Bergman, 1990; Mackie et al., 1979).

1.3.9 Concentration of Lactate

Concentration of lactate in the cecum and colon of horses previously maintained on hay increased as soon as 5 h following 30% and 50% dietary inclusion of barley (de Fombelle et al., 2001). Large intestinal samples from horses consuming 30% barley revealed an increase in concentration of lactate from 35.87 mg lactate/L of intestinal fluid at -24 h to 305.14 mg lactate/L of intestinal fluid at 29 h relative to the dietary change (de Fombelle et al., 2001). After increasing barley to 50% of the diet, lactate values increased from 70.13 mg lactate/L of intestinal fluid at -24 h to 280.34 mg lactate/L of intestinal fluid at 29 h post-dietary change (de Fombelle et al., 2001). Although there were numerical differences in lactate between the 30% and 50% barley treatment groups, there were large standard errors for the concentration of lactate in both dietary treatments, which made distinguishing differences between the concentration of lactate based on the amount of barley difficult (de Fombelle et al., 2001). The amount of barley supplied did not exceed the reported limit of pre-cecal digestion of starch (3.4 g starch·kg BW^{-1}·$meal^{-1}$; Potter et al., 1992), and the values for lactate are similar to those obtained in horses receiving 1.26 g starch·kg BW^{-1}·$meal^{-1}$ in a study by Medina et al. (2002). Horses fed a diet containing 1.26 g starch·kg BW^{-1}·$meal^{-1}$ for 21 d prior to a 12 h sampling period exhibited less variation in the concentration of lactate in the cecum and colon than horses fed 3.35 g starch (balanced equally with NDF)·kg BW^{-1}·$meal^{-1}$ in each of 2 meals/d for 21 d prior to a 12 h sampling period (Medina et al., 2002). A lesser concentration of lactate was observed in the cecum (167.9 mg lactate/L cecal fluid) and colon (116.5 mg lactate/L colonic content) of horses receiving 1.26 g starch·kg BW^{-1}·$meal^{-1}$ during a 12 h period compared to horses receiving 3.35 g starch (balanced equally with NDF)·kg BW^{-1}·$meal^{-1}$ (Medina et al., 2002). Lactic acid in the cecum of horses acclimated to 3.35 g starch (balanced equally with NDF)·kg BW^{-1}·$meal^{-1}$ in each of 2 meals/d reached a 407.7 mg lactate/L of cecal fluid over a 12 h period while the concentration of lactate in the colon of those horses was 303.2 mg lactate/L of colonic contents (Medina et al., 2002).

Horses fed a pelleted meal containing 286 g starch/100 kg BW had a greater concentration of D- and L-lactate in the small colon than horses fed pellets containing a lesser

amount of starch (0.74 g/kg BW; de Fombelle et al., 2003). Regardless of dietary treatment, there was an increased ratio of D-lactate:L-lactate in the hindgut of horses when compared to the foregut (de Fombelle et al., 2003). In accordance with findings by de Fombelle et al. (2003), Respondek et al. (2008) reported that colonic concentrations of D-lactate, but not L-lactate, increased 8-fold 5 h following inclusion of 0.28% BW starch in the form of barley, and D-lactate remained elevated until 29 h post-dietary starch increase (Respondek et al., 2008).

1.3.8.2 Volatile Fatty Acids

Volatile fatty acids consist of a short chain of 1 to 7 carbon atoms in either a straight or branched-chain (Bergman, 1990). Typically, VFA are present in the anionic state due to the nearly neutral pH of the gastrointestinal tract (Bergman, 1990). Although there are several VFA, of main concern are those comprising the majority: acetate, propionate, and butyrate (Bergman, 1990; Medina et al., 2002; Respondek et al., 2008). Formation of VFA occurs as a result of microbial fermentation of carbohydrates that reach the hindgut (Argenzio, 1974; Bergman, 1990; Shirazi-Beechey, 2008). Volatile fatty acids undergo glycolysis via the Embden-Meyerhoff pathway to result in pyruvate formation. Pyruvate is rapidly and primarily converted into acetic, propionic, and butyric acid, with little lactate formation under normal conditions in the hindgut. Acetate and butyrate formation is interconvertible and occurs via a separate pathway from the acrylate and succinate pathways utilized to form propionate (Bergman, 1990). The majority of butyrate is metabolized in the colonic epithelium and is its primary source of energy. Metabolism of propionate predominantly occurs in the liver where it is utilized for gluconeogenesis, and acetate is metabolized in peripheral tissues (Bergman, 1990). Energy yielded in the ruminant from the fermentation of glucose and subsequent metabolism of VFA varies, with 36 ATP yielded from 1 molecule of glucose that was converted to propionate prior to metabolism, 27 ATP yielded from 1 molecule of glucose that was converted to butyrate prior to metabolism, and 20 ATP yielded from 1 molecule of glucose that was converted to acetate prior to metabolism. Acetate and butyrate share a common pathway, and formation of acetate from butyrate is favorable for the animal as it results in a net gain of ATP, which can be used directly as a source of energy by peripheral tissues (Bergman, 1990).

Volatile fatty acids are typically present in the hindgut at a concentration of 100 mM (Rechkemmer et al., 1988). Production of VFA or other monocarboxylates is dependent on the interrelated composition of the microbial population and the diet (Daly et al., 2001). Mackie and

Wilkins (1988) cited a molar proportion of acetate:propionate:butyrate of 85:10:3 in the hindgut of horses on a natural, unimproved grass diet to be indicative of fibrolytic fermentation. However, inclusion of dietary concentrates at levels beyond small intestinal digestive and absorptive capacity leads to increased escape of concentrates to the hindgut. Shifts in the hindgut's microbial population following a carbohydrate overload, particularly a decrease in cellulolytic bacteria and concurrent increase in amylolytic bacteria, causes the VFA profile and other biochemical parameters to change (Berg et al., 2005; de Fombelle et al., 2003). Diets containing greater concentrations of starch and fructans tend to lead to increased concentrations of VFAs in the hindgut (Berg et al., 2005; de Fombelle et al., 2003). Although, in the event that the diet is not delivering starch beyond the digestive capacity of the small intestine or that the starch is balanced with NDF, an increase in VFA concentration may not be observed with the addition of dietary concentrates (de Fombelle et al., 2001; Medina et al., 2002). In these cases, as well as those that provide a greater amount of starch, a shift in the VFA profile is often observed, even if VFA concentration does not increase (de Fombelle et al., 2003; Respondek et al., 2008; Willard et al., 1977). The shift in VFA profile in response to an increase in dietary starch is typically characterized by a decrease in the concentration of acetate while the concentration of propionate tends to increase, causing a decrease in the acetate:propionate ratio in the hindgut (Medina et al., 2002; Respondek et al., 2008; Willard et al., 1977). Two of the more extreme shifts in the (acetate + butyrate)/propionate ratio that have been reported were decreases from 4.37 in the cecum of horses fed forage containing 1.26 g starch·kg BW^{-1}·$meal^{-1}$ to 3.06 in the cecum of horses fed 3.35 g starch (balanced equally with NDF)·kg BW^{-1}·$meal^{-1}$ and 4.25 in the colon of horses fed forage containing 1.26 g starch·kg BW^{-1}·$meal^{-1}$ to 2.68 in the colon of horses fed 3.35 g starch (balanced equally with NDF)·kg BW^{-1}·$meal^{-1}$ (Medina et al., 2002).

Acetate, propionate, and butyrate are absorbed in their protonated forms mainly through passive diffusion in the large intestine of the equine at rates similar to that of the rumen (Argenzio et al., 1974; Bergman, 1990). When VFA are in their anionic form, they may be transported across the apical membrane of human colonic epithelium by either a VFA anion/HCO_3^- exchanger, compete with Cl^- for a binding site at an anion exchanger, or be exchanged for a proton via a monocarboxylate transporter (Stein et al., 2000). Volatile fatty acids subsequently exit the basolateral membrane via monocarboxylate transporter 1 (MCT-1) while in the anionic form in the colon of humans and pigs (Ritzhaupt et al., 1998). Lactate is a

competitive inhibitor of MCT-1, as are acetate and propionate in the presence of butyrate (Daly et al., 2011; Ritzhaupt et al., 1998). Following a carbohydrate overload lactate is present at a much greater concentration in the hindgut than while horses are maintained on a fibrous diet (Medina et al., 2002). Increased concentrations of lactate cause decreased pH, which would lead to protonization of VFAs (Rechkemmer et al., 1988). One might assume that passive absorption would increase following VFA protonization, but, in fact, absorption of VFA is independent of pH (Rechkemmer et al., 1988). This is because the colonic epithelial surface in the guinea pig can maintain a pH microclimate near neutral pH despite alterations in luminal pH (Rechkemmer et al., 1986). If the equine is similar to guinea pigs, humans, and pigs passive absorption of VFA would decrease at a decreased luminal pH and competition with lactate for transport would increase. Thus, absorption of VFA into the epithelium following a carbohydrate overload would actually decrease (Ritzhaupt et al., 1998; Stein et al., 2000). Additionally, the absorption of butyrate, the primary energy source for colonic epithelial cells, decreases following a carbohydrate overload due to competition for MCT-1 (Ritzhaupt et al., 1998). Ultimately, decreased butyrate absorption may be cause for concern in regard to the health of colonic epithelium (Bergman, 1990).

1.3.8.3 pH

The pH of the hindgut is considered a reflection of the microbial profile, as well as an indicator of the presence of VFA and lactate (Goodson et al., 1988; Medina et al., 2002; Willard et al., 1977). Variation in hindgut pH is influenced by form and quantity of carbohydrate administered, which in turn affects production of VFA and lactate (Garner et al., 1978; Goodson et al., 1988; Medina et al., 2002). Minimum pH levels have been reported in the cecum and colon between 4 and 7 h following a carbohydrate challenge (Goodson et al., 1988; Medina et al., 2002; Willard et al., 1977). The pH of both sections of the large intestine have been reported to vary from neutral (pH 7.0) to slightly basic (pH 7.7) prior to dropping as low as 5.9 after a carbohydrate challenge (Goodson et al., 1988; Medina et al., 2002). Garner et al. (1978) observed a continual decline in cecal pH for 24 h following a laminitis-inducing carbohydrate overload, beginning at a pH of 7.18 ± 0.14 and ending at 4.14 ± 0.17. Concurrently, fecal pH decreased between h 8 and 16 following a carbohydrate overload from 6.8 to 4.3.

There has been greater diversity and greater concentrations of lactate-utilizing and amylolytic bacteria reported in the colon than the cecum following dietary changes, possibly due

to longer retention time in the colon (de Fombelle et al., 2001; Dougol et al., 2012; Drogoul et al., 2000; Muhonen et al., 2009). Researchers have observed that the feces and right dorsal colon contain a more diverse microbial population than the cecum, and feces have been reported to contain greater concentrations of lactate-utilizing and -producing bacteria than the right ventral colon (Dougol et al., 2012; Muller et al., 2008). Depressed pH is reflective of the greater density of lactate-producing bacteria in the feces, compared to the right ventral colon, but little difference in content of organic acids has been noted between the 2 compartments (Muller et al., 2008). De Fombelle et al. (2003) concur with Muller et al. (2008) that differences in pH exist between the upper and lower hindgut of horses fed either a fiber- or a starch-rich diet, and that decreased pH is inversely related to the concentration of lactate. Ultimately, fecal sampling may not be an accurate method to detect cecal disturbances (Dougol et al., 2012; Douthit et al., 2014; Schoster et al., 2013).

1.3.9 Acid Sensitivity or Tolerance

Shifts in the bacterial population following a carbohydrate overload occur largely due to differences in the ability of bacterial species to tolerate an acidic environment (Asanuma and Hino, 1996). Two groupings of acid-tolerant ruminal bacteria exist: those that are able to extrude protons at an acidic external pH, hence maintaining a neutral intracellular environment; and those that are able to change their fermentation pathway, but, in those cases, a reduction in intracellular pH occurs (Asanuma and Hino, 1996).

Miwa et al. (1997) determined that the cellulolytic bacteria *R. albus* and *F. succinogenes* contain less H^+-ATPase in their membranes than that of acid-tolerant *S. bovis* and *M. elsdenii* at a pH of 7.0, indicating these bacteria may have a limited capacity for proton extrusion. Accordingly, *R. albus* and *F. succinogenes* were less proficient in increasing H^+-ATPase activity in response to a decline in pH, while acid-tolerant bacteria increased H^+-ATPase activity (Miwa et al., 1997). Thus, the amount of H^+-ATPase present in the bacterial cell under neutral conditions and in response to an acidic environment may be a key factor in determining the microorganism's level of acid sensitivity or tolerance.

An in vitro study was conducted to compare the effect of pH on the growth of cellulolytic bacteria compared to *M. elsdenii*, a relatively acid-tolerant species (Miyazaki et al., 1992). Growth rates of *R. albus*, *R. flavefaciens*, and *F. succinogenes* were greatly depressed at low pH,

and each had maximum growth rates at pH 6.4, 6.4 to 6.8, and 6.8, respectively. Internal pH (pH_i) mimicked the extracellular decline linearly in each species, and there was a corresponding decrease in rate of growth. If cellubiose, the substrate for cellulolytic bacteria, was supplied, pH_i was maintained at approximately 6.0 despite decreases in extracellular pH (pH_e) to 5.6. The ability to maintain pH_i despite an extracellular decline may be a possible survival mechanism for bacterial species. Typically, maintenance of pH_i near neutrality is associated with tolerance to acidic pH_e (Miyazaki et al., 1992; Russell and Wilson, 1996). Maintaining a pH gradient across the cellular membrane prevents internal accumulation of anions, such as acetate, that may cause toxicity. Several acid-tolerant bacteria allow pH_i to decline with pH_e, but specialized enzymes are required to function at acidic pH_i (Miyazaki et al., 1992; Russell and Wilson, 1996). Lactate dehydrogenase is one such enzyme that functions at an acidic pH_i and is associated with acid-tolerance (Asanuma and Hino, 1996). Many lactate-producing bacteria possess lactate dehydrogenase, which is in accordance with their ability to thrive despite the harsh environment of the hindgut following a carbohydrate overload (Asanuma and Hino, 1996).

1.4 Yeast Additives

Yeast is a unicelluar fungus that has historically been used in the production of fermented foods, such as bread and alcohol (Rodrigues et al., 2005). Differing strains of yeast have differing capacities for respiration or fermentation, as well as differing substrate preferences, particularly in the presence of *Lactobacilli* (Gerekova et al., 2011; Rodrigues et al., 2005). Yeast and *Lactobacilli* can be symbiotic or inhibitory to one another, depending on substrates, metabolites, and pH during the fermentation of bread (Gerekova et al., 2011). Sugars are typically the primary carbon source for yeast, but yeast may also be involved in metabolism of amino acids, alcohols, and organic acids. Glucose ingested by yeast undergoes glycolysis to yield pyruvate. In anaerobic conditions, yeast has the capability to ferment pyruvate to acetate and ethanol (Rodrigues et al., 2005). The ability to compete with the microbial population naturally present in the gastrointestinal tract of animals for substrate may be of particular benefit to the horse following a carbohydrate overload. Additionally, consumption of yeast or yeast products provides supplemental protein and vitamins, particularly vitamin B_1 (Klose and Fevold, 1945; Parsons et al., 1945). The nutritional benefits of consuming yeast or its fermentative products

and the benefits of providing competition for lactate-producing bacteria have led researchers to use yeast and its derivatives as additives to livestock feed (Jouany et al., 2009).

Live yeast, yeast extract, yeast culture, and yeast fermentation products have been supplemented in horses and other species (Medina et al., 2002; Van Saun, 2008). *Saccharomyces cerevisie* and *Aspergillus oryzae* are the most commonly supplemented species of yeast in equine diets (Van Saun, 2008). The effects of supplementing live yeast have been more thoroughly studied than yeast extract, culture, or fermented products in horses and ruminants (NRC, 2007). The most notable effect of dietary yeast supplementation is the apparent ability to increase bacterial growth in the rumen (NRC, 2007). Research with horses has not yielded comparable evidence to support an increase in bacterial growth in the hindgut following dietary yeast supplementation (NRC, 2007). Instead, there are notable effects from yeast supplementation on digestibility and biochemical parameters in the equine (Jouany et al., 2008; Medina et al., 2002; Morgan et al., 2007).

Supplementing *S. cerevisiae* resulted in greater ADF digestibility for diets containing 1.2 g starch/kg BW or 3.1 g starch/kg BW (Jouany et al., 2008). When horses consuming 1.2 g starch/kg BW were fed the yeast supplement, an increased intake of DM, OM, and NDF also tended to occur (Jouany et al., 2008). Yearling horses fed a mixed diet with a yeast culture supplement had greater N, ADF, DM, NDF, hemicellulose, and cellulose digestibility as compared to pre-supplementation digestibility in the same horses (Glade and Sist, 1988). Morgan et al. (2007) reported improved digestibility of DM and NDF of poor quality forages in horses supplemented with *S. cerevisiae*, but they did not observe similar benefits when horses were fed better quality forage. Despite the findings by Morgan et al. (2007), some researchers have concluded that yeast supplementation improves overall fiber digestibility of hay and ultimately does so by stimulating activity of fibrolytic enzymes of microbial origin (Jouany et al., 2009).

Improvements in biochemical parameters have been observed as well. Medina et al. (2002) and Lattimer et al. (2007) reported an increase in the concentration of acetate in the cecum, colon, and feces (in vitro) of horses consuming a diet supplemented with *S. cerevisiae*. Lactate concentrations in the cecum and colon were reduced in horses fed a diet containing 3.4 g starch/kg BW supplemented with yeast compared to horses fed the same diet without yeast, and there was a corresponding increase in cecal pH (Medina et al., 2002). The increased concentration of acetate, decreased concentration of lactate, and increased pH are in accordance

with anaerobic metabolism of glucose to pyruvate and acetate by yeast. In both studies the yeast likely competed with lactate-producing bacteria for glucose. This would leave less glucose available for metabolism to lactate by lactate-producing bacteria, more metabolism of glucose to acetate, and ultimately a greater pH due to the increase in the weaker acid, acetate, relative to the stronger acid, lactate. Colonic pH did not increase similarly to cecal pH, which may be because yeast more readily colonizes the cecum than the colon (Jouany et al., 2009; Medina et al., 2002). The NRC for horses (2007) concludes that yeast supplementation may be beneficial, particularly when fed in conjunction with diets containing a large amount of starch.

There is a void in the literature regarding the effects of dietary supplementation of yeast fermentation products and products derived from the yeast cell wall, such as mannan oligosaccharides and β-glucans, in the equine (Gurbuz et al., 2010). One study analyzed the effects of supplementing mannan oligosaccharides with an equine diet containing 0.6% BW concentrate and 1.2% BW alfalfa hay on nutrient digestibilities, fecal pH, and fecal VFA concentrations. There were no differences between the group receiving the concentrate and alfalfa diet without mannan oligosaccharides and the group fed the same diet, but supplemented with mannan oligosaccharides (Gurbuz et al., 2010). Most research in this area has been conducted in swine and poultry utilizing yeast cell wall constituents (Biggs and Parsons, 2008; Shanmugasundaram et al., 2013; Sweeney et al., 2012). Researchers suggest that yeast cell wall products confer benefits, particularly in the reduction of potentially pathogenic bacteria. *Escherichia coli, Salmonella,* and *Clostridium* populations have been reduced in the cecum of chicks and broilers following the addition of yeast products to the diet (Biggs and Parsons, 2008; Shanmugasundaram et al., 2013). The addition of purified β-glucans to a piglet diet resulted in reduced counts of Enterobacteriaceae and fewer pro-inflammatory markers in the colon (Sweeney et al., 2012). Reduction of these potentially pathogenic bacteria in the cecum and colon, if also applicable in the horse, may simply provide health benefits to the equine, if not nutritional benefits.

1.5 Summary

In conclusion, abruptly increasing the level of starch in the equine diet or feeding levels of starch that exceed the digestive capacity of the foregut can culminate in alterations of the microbial population, and subsequently, biochemical parameters in the hindgut. Although

preventing a carbohydrate overload is ideal, it is not always possible. In the event of a carbohydrate overload, dietary prebiotics may prove to be useful in stimulating the growth of beneficial bacteria and ameliorating negative effects in the hindgut. Thus, the following experiments were conducted to determine whether the addition of a dietary prebiotic would decrease the severity of biochemical alterations in the cecum of horses following an abrupt dietary change.

Chapter 2 - The effect of abrupt dietary alterations with and without a proprietary supplement on biochemical parameters in the cecum of the equine

2.1 Abstract

Starch has been shown to bypass pre-cecal digestion when fed in excess of the equine foregut's capacity for digestion (3.4 g starch/kg BW), thus impacting the microbial population, and subsequently, biochemical parameters in the hindgut (de Fombelle et al., 2001; Potter et al., 1992). Deleterious alterations in the hindgut environment have been shown to result in colic and laminitis (Garner et al., 1977; Kaya et al., 2009). This study was designed to test the effects of a proprietary supplement on biochemical parameters in the cecum following an abrupt increase in dietary starch. Nine Quarter horses previously fitted with cecal cannulae were utilized for 3 consecutive 22-d experiments, each separated by 2 d of rest. During Exp. 1 and 2, horses were acclimated to the same baseline ration for 21 d, followed by a concentrate challenge on d 22. The acclimation ration consisted of a morning meal of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO; 1.07 g starch/kg BW) fed with 0.75% BW prairie grass hay and an evening meal of 0.75% BW prairie grass hay. Cecal fluid was obtained through cecal cannulae every 4 h following the morning meal on d 19 and continued through the morning of d 23. On d 22 of Exp. 1, horses were fed a morning meal consisting of 1.0% BW concentrate (2.12 g starch/kg BW; Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO). Cecal pH was decreased at h 12 following the concentrate challenge on d 22 compared to the same time point during the acclimation period ($P = 0.009$). On d 22 of Exp. 2, horses were fed a morning meal consisting of 1.25% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO; 2.67 g starch/kg BW). Cecal pH increased ($P = 0.02$) at h 4 and decreased ($P \leq 0.002$) at h 12 and 20 following the concentrate challenge on d 22 in comparison to cecal pH recorded at comparable time points during the acclimation period. The acclimation period of Exp. 3 was similar to that of Exp. 2, differing in the respect that horses were assigned to either a treatment (n = 5) or control (n = 4) group and were fed either a proprietary supplement or a placebo, respectively, in an alfalfa pellet medium that was top-dressed on the morning concentrate daily for the first 21 d (10 g pellets/45.5 kg BW). On d 22 both groups were provided

with a 1.25% BW concentrate meal (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO; 2.67 g starch/kg BW) without any proprietary supplement, placebo, or hay. Horses acclimated to the proprietary ingredient demonstrated increased ($P = 0.009$) cecal pH at h 4, a concurrent decrease ($P = 0.02$) in cecal lactate, an increased ($P \leq 0.006$) ratio of (acetate+butyrate)/propionate at h 8 and 16, and decreased ($P < 0.05$) concentrations of individual and total VFA in comparison to the control group at h 24 following the concentrate challenge.

2.2 Introduction

The equine is a non-ruminant herbivore that evolved to graze fibrous rangeland grasses that consist largely of structural carbohydrates (SC; Dyer et al., 2009). Due to the inability of intestinal enzymes to digest SC, the equine must rely on an endogenous microbial population located in its complex hindgut to utilize these feedstuffs (Costa and Weese, 2012; Daly et al., 2001). This microcosm contains a diverse array of microbes that degrade SC into volatile fatty acids (VFA) which are used as a primary energy source for the horse (Argenzio, 1974; Bergman, 1990; Julliand, 1999). Today, decreased pasture access or increased energy requirements necessitate the introduction of more energy dense non-structural carbohydrates (NSC) to the ration of many horses. Most NSC, particularly starch, are digested in the foregut; however, inclusion of starch beyond small intestinal capacity for its digestion (3.4 g/kg BW; Potter et al., 1992) leads to increased starch passage to the hindgut (Gray, 1992; Varloud et al., 2004).

If appreciable quantities of starch reach the hindgut, the native amylolytic microbial population rapidly ferments starch into lactate, acetate, propionate, and butyrate (Argenzio, 1974; Bergman, 1990). Fibrolytic bacteria, responsible for degradation of SC, and lactate-utilizing bacteria, which convert lactate to VFA, are often unable to keep pace with lactate-producing microbes (Counotte et al., 1981; Daly et al., 2012; Julliand, 2005; Medina et al., 2002; Ushida et al., 2002). Consequently, lactate accumulates in the hindgut and, due to its acidic nature, causes a decline in pH (Mackie et al., 1978; Willard et al., 1977). Compounding the acidifying effect of the accumulation of lactate is an increase in concentrations of total VFAs (Mackie et al., 1978; Bergman, 1990). The combined depressive effect of lactate and VFA on pH leads to suppressed growth of acid-sensitive microbes, such as the fibrolytic population and certain lactate-utilizing bacteria (de Fombelle et al., 2003; Goad, 1998; Russell, 1998). This

results in a shift from a predominantly fibrolytic Gram-negative population to a predominantly amylolytic Gram-positive population (de Fombelle et al., 2003; Respondek, 2008). Some researchers have suggested the die-off of Gram-negative bacteria, which results in the release of endotoxins, is part of the etiology of laminitis (Garner et al., 1978). Other researchers have suggested that the predominant amylolytic bacterial populations present following a carbohydrate overload, namely *Streptococci* and *Lactobacilli*, are responsible for the decarboxylation of amino acids in the hindgut, which yields vasoactive amines that ultimately travel through systemic circulation to act on the hoof to cause laminitis (Bailey et al., 2003).

The initial objective of these studies was to establish a feeding level of dietary starch that, when introduced abruptly into the equine ration, would be sufficient to decrease cecal pH without leading to laminitis. Following establishment of a protocol that would accomplish this objective, a proprietary dietary ingredient was tested to determine whether it would ameliorate effects of the previously established dietary challenge on cecal pH, lactate, and concentrations of VFA.

2.3 Materials and Methods

2.3.1 Objective

Three consecutive 22-d studies were performed, with each study separated by 2 d of rest. The objective of the first 2 studies was to determine the level of concentrate required to alter cecal pH dynamics when abruptly increased in the equine ration. After an appropriate concentrate level had been established to meet this objective in Exp. 2, Exp. 3 was designed to test whether a proprietary supplement would ameliorate cecal changes when the dietary conditions of Exp. 2 were replicated.

2.3.2 Animals and Housing

Animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Kansas State University prior to implementation.

Nine Quarter Horses, 4 mares and 5 geldings, previously fitted with permanent cecal cannulae (Beard et al., 2011) were utilized for these studies. Body weights ranged from 455 kg to 602 kg. Horses were housed individually in 3.66 m by 3.66 m stalls bedded with pine shavings, supplied a white salt block, and provided *ad libitum* access to water. During the first 18 d of each

experiment, horses were turned out together for exercise and socialization for 4 to 6 h/d into a dry lot with access to water .

2.3.3 Acclimation Rations

2.3.3.1 Experiment 1

All horses were fed an acclimation ration consisting of a textured sweet feed concentrate at 0.5% BW (Omolene 200; Purina Animal Nutrition, LLC, Gray Summit, MO; 1.07 g starch/kg BW; Table 2.1) at 0700 and 1.5% BW native prairie grass hay (Table 2.1.; hay 1) divided equally and fed at 0700 and 1930 for 21 d. During Exp. 1 the morning meal consisted of 1.105 g starch/kg BW while the evening meal contained 0.0314 g starch/kg BW which resulted in 1.1364 total g of starch/kg BW.

Table 2.1. Chemical analyses of feedstuffs utilized during Experiments 1, 2, and 3

Proximate Analyses, DM basis, %	Prairie Grass Hay 1[1]	Prairie Grass Hay 2	Concentrate[2]
DM	93.12	93.41	93.09
Ash	7.73	6.8	10.10
NDF	66.38	68.23	41.39
ADF	40.15	39.27	6.63
Starch	0.45	0.83	23.08
CP	4.42	4.35	18.31
Gross energy (Cal/g of DM)	4195	4233	4337

[1]Prairie grass hay 1 was fed at 1.5% BW/d during Exp. 1 while 0.75% BW/d was provided of both Prairie Grass Hay 1 and Prairie Grass Hay 2 (1.5% BW/d total) during Exp. 2 and 3 due to a shortage of hay.

[2]Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO fed at 0.5% BW during acclimation periods and 1.0% BW (Exp. 1) and 1.25% BW (Exp. 2 and 3).

2.3.3.2 Experiment 2

The acclimation ration during Exp. 2 was identical to that provided during Exp. 1, except that 2 batches of hay were utilized. Horses were fed a textured sweet feed concentrate at 0.5%

BW (Omolene 200; Purina Animal Nutrition, LLC, Gray Summit, MO; 1.07 g starch/kg BW; Table 2.1) at 0700 and 1.5% BW native prairie grass hay (Table 2.1) divided equally between meals at 0700 and 1930 (0.75% BW/meal) as well as hay batch (0.375% BW each hay at each meal). Inclusion of Prairie Grass Hay 2 during Exp. 2 resulted in 1.119 g starch/kg BW in the morning meal and 0.04468 g starch/kg BW in the evening meal. Consequently, the total daily starch was 1.164 g/kg BW.

2.3.3.3 Experiment 3

Horses were blocked by gender and BW in Exp. 3 and randomly assigned to receive either a placebo (control, CON; n = 4) or proprietary supplement (PS; n = 5) containing products of yeast fermentation. Alfalfa pellets were provided as the placebo and acted as a carrier for the proprietary supplement. Two geldings and 2 mares were allotted to CON, while 3 geldings and 2 mares were allotted to PS (Table 2.2). The feeding regimen throughout Exp. 3 differed from that of Exp. 1 and 2 only in that there was the addition of a proprietary supplement or placebo pellet (10 g pellets/45.5 kg BW) top dressed on the concentrate throughout the 21-d acclimation period.

Table 2.2. Assignment of horses to treatment groups receiving either a proprietary supplement or placebo pellet[1]

PS		CON	
Gender	BW (kg)	Gender	BW (kg)
Mare	602	Mare	531
Gelding	570	Gelding	582
Mare	475	Mare	499
Gelding	510	Gelding	526
Gelding	501		
Mean weight	531.6		534.5

[1]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

2.3.4 Concentrate Challenges

During each experiment, the amount of concentrate offered to each horse was increased on d 22. This large concentrate meal was termed "concentrate challenge," as it was designed to challenge the hindgut environment with an abrupt increase in dietary starch without inducing a colic or laminitic situation. Each animal was monitored for temperature, pulse, respiration, fecal consistency, and hoof capsule temperature for 24 h after the concentrate challenge of each experiment. Abnormalities were not observed.

2.3.4.1 Experiment 1

The concentrate challenge administered on d 22 of Exp. 1 consisted of doubling the amount of concentrate in the morning meal from 0.5% BW to 1.0% BW (2.12 g starch/kg BW). Hay was not fed on the morning of the challenge, but 0.5% BW hay (0.0209 g starch/kg BW) was provided at the evening feeding (1930). Twenty-four hours following the challenge and after the final collection of cecal fluid, 1% BW hay was fed and no additional concentrate was offered.

2.3.4.2 Experiment 2

As during Exp. 1, at 0700 on d 22 a concentrate challenge was administered, this time consisting of 1.25% BW concentrate (2.67 g starch/kg BW). Once again, hay was not provided with the morning meal, but horses were fed 0.5% BW hay (0.0298 g starch/kg BW) at h 12 and 1.0% BW hay 24 h following the challenge and after the final collection of cecal fluid.

2.3.4.3 Experiment 3

The same dietary protocol established in Exp. 2 was followed during Exp. 3. Control and PS pellets were not offered on the day of or following the concentrate challenge.

2.3.4.4 Rest Periods

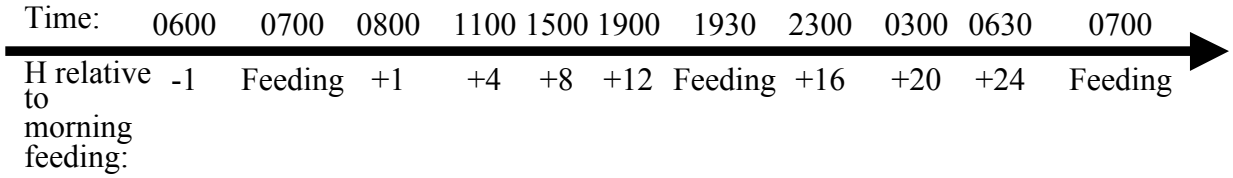
During the 2-d rest periods between each experiment 2% BW native prairie grass hay was provided and divided evenly between a morning and evening meal.

2.3.6 Experimental Sampling Period

Cecal fluid samples were collected during each experiment's acclimation period from d 19 to 21 and for 24 h following the concentrate challenge on d 22 of each experiment. Approximately 50 mL of cecal fluid was collected at h -1 (d 19 only), 1, 4, 8, 12, 16, 20, and 24

relative to the feeding of the morning meal but prior to the next morning meal (Fig. 2.1). After removing the plugs from the cannulae, cecal fluid was collected by gravity into 100-mL specimen cups. In Exp. 1, unfiltered cecal samples created challenges because they contained undigested feedstuffs that caused blockages in pipet tips. Consequently, for Exp. 2 and 3, cecal fluid was filtered through cheesecloth and into the specimen cup. Filtering cecal fluid enabled quicker aliquotting, thus reducing the number of metabolic reactions that might have occurred prior to deproteinization. pH of cecal fluid was measured immediately after collection using a handheld pH meter (Thermo Scientific Orion 3 Star Portable pH Meter, Waltham, MA; Accument probe). After pH was recorded, 10 aliquots of cecal fluid, measuring 1-mL each, were mixed with 0.25 mL meta-phospohoric acid in microcentrifuge tubes for deproteinization. These aliquots were frozen at -20°C until lactate analyses could be performed.

Figure 2.1. Experimental timeline outlining the daily schedule for the collection of cecal fluid and fecal material from d 19 to 22 of each experiment



2.3.7 Analyses of Lactate and VFA

Deproteinized samples of cecal fluid were thawed in duplicate and utilized for lactate analyses. A colorimetric assay (Baker and Summerson, 1941) was performed to measure concentrations of lactate in cecal samples obtained at all sampling time points during Exp. 3. Deproteinized samples of cecal fluid collected at h 4, 8, 12, 16, 20, and 24 relative to the morning meals on d 19 to 22 of Exp. 3 also were thawed and transferred to vials for VFA analysis. Cecal fluid was analyzed in duplicate to determine concentrations of VFAs by gas chromatography (model 5890, Hewlett-Packard, Avondale, PA) utilizing N_2 carrier gas (80 mL/min) and a flame-ionization detector. The column (6' x ¼", 4 mm ID glass, Supelco #1-1965; 10% SP-1200/1% H_3PO_4 packing) temperature was 130°C while the temperature of the detector was 250°C.

2.3.8 Statistical Analyses

The Mixed procedure (SAS version 9.3, Cary, NY) was used to determine whether cecal pH at h -1 and h 1 of all experiments were different. Following this analysis, h -1 was removed from the data set as h -1 samples were collected only on d 19 of each experiment; thus there were only 9 data points available at this time for each experiment. Secondary analysis of cecal pH at h 1, 4, 8, 12, 16, 20, and 24 following the morning meal was performed for each experiment. Cecal pH, cecal lactate (Exp. 3), and cecal VFA (Exp. 3) were analyzed utilizing the GLIMMIX procedure (SAS version 9.3, Cary, NC; some data were not normally distributed) to evaluate the effect of ration, sampling time point, treatment (Exp. 3), and their interactions on each response variable. Data were analyzed with and without gender during each experiment, as there had not been previous reports to indicate whether gender has an effect on the response variables analyzed. However, gender could not be included in the model for lactate analyses (Exp. 3) because the data would not converge after 1000 iterations. The Satterthwaite degrees of freedom method was performed for Exp. 1 and 3, while the containment degrees of freedom method was utilized to account for G-side random effects for Exp. 2. Least square means were calculated and the F-test was utilized to test the effect of each response variable with significance determined at $P < 0.05$.

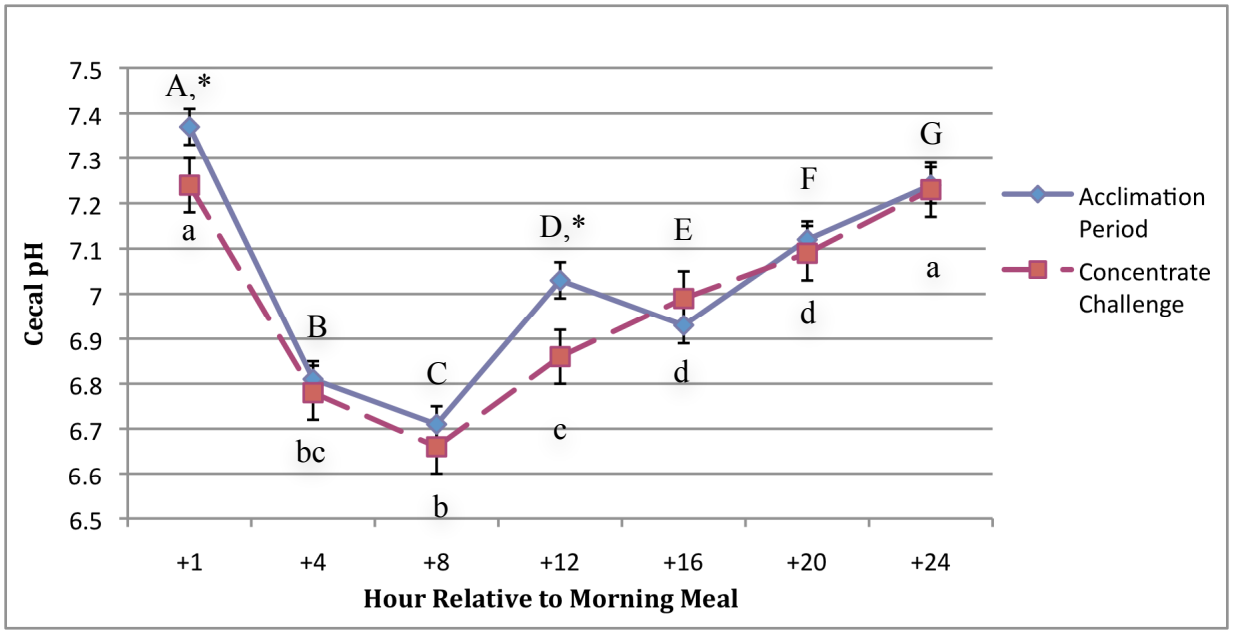
2.4 Results and Discussion

2.4.1 Experiment 1

Cecal pH values recorded across all days and time periods of Exp. 1 remained between 6.66 and 7.37 during both the acclimation and challenge periods (Fig. 2.2), which is well within the range of cecal pH values previously reported following a forage-only meal (Goodson et al., 1988). Cecal pH at h -1 on d 19 was 7.31, which was less than cecal pH at h 1 relative to the morning meal on the same day (7.41; $P = 0.006$). Despite this difference, cecal pH values at h 1 following the morning meals during both the acclimation and challenge periods (d 19 to 22) were similar to values reported by Medina et al. (2002) and Willard et al. (1977) in cannulated horses 1 h following a concentrate meal. Cecal pH at h 1 after the morning meals during the acclimation period (7.37 ± 0.04) was greater than the pH at h 1 after the concentrate challenge (7.24 ± 0.06; $P = 0.05$). Although intuitively one would not expect to observe effects as soon as 1 h following the morning meal, researchers have demonstrated that within 1.5 h after a meal a polyethylene

glycol marker can reach the cecum of ponies and concentration of VFA increases (Argenzio et al., 1974). Their experiment illustrated the rapid transit of liquid phase digesta to the cecum following a meal. Thus, the difference in cecal pH noted in the current experiment at h1, in comparison to pre-prandial values, may have been due to liquid phase digesta from the morning meal reaching the cecum.

Figure 2.2. Mean cecal pH[1] in horses (n = 9) from d 19 to 21 on an acclimation ration[2] and following a concentrate challenge[3] on d 22.



[1]Cecal pH was recorded every 4 h for 24 h following each morning meal.

[2]The acclimation ration consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) with 0.75% native prairie grass hay fed in both the morning (1.1105 g starch/kg BW) and evening (0.0314 g starch/kg BW).

[3]The concentrate challenge consisted of 1.0% BW concentrate (2.12 g starch/kg BW; Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) without hay for the morning meal followed by an evening meal of 0.5% BW native prairie grass hay (0.0209 g starch/kg BW).

* Indicates a difference in cecal pH between the acclimation period and concentrate challenge at that time point relative to the morning meal ($P < 0.05$).

[A,B,C] Differing capital letters indicate a difference in cecal pH during the acclimation period between given time points ($P < 0.05$).

[a,b,c] Differing lowercase letters indicate a difference in cecal pH following the concentrate challenge between given time points ($P < 0.05$).

As expected, there was an effect of time on cecal pH ($P < 0.0001$). Similar patterns in cecal pH following the morning meal were observed during both the acclimation and challenge periods. Patterns were characterized by decreased cecal pH by h 4 following the morning meal, the minimum cecal pH detected was reached by either h 4 or 8, this was followed by an increase at h 12, and cecal pH returned to h 1 values by h 24 only following the concentrate challenge. Medina et al. (2002) and Willard et al. (1977) observed similar patterns in cecal pH during the first 11 to 12 h after feeding. Typically, passage rate of nylon mobile bags through the pre-cecal tract is approximately 6 h (de Fombelle et al., 2004). This may explain why cecal pH in the current experiment decreased from the time of meal feeding until h 8, as the contents of the meal had presumably reached the cecum and acidic products of fermentation, it would seem VFA and lactate, were being produced. Production of lactate and VFAs were likely greater following the concentrate challenge, as cecal pH at h 12 following the morning meals during the acclimation period (7.03 ± 0.04) was greater than at h 12 following the concentrate challenge (6.86 ± 0.061; $P = 0.009$).

The minimum mean cecal pH observed in this experiment (6.66 ± 0.059) occurred at h 8 following the concentrate challenge and was greater than the minimum values obtained by Medina et al. (2002; approximately 6.5) for cecally cannulated horses fed a diet containing 3.4 g starch/kg BW in a morning meal, Brokner et al. (2012; approximately 6.47) for cecally cannulated horses following a concentrate meal containing 2 g starch/kg BW, and Goodson et al. (1988; approximately 6.4) in a cecally cannulated pony consuming a forage ration. The minimum cecal pH obtained in the current experiment was similar to minimum values reported for horses fed a ration of hay (Brokner et al., 2012; approximately 6.63), and less than the minimum cecal pH recorded by Medina et al. (2002) and Willard et al. (1977) for horses consuming a meal of forage (approximately 6.95 and 6.75, respectively). Starch levels in the current experiment were similar to those fed by Brokner et al. (2012), and similarities in cecal pH were not unexpected, supporting the idea that starch is a large determinant in cecal pH. Because forage rations are the most natural feedstuff for the equine and are typically considered safe, the similarities in cecal pH between experiments feeding only forage and the current experiment suggest that the concentrate challenge used in this experiment did not elicit changes

in cecal pH that would be abnormal to the equine hindgut. A lack of clinical symptoms or any physiological evidence of gastrointestinal distress during the monitoring period in these horses supports this conclusion.

There was not an effect of ration by sampling time point on cecal pH ($P = 0.11$), which was the effect of primary interest. Despite the fact that the level of dietary starch administered during the concentrate challenge was within the limit reported for digestive capacity of the foregut (Potter et al., 1992), at h 12 cecal pH was less following the morning meal of the concentrate challenge (6.86 ± 0.061) than it was at the same time point during the acclimation period (7.03 ± 0.04; $P = 0.009$). Because pre-cecal digestibility of starch and sugar is not complete (Varloud et al., 2004), some starch will reach the hindgut. Potter et al. (1992) identified 3.4 g starch/kg BW as the upper limit for starch digestion in the foregut, beyond which starch flow to the hindgut increases dramatically. It was apparent that at levels fed in this experiment (2.12 g starch/kg BW), the starch that escaped pre-cecal digestion impacted hindgut pH despite being within the limits of pre-cecal digestive capacity as determined by Potter et al. (1992).

Immediately after cecal samples were collected at h 12, 0.75% BW hay was fed. A lesser cecal pH was observed during the acclimation period at h 16 following the morning meals (6.93 ± 0.04) compared to h 12 (7.03 ± 0.04; $P = 0.02$). Presumably, hay from the evening meal had reached the cecum by h 16 to cause the decrease in cecal pH observed at that time. Despite feeding 0.5% BW hay at h 12 on the day of the concentrate challenge, a similar decline in cecal pH was not observed from h 12 (6.86 ± 0.06) to 16 (6.99 ± 0.06). Instead, a differing pattern over time was detected, as cecal pH steadily increased from h 12 to 16 ($P = 0.05$) and was further increased by h 24, at which time cecal pH was similar to those values obtained at h 1 ($P = 0.9$). Because cecal pH was measured every 4 h, it is possible that alterations in cecal pH were simply unobserved as the smaller hay meal fed at h 12 following the concentrate challenge may have passed more quickly through the cecum (de Fombelle et al., 2004), elicited changes of smaller magnitude, and thus have been undetected 4 h following the hay meal.

Day nested within ration (Table 2.3), as well as day by sampling time point nested within ration (Table 2.4.), had an effect on cecal pH ($P < 0.001$). Although these effects were not a primary focus of this experiment, it is important to note that there were daily variations and day tended to have an effect on cecal pH throughout this and subsequent experiments. Despite feeding the acclimation diet for 18 d prior to sampling to allow time for gastrointestinal

acclimation, daily differences in cecal pH indicate that variables beyond those controlled in this study had an impact on biochemical parameters in the cecum.

Table 2.3. Mean cecal pH[1] (± SEM) in horses (n = 9) from d 19 to 21 on an acclimation ration[2] and following a concentrate challenge[3] on d 22

Day	Cecal pH
19	7.22 ± 0.040
20	6.95 ± 0.039
21	6.91 ± 0.039
22	6.98 ± 0.039

[1]Cecal pH was recorded at h 1, 4, 8, 12, 16, 20, and 24 relative to the morning meal.

[2]The acclimation ration consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed with 0.75% native prairie grass hay for the morning meal (1.1105 g starch/kg BW) followed by an evening meal of 0.75% native prairie grass hay (0.0314 g starch/kg BW).

[3]The concentrate challenge consisted of 1.0% BW concentrate (2.12 g starch/kg BW) without hay for the morning meal followed by an evening meal of 0.5% BW native prairie grass hay (0.0209 g starch/kg BW).

Table 2.4. Mean cecal pH (± SEM) in horses (n = 9) at each sampling time from d 19 to 21 of an acclimation ration[1] and following a concentrate challenge[2] on d 22

	Cecal pH						
Day	+1	+4	+8	+12	+16	+20	+24
19	7.41 ± 0.065^{aA}	7.02 ± 0.062^{bA}	6.88 ± 0.061^{cA}	7.17 ± 0.064^{dA}	7.17 ± 0.064^{bdeA}	7.4 ± 0.066^{aA}	7.52 ± 0.067^{aA}
20	7.55 ± 0.067^{aA}	6.74 ± 0.06^{bcB}	6.68 ± 0.059^{bB}	6.85 ± 0.061^{cdB}	6.79 ± 0.060^{cB}	6.97 ± 0.062^{dB}	7.11 ± 0.063^{eB}
21	7.16 ± 0.064^{aB}	6.67 ± 0.059^{bB}	6.59 ± 0.059^{bB}	7.06 ± 0.063^{aceA}	6.84 ± 0.061^{dBC}	6.99 ± 0.062^{eB}	7.10 ± 0.063^{aeB}
22	7.24 ± 0.064^{aeB}	6.78 ± 0.060^{bcB}	6.66 ± 0.059^{bB}	6.86 ± 0.061^{cB}	6.99 ± 0.062^{dC}	7.09 ± 0.063^{dB}	7.23 ± 0.064^{eB}

[1]The acclimation ration consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed with 0.75% native prairie grass hay for the morning meal (1.1105 g starch/kg BW) followed by an evening meal of 0.75% native prairie grass hay (0.0314 g starch/kg BW).

[2]The concentrate challenge consisted of 1.0% BW concentrate (2.12 g starch/kg BW) without hay for the morning meal followed by an evening meal of 0.5% BW native prairie grass hay (0.0209 g starch/kg BW).

[a,b,c] Differing lowercase letters within a row are indicative of a difference in cecal pH ($P < 0.05$).

[A,B,C] Differing capital letters within a column are indicative of a difference in cecal pH ($P < 0.05$).

When dietary starch was abruptly increased from 1.06 g starch/kg BW during the acclimation period to 2.12 g starch/kg BW during the concentrate challenge, gender, gender by ration, gender by day nested within ration, gender by sampling time point, gender by ration by sampling time point, and gender by day by sampling time point nested within ration did not have an effect on cecal pH ($P \geq 0.30$).

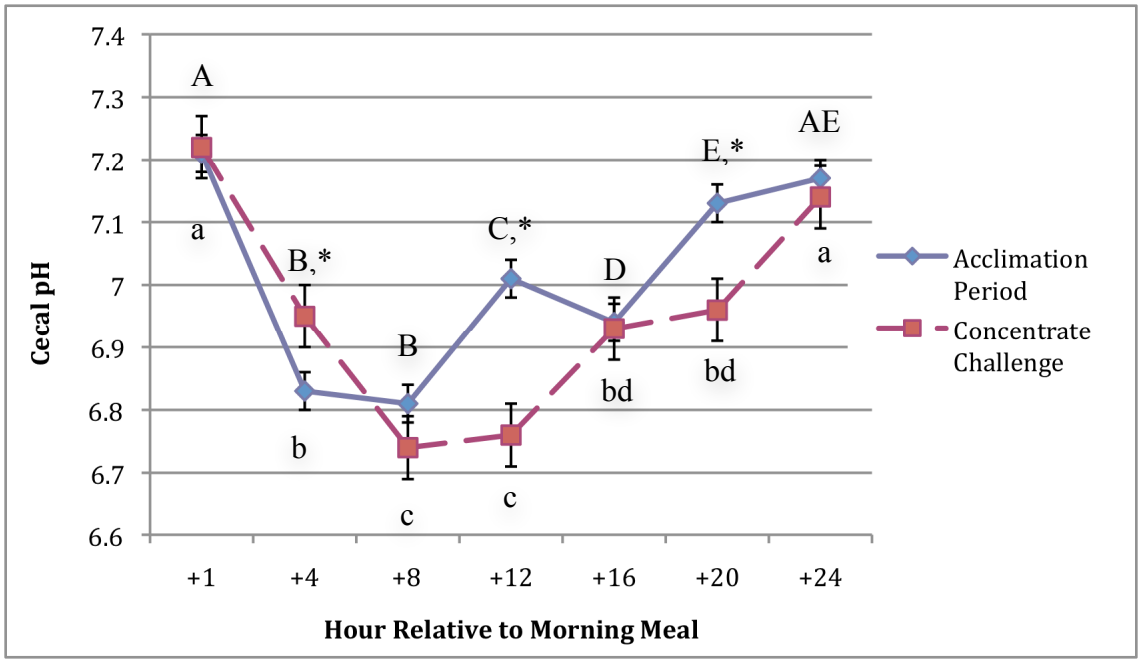
2.4.2 Experiment 2

As with Exp. 1, sampling time point had an effect on cecal pH during Exp. 2 ($P < 0.0001$), as meal consumption appeared to impact cecal dynamics. As anticipated, and unlike results from Exp. 1, diet had an effect on cecal pH, with a decrease in mean cecal pH following the concentrate challenge (6.95 ± 0.02) compared to the mean cecal pH during the acclimation period (7.01 ± 0.01; $P = 0.01$).

Contrary to results observed during Exp. 1, ration by sampling time point had an effect on cecal pH in Exp. 2 (Fig. 2.3; $P < 0.001$) and there was not a difference between cecal pH at h -1 and 1 ($P > 0.84$). Cecal pH during the acclimation period of Exp. 2 followed a similar pattern to Exp. 1. During Exp. 2 cecal pH was slightly above neutral (7.21 ± 0.03) at h 1 and decreased by h 4 to 6.83 ± 0.03 ($P < 0.0001$). Minimum cecal pH was reached at h 4 and 8 (6.83 ± 0.03 and 6.81 ± 0.03, respectively), and, excluding a decline in cecal pH from h 12 (7.01 ± 0.03) to 16 (6.95 ± 0.03; $P = 0.05$) presumably caused by hay consumption at h 12, a gradual increase in cecal pH persisted until h 24 at which point mean cecal pH was similar to pre-prandial values ($P > 0.05$). Cecal pH values at h 1 following the morning meal during the acclimation and challenge periods were similar (7.21 ± 0.03 vs. 7.21 ± 0.05, respectively; $P > 0.98$). However, differences presumably due to dietary treatments became apparent at h 4 following the morning meal. While cecal pH decreased from h 1 to 6.95 ± 0.05 by h 4 following the concentrate challenge ($P < 0.0001$), the value was greater than mean cecal pH at h 4 following the morning meals during the acclimation period (6.83 ± 0.03; $P = 0.02$). Minimum values following the concentrate challenge were reached at h 8 and 12 (6.74 ± 0.04 and 6.76 ± 0.04, respectively), a 4 h delay compared to the minimum cecal pH values recorded during the acclimation period. Although cecal pH increased from h 12 to 16 (6.93 ± 0.05; $P < 0.0001$) and was no different than cecal pH at h 16 during the acclimation period ($P = 0.79$), cecal pH by h 20 following the concentrate challenge (6.96 ± 0.05) was again less than cecal pH at h 20 following the morning meals during the acclimation period (7.13 ± 0.03; $P = 0.002$). Perhaps the concentrate challenge meal, due to its larger size, was retained in the foregut for a longer period of time than the morning meals provided during the acclimation period (de Fombelle et al., 2004). This may have caused a 4 h lag between the dietary treatments, evidenced by differences noted at h 4 after the morning meals and postponed minimum cecal pH detected in horses following the concentrate challenge. Differences between cecal pH at h 20 could be attributed to increased residual lactate resulting

from an increased influx of starch, increased retention time in the cecum, and increased microbial fermentation following the concentrate challenge compared to during the acclimation period. However, lactate was not measured during Exp. 2.

Figure 2.3. Mean cecal pH[1] in horses (n = 9) from d 19 to 21 on an acclimation ration[2] and following a concentrate challenge[3] on d 22.



[1]Cecal pH was recorded every 4 h for 24 h following the morning meal.

[2]The acclimation ration consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) with 0.75% native prairie grass hay fed in both the morning (1.119 g starch/kg BW) and evening (0.04468 g starch/kg BW).

[3]The concentrate challenge consisted of 1.25% BW concentrate (2.67 g starch/kg BW; Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) without hay followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

* Indicates a difference in cecal pH during the acclimation period and concentrate challenge at that h relative to the morning meal ($P < 0.05$).

[A,B,C] Differing capital letters indicate a difference in cecal pH during the acclimation period between given time points ($P < 0.05$).

[a,b,c] Differing lowercase letters indicate a difference in cecal pH following the concentrate challenge between given time points ($P < 0.05$).

Cecal pH during acclimation and challenge periods ranged from a minimum of 6.74 ± 0.045 (h 8 following the concentrate challenge) to a maximum of 7.21 ± 0.048 (at h 1 following the morning meal during the challenge period) in this experiment, which is similar to cecal pH

ranges observed by others following forage meals fed to cannulated horses (Brokner et al., 2012; Willard et al., 1977) and a cannluated pony (Goodson et al., 1988). Despite a modified post-prandial pattern of cecal pH changes following the concentrate challenge, because the range of cecal pH obtained in Exp. 2 is similar to cecal pH values reported in forage-fed horses and ponies, and because no clinical manifestations of gastrointestinal upset were noted, the alterations in cecal pH following an abrupt increase from 1.06 g starch/kg BW to 2.67 g starch/kg BW appear to be well-tolerated by horses.

Dietary starch was supplied in the concentrate challenge of this experiment at 2.67 g starch/kg BW, which is within the quantity of starch the equine foregut is reportedly capable of digesting (Potter et al., 1992). Because small intestinal digestion of starch is not 100% (Varloud et al., 2004), some starch likely reached and was fermented in the cecum during this experiment. Potter et al. (1992) did not acclimate horses to a ration for an extended period of time, which, according to Dyer et al. (2009), is required for small intestinal adaptation, which would be necessary to determine absolute maximal pre-cecal digestive capacity. Other limitations in comparing the results of the study performed by Potter et al. (1992) to the current study were that only 2 horses were utilized and abrupt increases in starch were not performed, thus limiting confidence in their conclusion that feeding less than 3.4 g starch/kg BW during an abrupt increase in dietary starch will not cause a perturbation in the hindgut. Findings from the current study, coupled with those of Potter et al. (1992) and Dyer et al. (2009) begs the question: at what point does an abrupt increase in dietary starch induce sufficient changes in biochemical parameters to threaten the hindgut environment? There is a void in the literature regarding threshold values for biochemical parameters in the cecum of the equine above which virtually assure that the horse will develop laminitis. The same is true regarding abrupt increases in dietary starch; threshold values have not been determined to delineate a safe versus an unsafe increase in dietary starch. It would seem prudent to establish a definition of laminitis in regards to biochemical parameters in the cecum, thus allowing the determination of what point starch overload becomes an issue for the health of the horse.

Gender had an effect on cecal pH, as mares (7.02 ± 0.02) exhibited greater cecal pH than geldings (6.95 ± 0.02; $P = 0.04$) across all time points. Although collection day nested within diet irrespective of gender did not have an effect on cecal pH, indicating daily variation was minimal, differences were apparent when the effect of gender by day nested within diet on cecal

pH was analyzed (Table 2.5; $P < 0.01$). Mares displayed greater daily variation during the acclimation period and a greater pH following the concentrate challenge than geldings, which could be partially attributed to the fact 4 mares were used, while 5 geldings were included in the study, or, perhaps, that there are differences between the equine genders that have not yet been identified. Gender by diet, gender by sampling time point, gender by diet by sampling time point, and gender by day by sampling time point nested within diet did not have an effect on cecal pH.

Table 2.5. Mean cecal pH[1] (± SEM) in mares (n = 4) and geldings (n = 5) from d 19 to 21 of an acclimation ration[2] and following a concentrate challenge[3] on d 22

	Cecal pH	
Day	Mares	Geldings
19	6.97 ± 0.030	7.0 ± 0.027
20	7.03 ± 0.030	6.99 ± 0.027
21	7.11 ± 0.031	6.98 ± 0.027
22	7.0 ± 0.030	6.91 ± 0.027

[1]Cecal pH was recorded at h 1, 4, 8, 12, 16, 20, and 24 relative to the morning meal.

[2]The acclimation ration consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) with 0.75% native prairie grass hay fed in both the morning (1.119 g starch/kg BW) and evening (0.04468 g starch/kg BW).

[3]The concentrate challenge consisted of 1.25% BW concentrate (2.67 g starch/kg BW; Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) without hay for the morning meal followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

2.4.3 Experiment 3

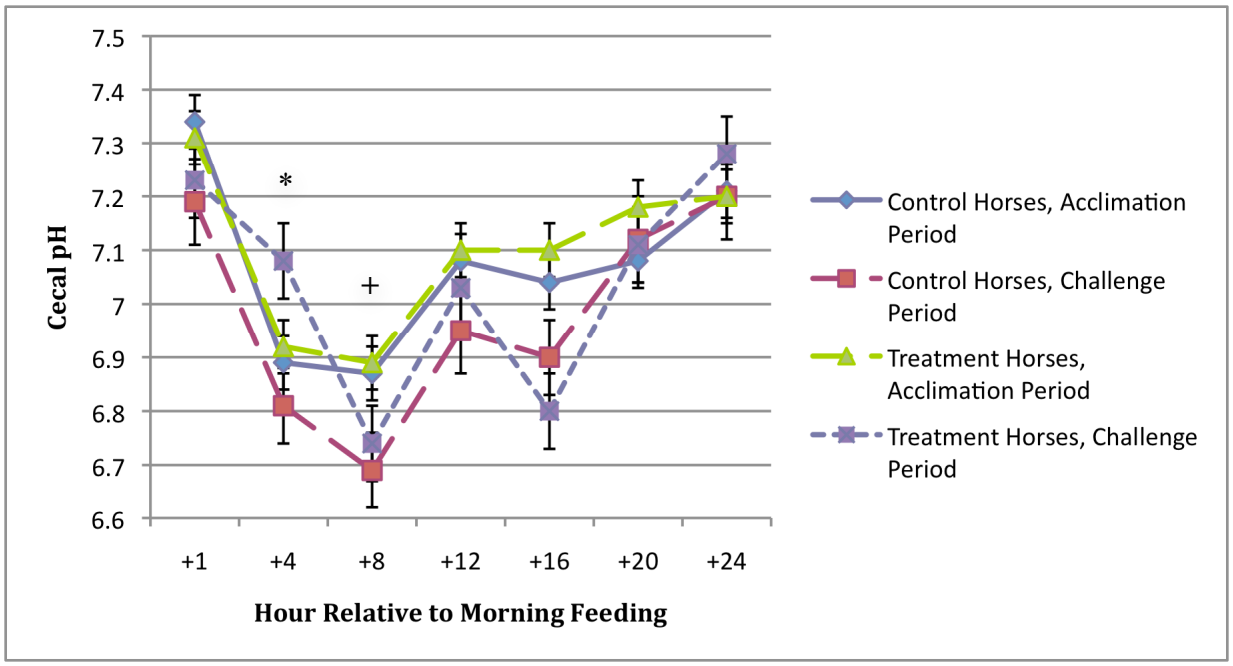
As anticipated and similar to results obtained during Exp. 2, ration, sampling time point, and ration by sampling time point all had an effect on cecal pH during Exp. 3 ($P \leq 0.003$). Cecal lactate, total VFA, the ratio of (acetate+butyrate)/propionate, acetate, propionate, butyrate, valerate, and isovalerate were also impacted by these parameters ($P < 0.05$). Effects of ration and ration by sampling time point on each parameter ultimately confirm that abruptly increasing starch from 1.06 g/kg BW to 2.67 g/kg BW is sufficient to induce alterations in the hindgut, which were necessary to test the potential value of adding a proprietary supplement. Treatment

by ration by sampling time point did not have an effect on any variables ($P \geq 0.13$), excluding isovalerate ($P = 0.01$).

Cecal pH ranged from a minimum of 6.69 ± 0.07 (h 8 following the concentrate challenge in the CON group; Fig. 2.4) to a maximum of 7.34 ± 0.05 (h 1 following the morning meals during the acclimation period for CON horses). At h 1 following the morning meals during both the acclimation period and concentrate challenge, cecal pH values were slightly above neutral for for horses in both the CON and PS groups, and they were similar to cecal pH values obtained in Exp. 2. By h 4 following the morning meals of the acclimation period for the CON (6.87 ± 0.05) and PS (6.92 ± 0.05) group, and by h 4 following the concentrate challenge in the CON group (6.81 ± 0.07), cecal pH decreased compared to h 1 values ($P < 0.0001$). However, horses in the PS group had greater cecal pH at h 4 following the concentrate challenge (7.08 ± 0.07) in comparison to CON horses (6.81 ± 0.07; $P = 0.009$), which coincided with less cecal lactate [1.25 ± 0.55 mmol/L (PS) vs. 6.29 ± 3.1 mmol/L (CON); $P = 0.02$; Fig. 2.5]. The minimum cecal pH observed was reached at h 4 and maintained at h 8 following the morning meals during the acclimation period for horses in the CON [6.89 ± 0.05 (h 4) v. 6.87 ± 0.05 (h 8); $P = 0.78$] and PS [6.92 ± 0.05 (h 4) v. 6.89 ± 0.05 (h 8); $P = 0.51$] groups and at h 8 following the concentrate challenge of the CON group [6.81 ± 0.07 (h 4) v. 6.69 ± 0.07 (h 8); $P = 0.21$], while the initial minimum cecal pH following the concentrate challenge of the PS group was observed at h 8 (6.74 ± 0.07) followed by a second equally depressed cecal pH at h 16 (6.8 ± 0.07; $P = 0.049$). For horses in CON and PS groups, cecal pH at h 8 following the concentrate challenge was less than the cecal pH observed at h 8 following the morning meals during the acclimation period ($P \leq 0.04$). This coincided with increased cecal lactate in PS (20.93 ± 10.27) and CON (18.93 ± 8.3; $P < 0.001$) groups at h 8 following the concentrate challenge. There were no differences in minimum cecal pH and maximum cecal lactate observed between the PS and CON groups following the concentrate challenge ($P > 0.05$). By h 12 following the morning meals, cecal pH in each treatment and dietary group combination had increased above their respective h 8 values ($P \leq 0.007$), while cecal lactate at h 12 following the concentrate challenge was greater for both CON (1.14 ± 0.56 mmol/L) and PS (0.72 ± 0.32 mmol/L) horses compared to values at h 12 following morning meals during the acclimation period (0.17 ± 0.06 and 0.11 ± 0.04, respectively; $P < 0.0001$). There were differences between cecal VFA after morning meals during the acclimation period compared to those obtained following the concentrate challenge, as

total VFA, acetate, propionate, butyrate, valerate, isovalerate, and isobutyrate (Fig. 2.6, 2.7, 2.8, 2.9, 2.10, 2.11, and 2.12) increased in horses in both the PS and CON treatment groups at h 12 and 16 following the concentrate challenge compared to values obtained following morning meals during the acclimation period ($P < 0.05$). Possibly, the difference in total and individual VFA concentration in the cecum between the acclimation and challenge periods of both groups is that, due to the larger meal size, more substrate was available for fermentation following the concentrate challenge than during the acclimation period, ultimately resulting in continued fermentation and accumulation of VFA at h 12 and 16. Cecal lactate was greater following the concentrate challenge in the CON group at h 16 (0.25 ± 0.12 mmol/L; $P = 0.05$;) and at h 20 (0.16 ± 0.08 mmol/L; $P = 0.02$;) than following the morning meals during the acclimation period for the same horses (0.11 ± 0.04 mmol/L and 0.05 ± 0.02 mmol/L, respectively). At h 24 following the concentrate challenge, horses in the PS group had greater cecal lactate (0.14 ± 0.06 mmol/L) compared to the same horses at h 24 during the acclimation period (0.04 ± 0.01 mmol/L; $P = 0.0006$). However, there were no observable differences in pH at those times (h 16, 20, and 24; $P \geq 0.07$). There was a difference noted in the pattern of cecal pH observed over time between experiments 2 and 3. From h 12 to h 16 following the morning meals during the acclimation period of Exp. 3, cecal pH did not decline ($P \geq 0.57$), although a decline at h 16 was noted during Exp. 2 ($P = 0.05$). There was also a difference in the pattern of cecal pH over time between treatment groups in Exp. 3. At h 16 following the concentrate challenge, cecal pH in the PS group was comparable to the pH noted at h 8, yet values remained similar to cecal pH of the CON group at h 16 following the concentrate challenge ($P = 0.36$). This decrease in pH did not correspond with increased cecal lactate at h 16 compared to the h 12 value ($P = 0.70$).

Figure 2.4. Mean cecal pH[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Cecal pH was recorded every 4 h for 24 h following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

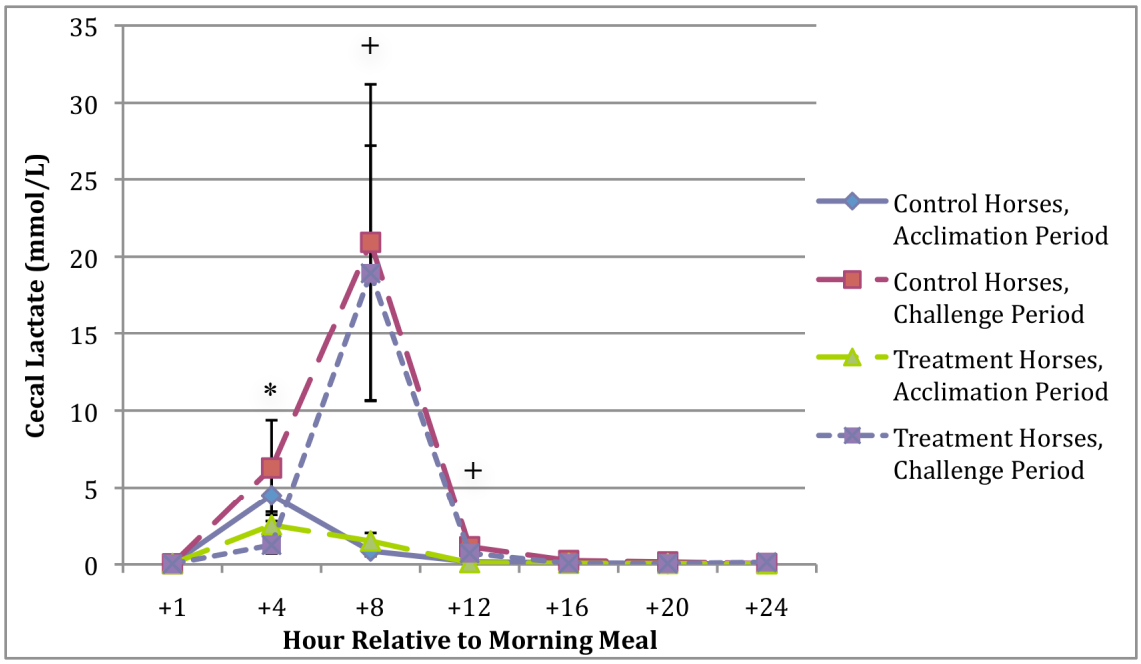
[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Figure 2.5. Mean cecal lactate[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Cecal lactate was recorded every 4 h for 24 h following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

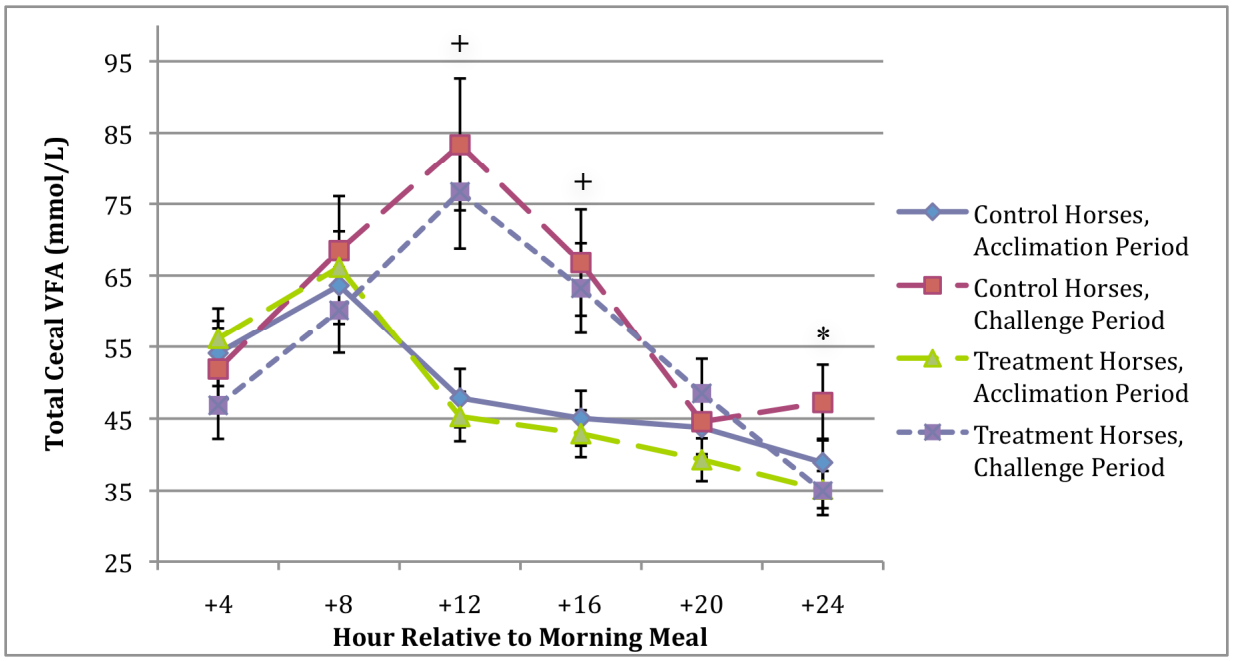
[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Figure 2.6. Mean total cecal VFA[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Total cecal VFA was recorded every 4 h from h 4 to 24 following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

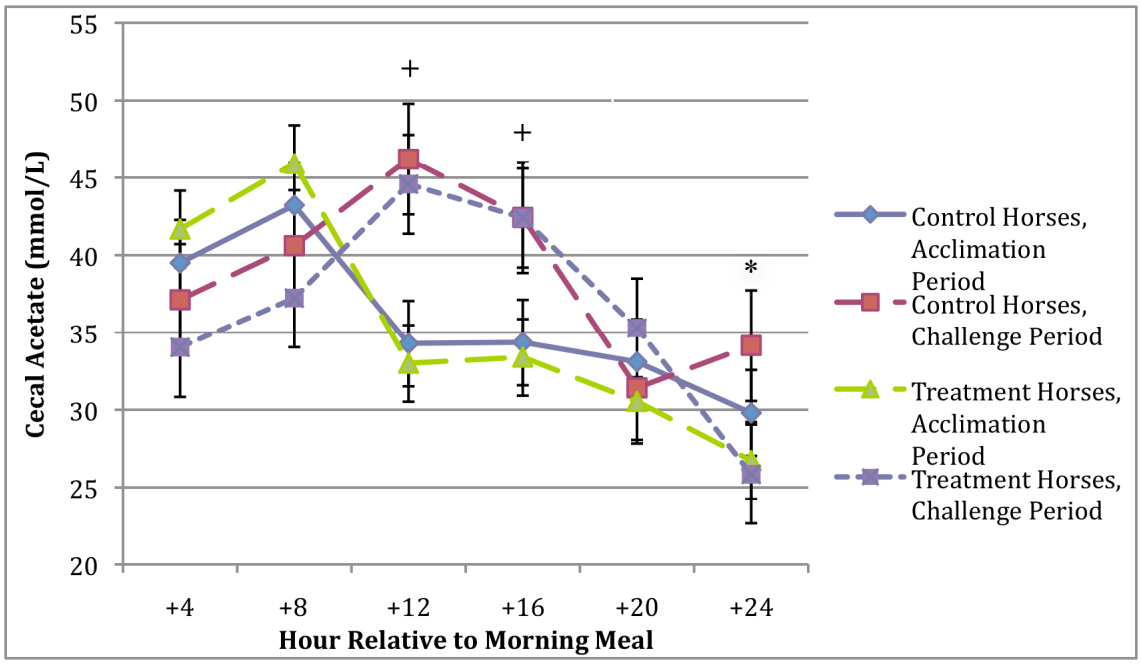
[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Figure 2.7. Mean cecal acetate[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Cecal acetate was recorded every 4 h from h 4 to 24 following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

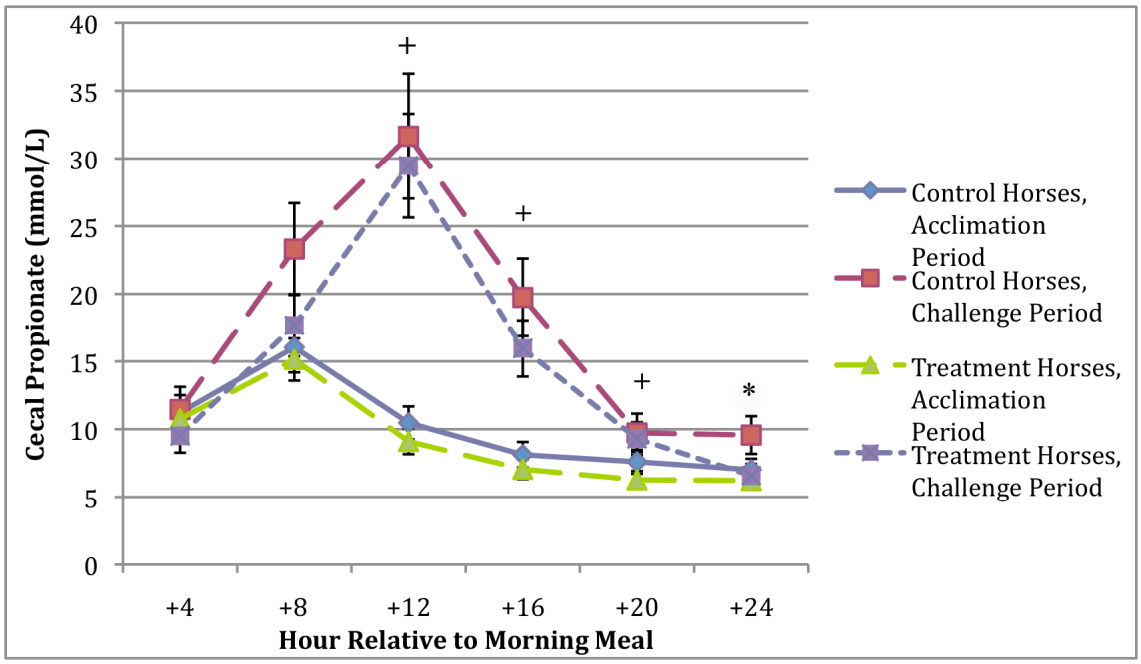
[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Figure 2.8. Mean cecal propionate[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Cecal propionate was recorded every 4 h from h 4 to 24 following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

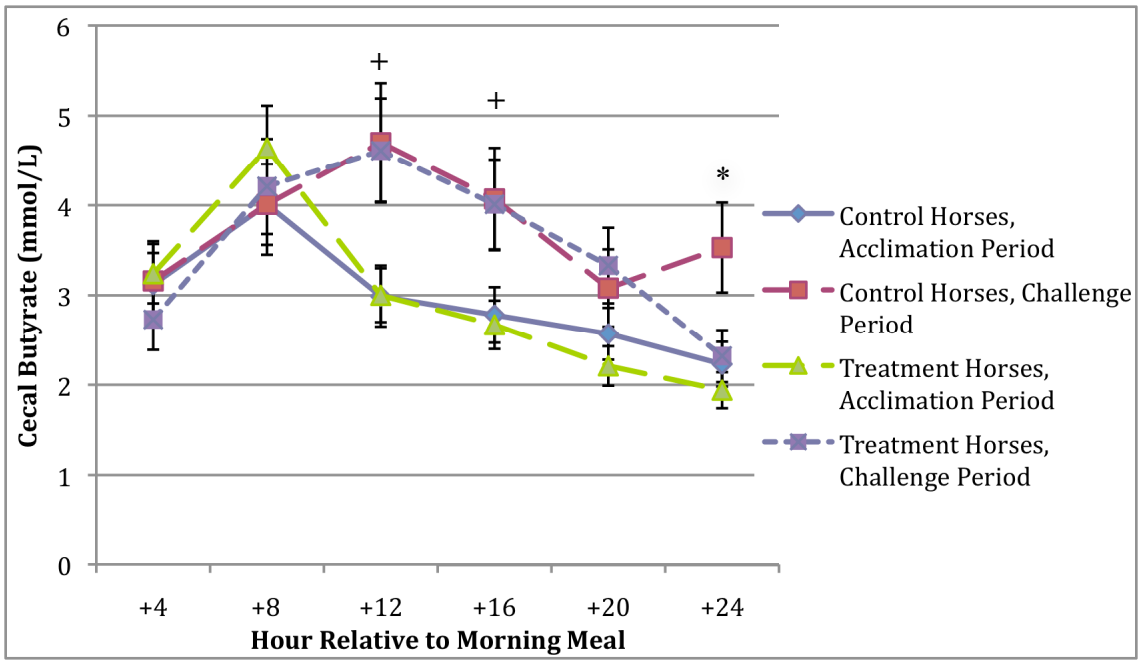
[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Figure 2.9. Mean cecal butyrate[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Cecal butyrate was recorded every 4 h from h 4 to 24 following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

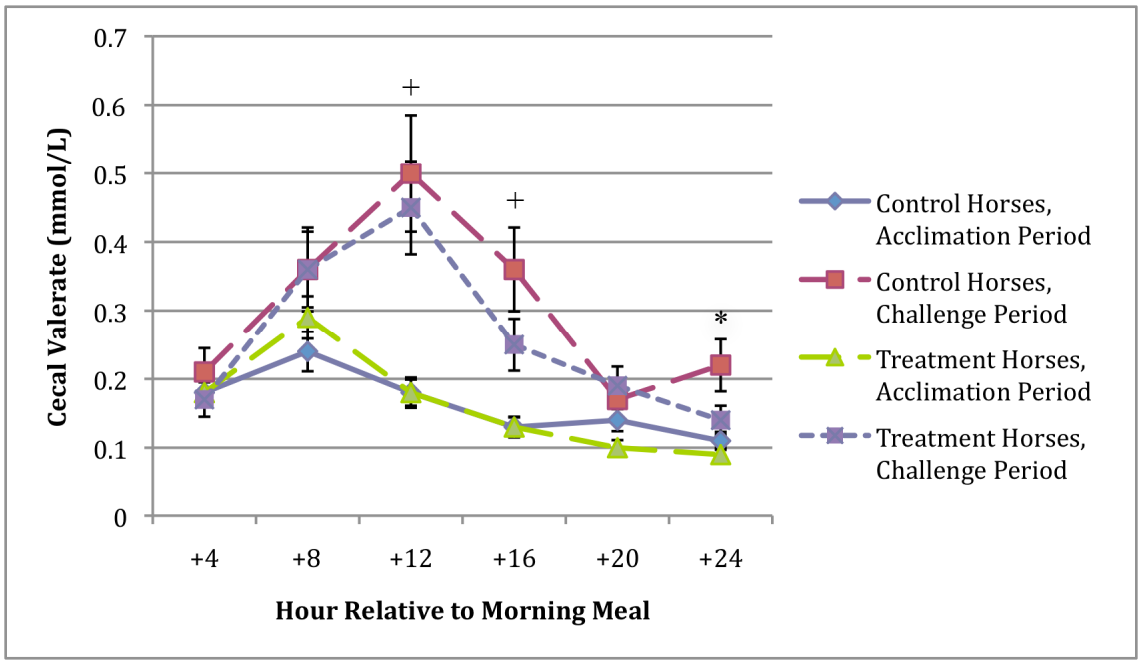
[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Figure 2.10. Mean cecal valerate[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Cecal valerate was recorded every 4 h from h 4 to 24 following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

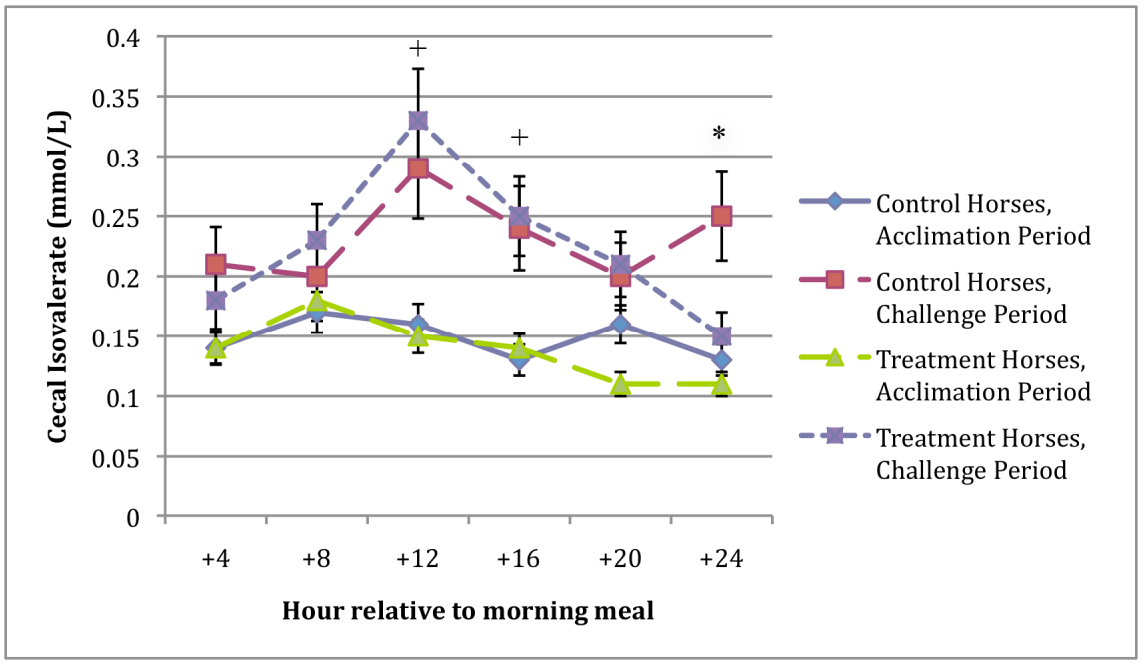
[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Figure 2.11. Mean cecal isovalerate[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Cecal isovalerate was recorded every 4 h from h 4 to 24 following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

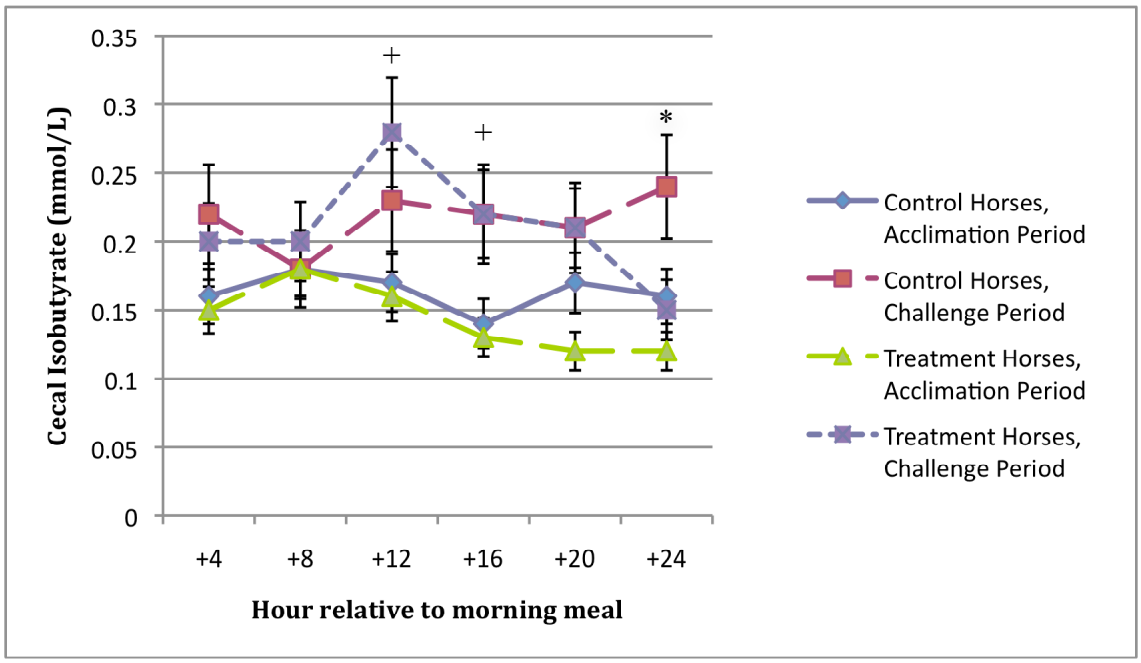
[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Figure 2.12. Mean cecal isobutyrate[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Cecal isovalerate was recorded every 4 h from h 4 to 24 following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Medina et al. (2002) supplemented live yeast to horses fed a meal containing 3.4 g starch/kg BW and observed lesser concentrations of lactate in the cecum for 12 h following the meal compared to horses fed the same meal, but without live yeast. Live yeast was not fed in the current study, but instead a product of yeast fermentation was. While it is unknown whether live yeast and yeast fermentation products affect the cecum in a similar manner, there were additional differences (diet, individual horses used, etc.) in the current experiment that, in conjunction with differences in the supplement utilized, render it impossible to directly compare the results obtained herein to those of Medina et al. (2002). Ultimately, the greatest concentration of cecal lactate observed throughout Exp. 3 occurred at h 8 following the concentrate challenge for the CON group (20.93 ± 10.27 mmol/L) and was similar to the concentration of lactate reported in horses fed a ration containing primarily forage that contained 2.52 g starch/kg BW (Medina et al., 2002). While starch was not fed at a level sufficient to induce serious digestive distress in this experiment, our ability to detect any benefit of feeding this proprietary supplement likely would be enhanced by inducing pronounced digestive distress or potentially by increasing the dose of the proprietary supplement.

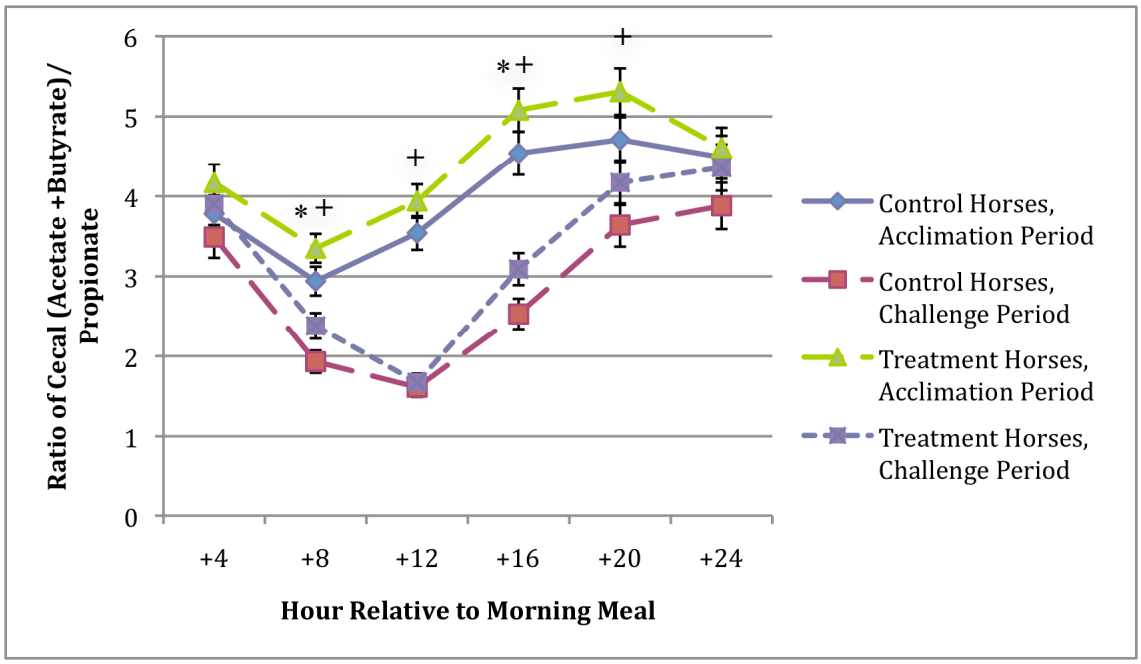
Total cecal VFA ranged from 35.09 ± 2.7 mmol/L at h 8 following the concentrate challenge for the PS group to 83.33 ± 9.86 mmol/L at h 12 following the concentrate challenge for the CON group in Exp. 3 (Fig. 2.6). The maximum value obtained for mean total VFA in each group was observed at h 12 following the concentrate challenge (CON 83.33 ± 9.86 mmol/L v PS 79.58 ± 8.59 mmol/L), and were similar to values reported by de Fombelle et al. (2001) while horses were fed an all-forage diet. Presumably, the similarities in total cecal VFA between the current study and those reported by de Fombelle et al. (2001) are due to conversion of some starch reaching the hindgut into lactate rather than directly into VFA in the current experiment. Additionally, because the concentration of VFA in the cecum is a function of both production and absorption, of which absorption has been demonstrated to increase linearly as the concentration of VFA increases (Bergman, 1990), it is likely that increased VFA were absorbed effectively by the cecum rather than accumulating beyond tolerable levels, hence, the similarity

to forage-fed horses. However, studies have not examined the rate of VFA production and absorption following an abrupt increase in dietary starch.

Individual VFA dynamics were similar to each other, with concentration of VFA present in the hindgut being the primary variant as they ordered from numerically greatest to least concentration: acetate > propionate > butyrate > valerate > isovalterate ≅ isobutyrate. Generally values across all ration by treatment combinations within each VFA category were similar to one another at h 4 and 8, followed by an increase in both CON and PS groups at h 12 and 16 following the concentrate challenge compared to the same time points during the acclimation period. By h 20 following the concentrate challenge concentration of individual VFAs were again similar to values recorded during the acclimation period for PS and CON groups. Alterations in the VFA profile occurred at h 24 following the concentrate challenge in the CON group, such as an increase in cecal total VFA, butyrate, valerate, isovalerate, and isobutyrate, in comparison to horses in the PS group. However, these values noted at h 24 were less than the maximum values obtained for each respective variable throughout Exp. 3 and did not coincide with decreased cecal pH or outward manifestations of a disturbance in the hindgut. Thus, the differences at h 24, although statistically significant, did not appear to cause a disturbance in hindgut health. The fermentation of fibrous material appeared to be altered following the concentrate challenge in the CON group compared to the PS group, as the ratio of (acetate+butyrate)/propionate was decreased at h 8 and h 16 for the CON group in comparison to the PS group, although individual VFAs did not change ($P \leq 0.05$; Fig. 2.13). Ration also had an effect on the ratio of (acetate+butyrate)/propionate, as the ratio was decreased following the concentrate challenge at h 8, 12, 16, and 20 compared to the respective values during the acclimation period ($P < 0.05$). A decreased ratio of (acetate+butyrate)/propionate is indicative of impaired fermentation of fiber, as fiber is preferentially fermented to acetate rather than propionate. Thus, the addition of this proprietary supplement to a morning meal of concentrate during this acclimation period may have enhanced subsequent fermentation of fiber following the concentrate challenge. Other studies, both in vivo and in vitro, utilizing yeast preparations and extracts have reported few differences in VFA profile following addition of said yeast additives to cecal fluid or to the diets of horses fed concentrates (McDaniel et al., 1993; Medina et al., 2002). Conversely, Jouany et al. (2008; 2009) reported that supplementation of a live yeast

culture improved fiber degradation, which is in accordance with our results, as the (acetate+butyrate)/propionate ratio improved in both studies with supplementation.

Figure 2.13. Ratio of mean cecal (acetate+butyrate)/propionate[1] in horses assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.



[1]Cecal (acetate+butyrate)/propionate was recorded every 4 h from h 4 to 24 following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

*Indicates a difference between the CON and PS group following the concentrate challenge ($P < 0.05$).

+Indicates a difference between the acclimation and challenge periods for PS and CON groups ($P < 0.05$).

Treatment by gender by ration had an effect on cecal pH ($P = 0.03$; Table 2.6). Gender and its interaction with several variables had an unexplained effect on certain VFA analyses (Table 2.7). Geldings exhibited greater overall cecal butyrate and propionate ($P = 0.05$) and a lesser pH following the concentrate challenge for the CON group ($P < 0.05$) than did mares, which has not been reported previously but concurs with the cecal pH data obtained during Exp. 2. Addition of the proprietary supplement to the ration of geldings during the acclimation period increased cecal pH following the concentrate challenge compared to cecal pH of geldings in the CON group ($P < 0.05$).

Table 2.6. Mean cecal pH[1] (± SEM) in geldings (n = 5) and mares (n = 4) assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.

	Mares, CON	Geldings, CON	Mares, PS	Geldings, PS
Acclimation period	7.11	7.04	7.13	7.07
Concentrate challenge	7.06^{A}	6.90^{*+B}	7.02^{*A}	7.06^{+A}

[1]Cecal pH was recorded every 4 h until 24 h following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

* Indicates a difference within the column ($P < 0.05$).

$^{+}$ Indicates a difference between treatment groups within gender ($P < 0.05$).

^{A,B} Indicates a difference between genders within the treatment group ($P < 0.05$).

Table 2.7. The effect of gender and its interaction with diet, sampling time, and treatment on individual VFAs in the cecum of geldings (n = 5) and mares (n = 4) assigned to the control (CON; n = 4) and proprietary supplement (PS; n = 5) group[2] from d 19 to 21 of an acclimation diet[3] and following a concentrate challenge[4] on d 22.

Response Variable	Effect				
	Gender	Diet x Gender	Diet x Sampling time x Gender	Treatment x Diet x Gender	Treatment x Diet x Sampling time x Gender
(Acetate + Butyrate)/Propionate	(P = 0.0052)	(P = 0.0084)	(P = 0.0078)		(P = 0.0007)
Propionate	(P = 0.05)				
Butyrate	(P = 0.05)				
Valerate		(P = 0.02)			
Isovalerate		(P = 0.02)		(P = 0.04)	

[1]Cecal VFA was recorded every 4 h from h 4 to 24 following the morning meal.

[2]Alfalfa pellets were administered as a placebo to horses in the control group (CON) or used as the carrier for a proprietary supplement containing products of yeast fermentation (PS treatment group).

[3]The acclimation diet consisted of 0.5% BW concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) top-dressed with either a CON or PS pellet (10 g/100 kg BW) and fed with 0.75% native prairie grass hay for the morning meal (1.119 g starch/kg BW). This was followed by an evening meal of 0.75% native prairie grass hay (0.04468 g starch/kg BW).

[4]The concentrate challenge consisted of 1.25% BW of the same concentrate (Omolene 200, Purina Animal Nutrition, LLC, Gray Summit, MO) fed without hay and without either pellet (CON or PS) for the morning meal (2.67 g starch/kg BW). This was followed by an evening meal of 0.5% BW native prairie grass hay (0.0298 g starch/kg BW).

2.4.4 Implications

Based on the results obtained during the 3 experiments presented herein, abruptly increasing the level of dietary starch from 1.119 g starch/kg BW to 2.67 g starch/kg BW results in alterations of the biochemical profile in the equine cecum. This model appears to induce changes in the hindgut, but it does not elicit clinical symptoms of hindgut dysfunction, which could be beneficial for future research attempting to study abrupt dietary alterations without harming horses. Although horses participating in these experiments did not show clinical signs of gastrointestinal distress, establishment of threshold biochemical values associated with digestive distress would be beneficial to confirm that the lack of clinical symptoms observed in this study was in fact due to a lack of damage to the hindgut, particularly following an abrupt increase in starch. Differences noted in cecal pH between mares and geldings in these studies indicate that gastrointestinal differences may exist between genders, and results may need to be considered separately for the genders.

During Exp. 3, the addition of the proprietary supplement to the ration of the equine decreased the concentration of lactate, increased pH, and decreased the concentration of VFA observed at specific time points in the cecum following an increase of dietary starch. However, the aforementioned biochemical parameters of all horses maintained a cecal environment similar to that reported in horses fed forage only rations, regardless of whether they received the proprietary supplement. Therefore, the supplement was likely not necessary in the case of increasing starch from 1.119 g starch/kg BW to 2.67 g starch/kg BW as none of the horses in these experiments experienced significant hindgut disturbances. To determine the extent of the supplement's usefulness, a more significant starch challenge may be required.

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